The 28th Annual Meeting of
Japan Society of Gene and Cell Therapy

JSGCT2022

Program & Abstracts

Date

Live Conference: July 14 – 16, 2022

Venue

Hakata International
Exhibition Hall & Conference Center
2-22-15 Toko Hakata, Fukuoka city, Fukuoka 812-0008, Japan

The 28th Annual Meeting of Japan Society of Gene and Cell Therapy 2022

Meeting President
Yoshikazu Yonemitsu

Member of the JSGCT Committee

Chairman of the Board of Director (BOD)
Ryuichi Morishita

Vice Chairman of BOD
Takashi Okada & Akihiro Kume & Toshiyoshi Fujiwara & Yoshikazu Yonemitsu

President-Elect
Hideki Mochizuki

Members of BOD

Kazunori Aoki, Yasuhiro Ikeda, Eriko Uchida, Makoto Otsu, Torayuki Okuyama, Noriyuki Kasahara, Yumi Kanegae, Katsuto Tamai, Tomoki Todo, Masahiro Toda, Koichi Nakao, Hironori Nakagami, Takafumi Nakamura, Yasutomo Nasu, Hiroshi Fukuara, Hiroyuki Mizuguchi, Kohnosuke Mitani, Shin-ichi Muramatsu, Hideki Mochizuki, Ei Yamada, Masato Yamamoto

JSGCT2022 Scientific Committee Chair: Yoshikazu Yonemitsu

Basic Science
Makoto Otsu, Fuminori Sakurai, Tomoki Todo

Gene transfer vectors
Toya Ohashi, Takashi Okada, Yumi Kanegae, Hiromi Hayashita-Kinoh, Hiroyuki Mizuguchi, Junichi Mineno

Genetic Diseases
Torayuki Okuyama , Masafumi Onodera

Cancer
Kazunori Aoki, Tomoki Todo, Takafumi Nakamura , Yasutomo Nasu, Toshiyoshi Fujiwara, Masato Yamamoto

Neurologic, ophthalmic, and músculo-skeletal diseases
Kohnosuke Mitani, Shin-ichi Muramatsu, Hideki Mochizuki

Cell therapy
Katsuto Tamai, Hironori Nakagami , Yui Harada

Gene editing & others
Kohnosuke Mitani, Ken-ichi Wada

Clinical Studies & Regulatory Science
Yasuhiro Ikeda, Akihiro Kume

JSGCT2022 Secretariat Office Yui Harada

Graduate School of Pharmaceutical Sciences, Kyushu University
3-1-1 Maidashi, Higashi-ku, Fukuoka-shi, 812-8582, Japan
E-mail: jsgct2022@c-linkage.co.jp https://www.c-linkage.co.jp/jsgct2022/

Japan Society of Gene and Cell Therapy Administrative Office

Secretary General
Makoto Otsu

Vice Secretary General
Yumi Kanegae, Hironori Nakagami

Welcome Message from the President

Dear colleagues:

Welcome to JSGCT2022 in Fukuoka, JAPAN!

It is my great pleasure to have you all at JSGCT2022- 28th Annual Meeting of Japan Society of Gene and Cell Therapy.

The theme of the annual meeting is ‘Opening the 2nd Quarter for Gene and Cell Therapy’.

Gene therapy, which was initially launched to great fanfare from first clinical trial for ADA deficiency in 1989, did not demonstrate desired clinical results and had been plagued by a series of side effects since 1999. While many researchers have abandoned gene therapy, some who believed in its potential persevered in their studies, finally resulting in the approval of some epoch-making new gene therapy drugs. Our missions in the next decade should include to expand these successes to great fruits for human healthcare, beyond the scope of government, industry, and academia.

We all look forward to the enthusiastic and heated discussions that will be a perfect match for Hakata’s hot summer days.

With my warmest regards,

Yoshikazu Yonemitsu, M.D., Ph.D.
President of JSGCT2022
Professor, R&D Laboratory for Innovative Biotheraneutics
Kyushu University, Graduate School of Pharmaceutical Sciences
Fukuoka, JAPAN

お知らせ

▌開催期間

2022年7月14日（木）～16日（土）

▌会場

博多国際展示場＆カンファレンスセンター
〒812-0008 福岡市博多区東光2丁目22番15号

▌参加登録

「オンライン参加登録」のみとなります。
※会場で現金を伴う参加受付はございませんので、必ず事前に参加登録をお願いいたします。

参加カテゴリー	事前 2022年5月12日（木） ～7月 7日（木）	当日 2022年7月14日（木） ～16日（土）
会員	12,000円	15,000円
非会員	20,000円	30,000円
大学院生	3,000円	
学生[*1]	無料	
Get Together[*2] 予定：7月14日（木） 19:00～21:00 会場：アサヒビール園	3,000円（※学部学生：無料）	
懇親会[*2] 予定：7月15日（金） 19:00～21:00 会場：オリエンタルホテル福岡博多ステーション 3F YAMAKASA	5,000円（※学部学生：無料）	

*1 学生（学部生）の方は、学会期間［2022年7月14日（木）～16日（土）］に有効な学生証の提示が必要です。
*2 すべてのカテゴリーの方がお申込みいただけます。
なお、参加人数に制限がございますので、ご希望の方はお早目にお申込みください。
※参加費に含まれるもの：全てのセッションへの参加、企業展示・ピッチプレゼンテーション、
プログラム抄録集（PDF）

▌お支払い方法

お支払いはクレジットカード決済のみとなります。
VISA、MasterCard、JCB、AMEX、Diners Clubをご利用いただけます。

▌受付時間

受付場所： 博多国際展示場＆カンファレンスセンター 3F ロビー
受付時間： 7月14日（木） 8：00～18：00
7月15日（金） 7：30～18：00
7月16日（土） 7：30～14：30
※現地で現金を伴う参加受付はございませんので必ず事前に参加登録をお願いいたします。
※現地会場へご来場の際は、会場にご用意した「健康チェックシート」にご記入の上、受付にご提出ください。
健康チェックシートと引き換えに、ネームカードをお渡しいたします。

▌理事会

日　　時：7月13日（水） 17：00〜18：00
会　　場：博多国際展示場＆カンファレンスセンター　2F　209

▌評議員会

日　　時：7月14日（木）8：30〜9：30
会　　場：第3会場（博多国際展示場＆カンファレンスセンター　3F　304）

▌総会

日　　時：7月15日（木）13：00〜13：50
会　　場：第1会場（博多国際展示場＆カンファレンスセンター　3F　302・303）

▌展示

日　　時：7月14日（木） 9：30〜18：30　　※ピッチプレゼンテーション　10：30〜11：30／15：00〜15：30
　　7月15日（金） 8：30〜18：30　　※ピッチプレゼンテーション　10：30〜11：30／15：00〜15：30
　　7月16日（土） 7：30〜14：30
会　　場：企業展示会場（博多国際展示場＆カンファレンスセンター 3F　305・306）

▌コーポレートセミナー、ティーブレイクセミナーについて

整理券の配布はございません。
プログラム開始時間に直接会場へお越しください。

▌クローク

場　　所：博多国際展示場＆カンファレンスセンター　3F　ロビー
受付時間：7月14日（木） 8：00〜18：30
　　7月15日（金） 7：30〜19：00
　　7月16日（土） 7：30〜17：00

▌演者の皆様へ

・Plenary Session・一般演題
　発表言語：日本語・英語どちらでも可能です。
　スライド：日本語・英語どちらでも可能です。
・同時通訳はございません。
・プレゼンテーションデータは、PowerPoint 2013以降で作成してください。
・Macintosh PowerPointでプレゼンテーションデータを作成された場合は、表示上の問題を回避するために、Windows ベースの環境でプレゼンテーションが正しく機能するか事前にご確認いただくか、ご自身の PC を持参してください。
・画面レイアウト崩れを防ぐため、以下の推奨フォントをご使用ください。
　日本語……MSゴシック、MS Pゴシック、MS明朝、MS P明朝
　英　語……Arial、Arial Black、Helvetica、Century、Century Gothic、Times New Roman
・ご発表者全員の利益相反（COI）状態の開示をお願い申し上げます。
　演題発表時に発表スライドの最初のスライドで利益相反（COI）を開示ください。
※日本遺伝子細胞治療学会利益相反（COI）規定細則については本学術集会のホームページをご確認ください。
　様式を学会ホームページ「利益相反（COI）」ページからダウンロードすることができます。
・コンピュータウイルスの拡散を防ぐために、アップデートしたアンチウイルスソフトウェアを使用して、事前にプレゼンテーションファイルをスキャンしてください。

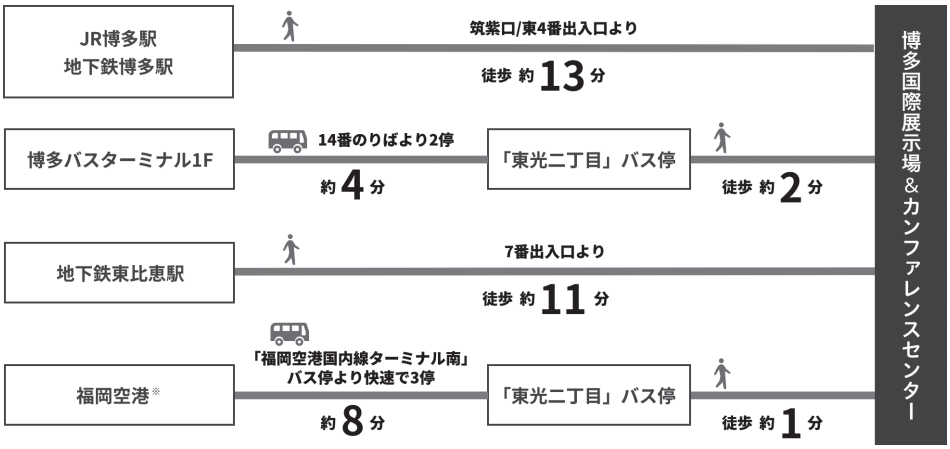
▌PC受付

・発表者はご自身の発表時間の30分前までに、PC受付にして試写をお済ませください。
受付場所：博多国際展示場＆カンファレンスセンター　3F　306付近
受付時間：7月14日（木）　8：00〜18：00
　　7月15日（金）　7：30〜18：00
　　7月16日（土）　7：30〜14：30
・発表データはUSBフラッシュメモリに保存してご持参の上、発表時間の30分前までにPC受付にて動作確認を行ってください。
・会場に用意するPCのアプリケーションは、Windows 10、PowerPoint 2013以降でございます。
・PowerPointプレゼンテーションにリンクされているすべての動画ファイルを、1つのフォルダに保存してください。ビデオファイルはWMVまたはMPEG1、MPEG4である必要があります
・Macintoshを使用している場合、またはPowerPointプレゼンテーションに動画が含まれている場合は、ご自身のPCとバックアップデータをご持参ください。
・PC受付でお預かりしたデータは、学会終了後、消去いたします。

▌PC本体持ち込みの際のお願い

・Macintoshをご使用の方は、ご自身のパソコンをお持ち込みください。
・省電力設定とスクリーンセーバーは、あらかじめ解除しておいてください。
・会場で用意するPC ケーブルコネクタの形状は、D-sub 15pin（ミニ）またはHDMIです。
変換コネクタを必要とする場合には必ずご自身でお持ちください。
・PC 受付にて動作確認後、セッション開始30分前までにPCをご自身で各会場左手前方のPC オペレーター席へお持ちください。
・発表終了後、PC オペレーター席にてPC をお返しいたします。

会場：博多国際展示場＆カンファレンスセンター

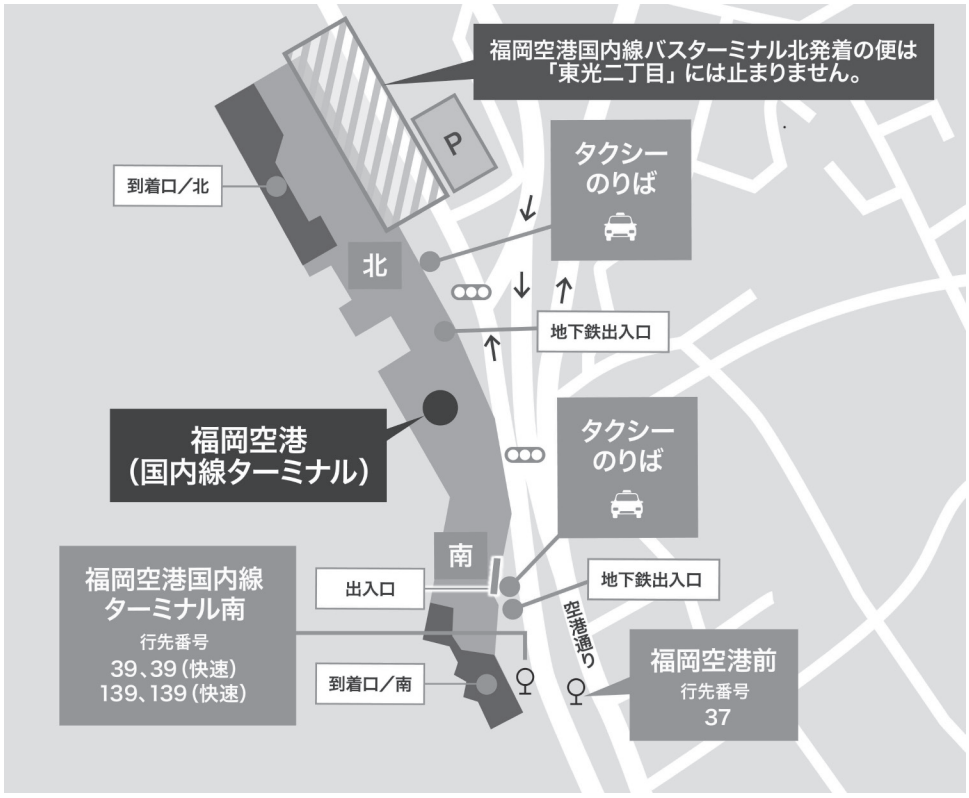


アクセスマップ

博多駅周辺マップ

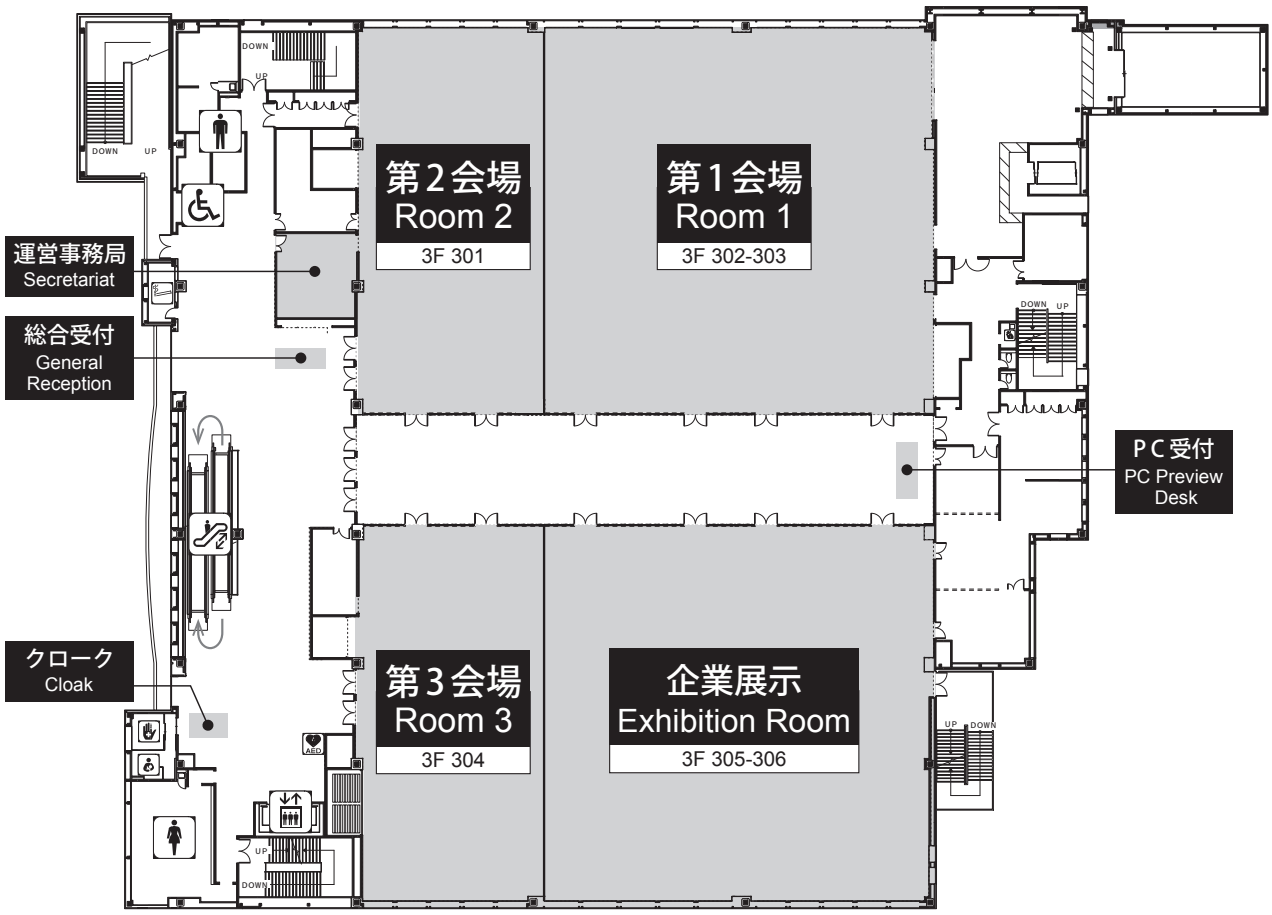
周辺マップ

福岡空港周辺マップ



施設周辺マップ

3F


第2会場
Room 2
3F 301
第1会場
Room 1
3F 302-303
運営事務局
Secretariat
総合受付
General
Reception
PC受付
PC Preview
Desk
クローク
Cloak
第3会場
Room 3
3F 304
企業展示
Exhibition Room
3F 305-306
DOWN
UP
AED

1日目　2022年7月14日（木）

	第1会場 3F　302・303	第2会場 3F　301	第3会場 3F　304	企業展示会場 3F　305・306
8:00				
30			8:30 ～ 9:30 **評議員会**	
9:00				
30	9:25 ～　**開会式**			
10:00	9:30 ～ 11:00 **Plenary Session** 座長：福原　浩、鐘ヶ江裕美 (E or J)	9:45 ～ 11:35 **シンポジウム 1** Ocular gene and cell therapy 座長：大橋　十也、池田　康博 演者：大家　義則 万代　道子 西口　康二 冨田　浩史 村上　祐介 (J)	9:45 ～ 11:09 **Oral Session I** Cell Therapy 座長：花園　豊、喜納　宏昭 (E or J)	**企業展示**
30				10:30 ～ 11:30 **ピッチプレゼンテーション**
11:00	11:00 ～ 11:50 **教育講演 1** 座長：谷　憲三朗　演者：津田　誠 (J)			
30				
12:00	12:00 ～ 12:50 **Corporate Seminar I** 座長：米満　吉和 演者：珠玖　洋 ミルテニーバイオテク㈱	12:00 ～ 12:50 **Corporate Seminar II** 座長：針金谷尚人 演者：Peiqing Zhang、堀内　賢一 日本ポール㈱	12:00 ～ 12:50 **Corporate Seminar III** 座長：多田　誠一 演者：保仙　直毅 ㈱スクラム	
30				
13:00	13:00 ～ 13:30 **理事長講演** 座長：衛藤　義勝　演者：森下　竜一(J)			**企業展示**
30	13:30 ～ 14:00 **特別講演** 座長：笠原　典之　演者：Anna Maria Ranzoni (E)	13:30 ～ 15:00 **シンポジウム 2** Gene Editing & Mitochondria Manipulation 座長：三谷幸之介、和田　健一 演者：Beverly Y. Mok 山田　勇磨 和田　健一 大森　司 Matthew Porteus (E or J)	13:30 ～ 14:42 **Oral Session II** Cancer① 座長：小賀健一郎、田澤　大 (E or J)	
14:00	14:00 ～ 16:00 **会長特別企画 1** ASGCT/ESGCJSGCT Joint Symposium 座長：笠原　典之、中村　貴史 演者：Hildegard Büning Juan A. Bueren Hans-Peter Kiem Guangping Gao 森下　竜一 (E)			
30				
15:00		15:00 ～ 15:30 **Tea break seminar I** 演者：四柳　雄一 ㈱キアゲン	15:00 ～ 15:30 **Tea break seminar II** 座長：原田　結　演者：石垣　靖人 ㈱フコク	15:00 ～ 15:30 ピッチプレゼンテーション
30		15:30 ～ 17:00 **シンポジウム 3** Vector Development 座長：岡田　尚巳、鐘ケ江裕美 演者：位高　啓史 小原　道法 喜納　裕美 Jessica M. Tate (E or J)	15:30 ～ 16:42 **Oral Session III** Cancer② 座長：久保　秀司、黒崎　創 (E or J)	
16:00	16:00 ～ 18:30 **理事長特別企画** COVID-19 vaccines as gene-based technologies 座長：森下　竜一、米満　吉和 演者：Melissa Moore 石井　健 森久保典子 田中　倫夫 中神　啓徳 荒木　康弘 (E or J)			
30			16:42 ～ 18:18 **Oral Session IV** Vectors① 座長：卜部　匡司、喜納　裕美 (E or J)	
17:00		17:00 ～ 18:30 **シンポジウム 4** Cancer Gene Therapy 1 座長：那須　保友、山本　正人 演者：Mizuho Sato-Dahlman 脇本　浩明 保仙　直毅 (E or J)		**企業展示**
30				
18:00				
30				
19:00				

※ 19:00～21:00　Get together（アサヒビール園）

2日目　2022年7月15日（金）

第1会場　3F　302・303

8:30 ～ 11:00
会長特別企画 2
再生医療等製品における薬価制度と早期・条件付承認制度を考える
座長：植田真一郎、米満　吉和
演者：白沢　博満
奥平　真一
加納　浩之
(J)

11:00 ～ 11:50
教育講演 2
座長：望月　秀樹　演者：山海　嘉之
(J)

12:00 ～ 12:50 **Corporate Seminar IV**
座長：山田　尚之
演者：近藤　昭彦、内田　和久
㈱シンプロジェン

13:00 ～ 13:50
総会

14:00 ～ 14:40 **会長講演**
座長：金田　安史　演者：米満　吉和
(J)

14:40 ～ 16:40
会長特別企画 3
Gene and Cell Therapy: Industrial Views
座長：山田　英、仲尾　功一
演者：廣瀬　徹
小林美穂子
志鷹　義嗣
峰野　純一
中條　光章
(J)

16:40 ～ 18:40
NATSJ-JSGCT Joint Symposium
Non-viral Therapeutics
座長：横田　隆徳、金田　安史
演者：横田　隆徳
和田　猛
宮田完二郎
木村　康義／望月　秀樹
中山　東城
(J)

第2会場　3F　301

8:30 ～ 10:00
シンポジウム 5
Oncolytic virus
座長：藤堂　具紀、藤原　俊義
演者：Khalid Shah
Chae-Ok Yun
奥山　隆平
小賀健一郎
田澤　大
(E)

10:00 ～ 11:30
シンポジウム 6
Young investigators session: explore a new era of gene therapy research
座長：櫻井　文教、大津　真
演者：二村　圭祐
柳生　茂希
髙橋　葉子
堀田　秋津
(J)

12:00 ～ 12:50 **Corporate Seminar V**
座長：奥山　虎之
演者：森下　竜一、村上　晶彦
アンジェス㈱

演者：荻田　伸夫
鳥海健太郎
サーモフィッシャー
サイエンティフィック㈱

15:00 ～ 15:30
Tea break seminar III

15:30 ～ 17:00
シンポジウム 7
Regulatory Sciences
座長：久米　晃啓、内田恵理子
演者：内田恵理子
岡本　圭祐／笹渕　美香
櫻井　陽
(J)

17:00 ～ 18:30
シンポジウム 8
Neuromuscular Disorders
座長：村松　慎一、望月　秀樹
演者：村松　一洋
三宅　紀子
Sandra P. Reyna
戸田　達史
(E or J)

第3会場　3F　304

8:30 ～ 9:42
Oral Session V
Genetic Diseases
座長：内山　徹、内田　直也
(E or J)

9:42 ～ 11:18
Oral Session VI
Vectors②
座長：中井　浩之、内田　直也
(E or J)

12:00 ～ 12:50 **Corporate Seminar VI**
座長：内山　徹
演者：等　泰道
キコーテック㈱

座長：春日　卓郎
演者：奥山　知之
Cytiva（グローバルライフサイエンステクノロジーズジャパン㈱）

15:00 ～ 15:30
Tea break seminar IV

15:30 ～ 16:30
Oral Session VII
Cancers③
座長：粕谷　英樹、池田　裕明
(E or J)

16:30 ～ 17:42
Oral Session VIII
Clinical Trials
座長：中沢　洋三、岡崎　利彦
(E or J)

企業展示会場　3F　305・306

企業展示

10:30 ～ 11:30
ピッチプレゼンテーション

企業展示

15:00 ～ 15:30
ピッチプレゼンテーション

企業展示

※ 19:00 ～ 21:00　懇親会（オリエンタルホテル 福岡博多ステーション 3F YAMAKASA）

3日目　2022年7月16日（土）

第1会場　3F　302・303

8:30 ～ 11:00
会長特別企画 4
CAR-T/TCR/NK and beyond：
Breakthrough technologies
for solid tumors
座長：珠玖　洋、原田　結
演者：池田　裕明
藤原　弘
金子　新
Katy Rezvani
原田　結
(E or J)

11:00 ～ 11:50
教育講演 3
座長：池田　康博　演者：髙橋　政代
(J)

12:00 ～ 13:00
Corporate Seminar VII
座長：榎　竜嗣
演者：平野　直人
タカラバイオ㈱

13:10 ～ 14:40
シンポジウム 11
Regenerative Medicine &
Cardiovascular diseases
座長：玉井　克人、中神　啓徳
演者：宮川　繁
尾池　雄一
玉井　克人
(J)

14:40 ～　**閉会式**

第2会場　3F　301

8:30 ～ 10:00
シンポジウム 9
Genetic diseases
座長：小野寺雅史、奥山　虎之
演者：内山　徹
小林　博司
小島　華林
荒川　玲子
(E or J)

10:00 ～ 11:30
シンポジウム 10
Cancer Gene Therapy2
座長：青木　一教、中村　貴史
演者：青木　一教
中村　貴史
玉田　耕治
中原　知美
(E)

12:30～
トレーニングコース受付

13:00 ～ 16:30
第 7 回
遺伝子治療臨床試験
トレーニングコース
～DNA/RNA ワクチン開発に向けて～
代表：久米　晃啓
講師：三ツ木元章
真木　一茂
岡田真由美

※事前参加登録制
本学術集会とは別に、
参加費が必要となります。

第3会場　3F　304

8:30 ～ 9:42
Oral Session IX
Neurogenic, Ophthalmic,
and Musculo-skeletal Diseases
座長：長野　清一、石井亜紀子
(E or J)

9:42～10:54
Oral Session X
Vectors ③
座長：平井　幸彦、水上　浩明
(E or J)

12:00 ～ 13:00
Corporate Seminar VIII
座長：山形　崇倫
演者：小俣　卓、山形　崇倫
ノバルティス ファーマ㈱

13:10 ～ 14:34
Oral Session XI
Basic Science
座長：柳生　茂希、喜納　裕美
(E or J)

企業展示会場　3F　305・306

企業展示

■Meeting Schedule

July 14 – 16, 2022

■Venue

Hakata International Exhibition Hall & Conference Center
2-22-15 Toko Hakata, Fukuoka city, Fukuoka 812-0008, Japan

■Registration

Online registration only.
No registration fee payment on site in cash is accepted.

Category	Pre-registration Fee & Date Until July 7th	On-site registration Fee & Date (From July 14th to 16th)
JSGCT Member	12,000 JPY	15,000 JPY
Non Member	20,000 JPY	30,000 JPY
Graduate Student	3,000 JPY	
Student *1	Free	
Get Together *2 July 14 (Thu.) 19:00 ~ 21:00 Venue: Asahi Beer Community Hall	3,000 JPY (Student: Free)	
Welcome Reception *2 July 15 (Fri.) 19:00 ~ 21:00 Venue: Oriental Hotel Fukuoka Hakata Station 3F YAMAKASA	5,000 JPY (Student: Free)	

*1 Student needs to adduce verification card.
*2 All categories can apply.
The number of participants is limited. Please apply as soon as possible.
※Registration Fee Includes: Participation in all sessions. Exhibition & Pitch presentation, Abstract Book (PDF)

■Method of Payment

Method of Payment: Credit Card only (VISA, MasterCard, JCB, AMEX, Diners Club are accepted).

■Reception Desk Hours

Reception desk is located on 3F Lobby.
July 14 (Thu.) 8:00 ~ 18:00
July 15 (Fri.) 7:30 ~ 18:00
July 16 (Sat.) 7:30 ~ 14:30

※Please submit a “Health Check Sheet” at the Reception desk when you come to the venue.
In exchange for the sheet, you will be given a “Name Card”.

■JSGCT Board Meetings

July 13 (Wed.) 17:00 ~ 18:00 (2F Room 209)

■Councilors Meeting

July 14 (Thu.) 8:30 ~ 9:30 (3F Room 304)

■Business meeting

July 15(Fri.) 13:00 ~ 13:50 (3F Room 302 & 303)

▮Exhibition

The Exhibition room is at 3F 305 & 306.

July 14 (Thu.) 9:30 ~ 18:30 ※Pitch presentation:10:30 ~ 11:30 / 15:00 ~ 15:30
July 15 (Fri.) 8:30 ~ 18:30 ※Pitch presentation:10:30 ~ 11:30 / 15:00 ~ 15:30
July 16 (Sat.) 7:30 ~ 14:30

▮Corporate Seminars / Tea break Seminars

No tickets will be distributed

▮Cloak

Cloak is located on 3F Lobby.
Be sure to keep valuables in your possession at all times.

July 14 (Thu.) 8:00 ~ 18:30
July 15 (Fri.) 7:30 ~ 19:00
July 16 (Sat.) 7:30 ~ 17:00

▮Instructions for speakers in oral presentation

1. The language for oral presentation is either Japanese or English.
2. No simultaneous interpretation will be provided.
3. Application software for preparing presentation data should be PowerPoint 2007/2010/2013/2016.
4. If you have prepared your presentation data on a Macintosh PowerPoint, please check that your presentation functions correctly in a windows-based environment, or bring your own PC in order to avoid display problems.
5. Use standard font (e.g. Arial, Helvetica, Times, Times New Roman) in preparing your presentation to avoid conversion errors.
6. All authors required to disclose any conflict of interest with sponsoring companies.
   For oral presentations, please include the slide disclosing the state of COI in your PowerPoint presentation after your title slide.
   (You can download sample template from Call for Abstract page on your website
   https://www.c-linkage.co.jp/jsgct2022/coi.html)
7. To avoid the possible spread of computer viruses, please scan your presentation files beforehand with update anti-virus software.

▮PC Preview Section

1. Please bring your presentation data in a USB flash memory on your own laptop PC, at least 30 minutes prior to your presentation to the PC Preview Section to complete review of presentation data.
2. On-site operating system will be Windows 10, PowerPoint 2007/2010/2013/2016.
3. Please place all video clips linked with the PowerPoint presentation into a single folder. Video file should be WMV or MPEG1, MPEG4.
4. If using a Macintosh or you're PowerPoint presentation includes moving images, please bring your own PC and back-up data to make your presentation.
5. Presentation data loaded on the conference PC will be completely deleted after your presentation by our staffs.

PC Preview Desk is located in front of Exhibition room on 3F.

July 14(Thu.) 8:00 ~ 18:00
July 15(Fri.) 7:30 ~ 18:00
July 16(Sat.) 7:30 ~ 14:30

▮Laptop users

1. Macintosh users are requested to bring your own PC.
2. Turn off any sleep functions and screen savers beforehand.
3. Cable connector used at the venue for image output is D-sub 15 pin connector or HDMI. Please bring your own connector conversion adapter if necessary.
4. After checking your data at the PC Preview Section, please bring your PC to the “Operating Desk” near the speakers’ podium in the session rooms.
5. Please pick up your PC at the Operation Desk after your presentation.

▮Time allocations for presentations

The speakers of Plenary Session will be given 15 minutes in total.
(12 min. talk followed by 3 min. discussion)
The speakers of Oral Sessions will be given 12 minutes.
(9 min. talk followed by 3 min. discussion)

Access

Venue: Hakata International Exhibition Hall & Conference Center

◆ Train
- 13 min. walk from “Chikushi gate” of JR Hakata Station
- 13 min. walk form “Exit East No.4” of Hakata Subway Station
- 11 min. walk from “Exit No.7” of Higashihie Subway Station

◆ Bus
- From JR Hakata Station

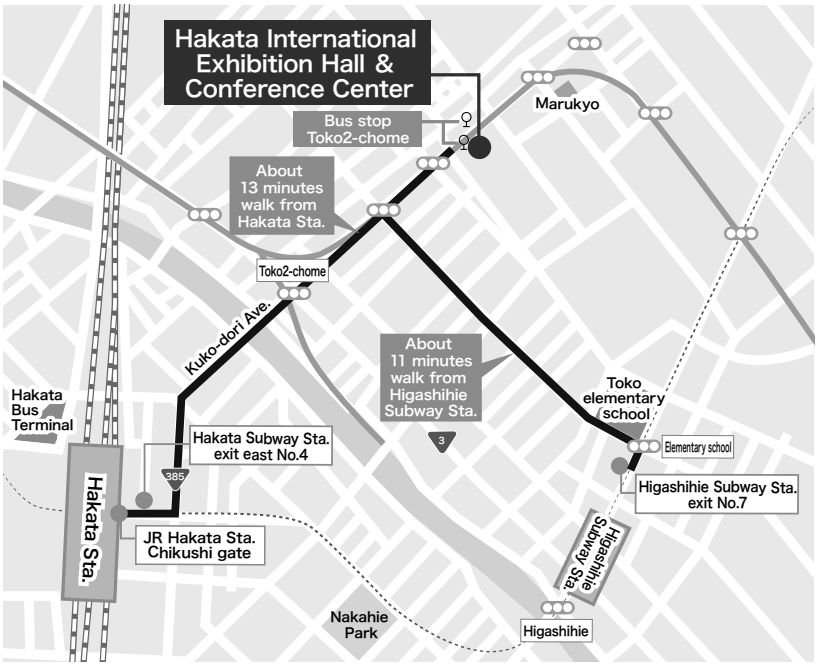
  2 min. walk form Bus stop “Toko2-chome”

  4 min. by bus from Bus stop “No.14” of Hakata Bus Terminal to “Toko2-chome”
- From Fukuoka Airport

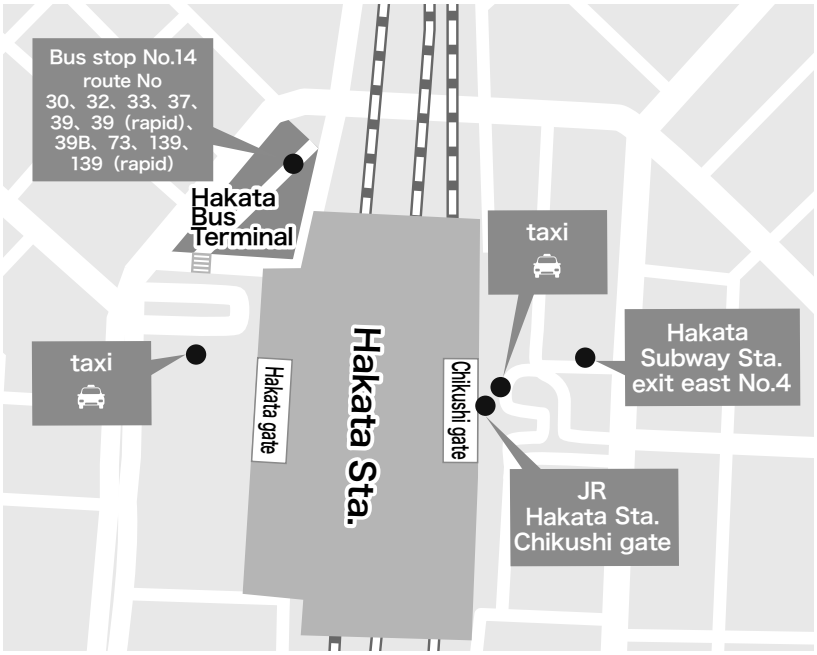
  1 min. walk form Bus stop “Toko2-chome”

  8 min. by bus from Bus stop “Fukuoka Domestic Bus Terminal South” to “Toko2-chome”

Access map

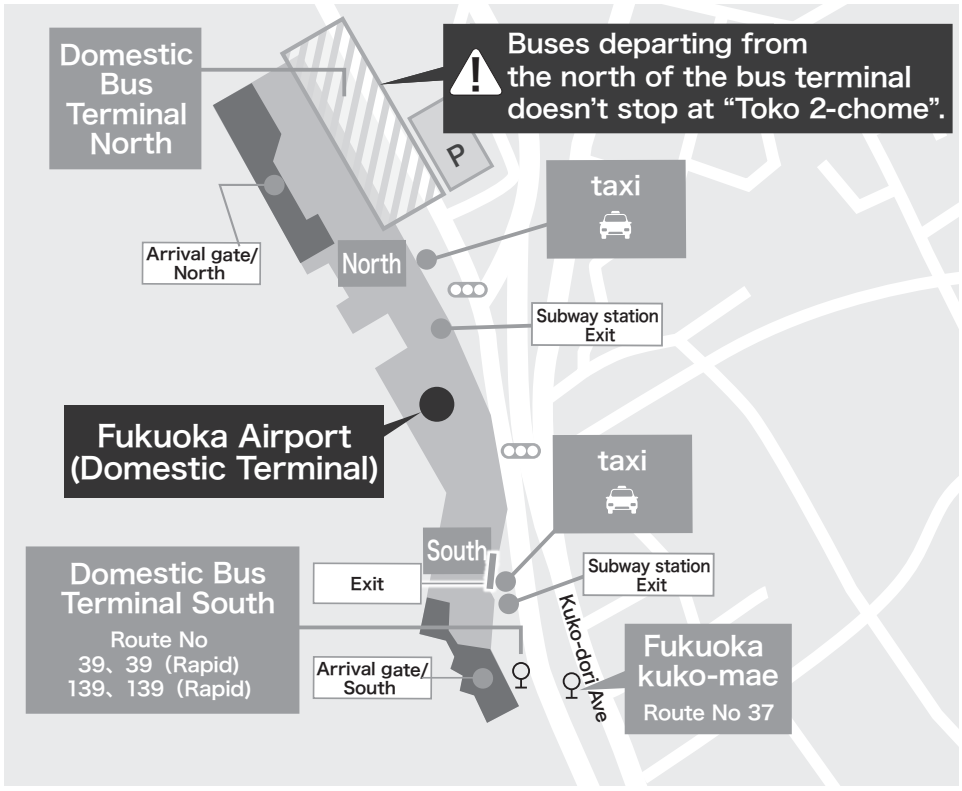


Hakata Station area map

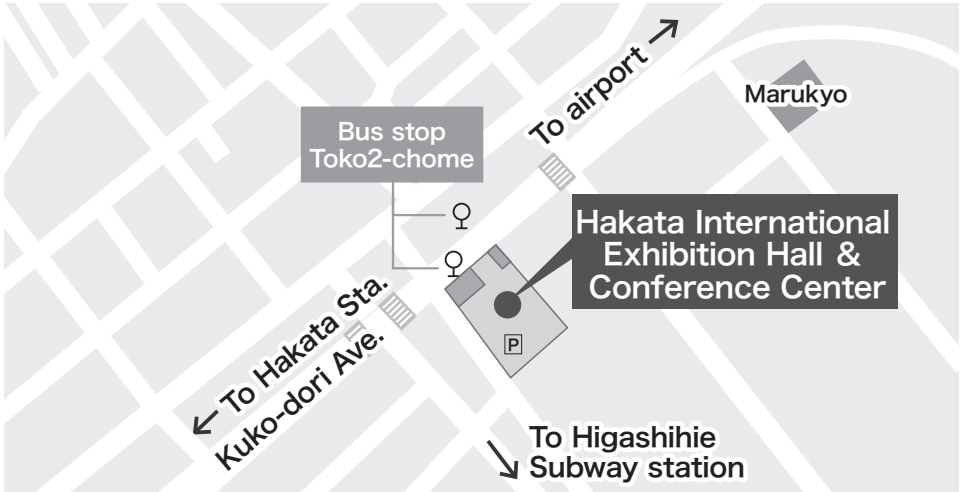


Map of Surrounding Areas

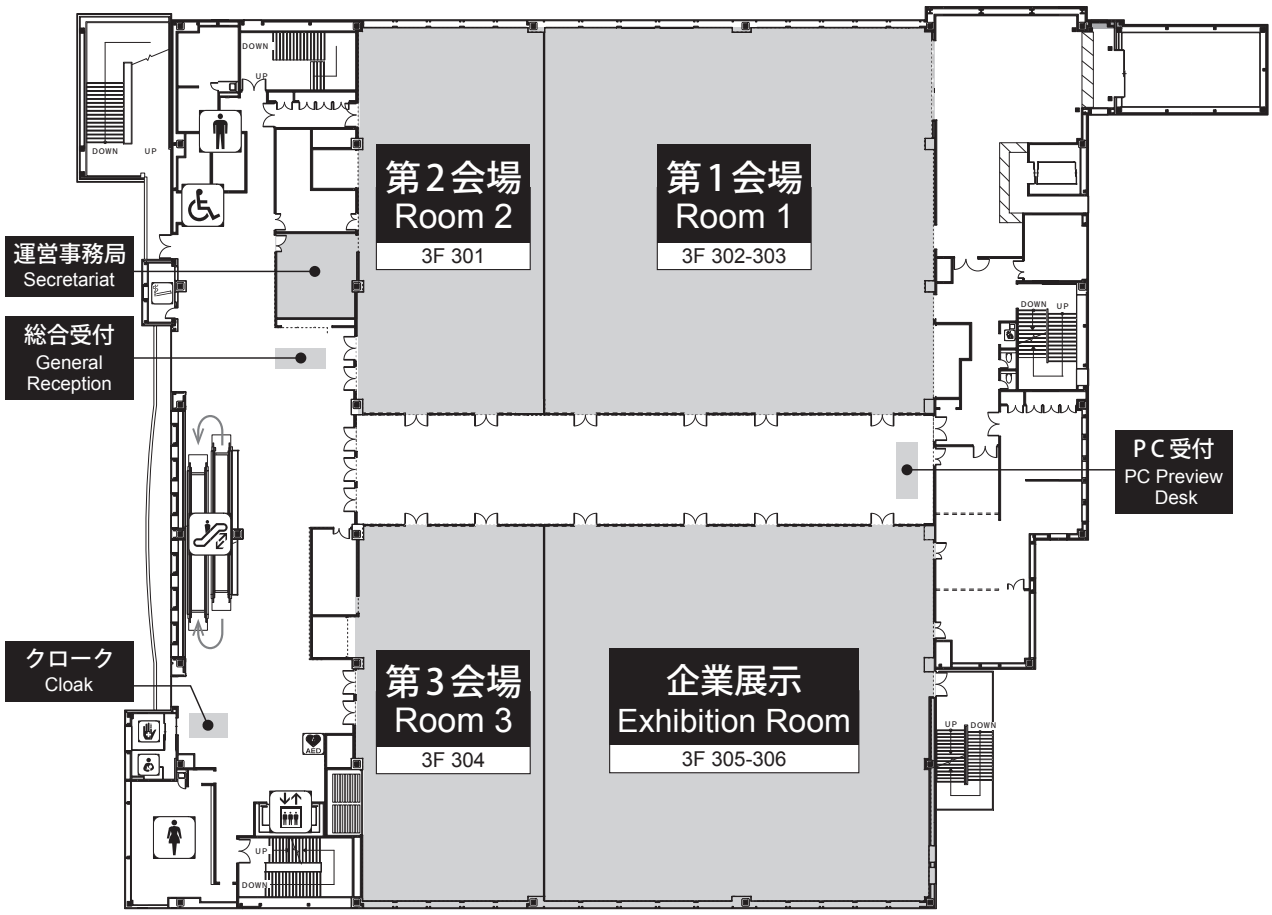
Fukuoka Airport area map



Venue area map



3F


第2会場
Room 2
3F 301
第1会場
Room 1
3F 302-303
運営事務局
Secretariat
総合受付
General
Reception
PC受付
PC Preview
Desk
クローク
Cloak
第3会場
Room 3
3F 304
企業展示
Exhibition Room
3F 305-306
DOWN
UP
AED

Day 1 July 14 (Thu.)

Room 1 (3F 302・303)

8:30–9:30
Councilor's meeting

9:25– **Opening Remarks**

9:30–11:00
Plenary Session
Chairs: Hiroshi Fukuhara/Yumi Kanegae
(E or J)

11:00–11:50
Educational Lecture 1
Chair: Kenzaburo Tani
Speaker: Makoto Tsuda
(J)

12:00–12:50
Corporate Seminar I
Chair: Yoshikazu Yonemitsu
Speaker: Hiroshi Shiku
(Miltenyi Biotec K.K.)

13:00–13:30 **JSGCT Chairman's Lecture** (J)
Chair: Yoshikatsu Eto
Speaker: Ryuichi Morishita

13:30–14:00 **Special Lecture** (E)
Chair: Noriyuki Kasahara
Speaker: Anna Maria Ranzoni

14:00–16:00
Presidential Special Program 1
ASGCT/ESGCJSGCT Joint Symposium
Chairs:
Noriyuki Kasahara/Takafumi Nakamura
Speakers: Hildegard Büning
Juan A. Bueren
Hans-Peter Kiem
Guangping Gao
Ryuichi Morishita
(E)

16:00–18:30
JSGCT Special Program
COVID-19 vaccines as gene-based technologies
Chairs:
Ryuichi Morishita/Yoshikazu Yonemitsu
Speakers: Melissa Moore
Ken J Ishii
Noriko Morikubo
Michio Tanaka
Hironori Nakagami
Yasuhiro Araki
(E or J)

Room 2 (3F 301)

9:45–11:35
Symposium 1
Ocular gene and cell therapy
Chairs: Toya Ohashi/Yasuhiro Ikeda
Speakers: Yoshinori Oie
Michiko Mandai
Koji Nishiguchi
Hiroshi Tomita
Yusuke Murakami
(J)

12:00–12:50
Corporate Seminar II
Chair: Dr. Naohito Hariganeya
Speakers: Peiqing Zhang/Kenichi Horiuchi
(Pall Corporation)

13:30–15:00
Symposium 2
Gene Editing & Mitochondria Manipulation
Chairs: Kohnosuke Mitani/Ken-Ichi Wada
Speakers: Beverly Y. Mok
Yuma Yamada
Ken-Ichi Wada
Tsukasa Ohmori
Matthew Porteus
(E or J)

15:00–15:30 **Tea break seminar I**
Speaker: Yuichi Yotsuyanagi
(QIAGEN K.K.)

15:30–17:00
Symposium 3
Vector Development
Chairs: Takashi Okada/Yumi Kanegae
Speakers: Keiji Itaka
Michinori Kohara
Hiromi Hayashita-Kinoh
Jessica M. Tate
(E or J)

17:00–18:30
Symposium 4
Cancer Gene Therapy 1
Chairs: Yasutomo Nasu/Masato Yamamoto
Speakers: Mizuho Sato-Dahlman
Hiroaki Wakimoto
Naoki Hosen
(E or J)

Room 3 (3F 304)

9:45–11:09
Oral Session I
Cell Therapy
Chairs: Yutaka Hanazono/Hiroaki Kinoh
(E or J)

12:00–12:50
Corporate Seminar III
Chair: Seiichi Tada
Speaker: Naoki Hosen
(Scrum Inc)

13:30–14:42
Oral Session II
Cancer①
Chairs: Ken-ichiro Kosai /Hiroshi Tazawa
(E or J)

15:00–15:30 **Tea break seminar II**
Chair: Yui Harada
Speaker: Yasuhito Ishigaki
(FUKOKU Co., Ltd.)

15:30–16:42
Oral Session III
Cancer②
Chairs: Shuji Kubo/Hajime Kurosaki
(E or J)

16:42–18:18
Oral Session IV
Vectors①
Chairs:
Masashi Urabe/Hiromi Hayashita-Kinoh
(E or J)

Exhibition Room (3F 305・306)

Exhibition

10:30–11:30
Pitch presentation

Exhibition

15:00–15:30
Pitch presentation

Exhibition

※ **19:00–21:00 Get together (Asahi Beer Community Hall)**

Day 2 July 15 (Fri.)

Room 1
3F 302・303

8:30–11:00
Presidential Special Program 2

Chairs:
Shinichiro Ueda/Yoshikazu Yonemitsu

Speakers: Hiromichi Shirasawa
Shinichi Okudaira
Hiroyuki Kanoh

(J)

11:00–11:50
Educational Lecture 2
Chair: Hideki Mochizuki
Speaker: Yoshiyuki Sankai
(J)

12:00–12:50 **Corporate Seminar IV**
Chair: Naoyuki Yamada
Speakers: Akihiko Kondo
Kazuhisa Uchida
(Synplogen Co.,Ltd.)

13:00–13:50
Business meeting

14:00–14:40 **President's Lecture**
Chair: Yasufumi Kaneda
Speaker: Yoshikazu Yonemitsu
(J)

14:40–16:40
Presidential Special Program 3
Gene and Cell Therapy: Industrial Views

Chairs: Ei Yamada/Koichi Nakao
Speakers: Tohru Hirose
Mihoko Kobayashi
Yoshitsugu Shitaka
Junichi Mineno
Mitsuaki Chujo

(J)

16:40 ～ 18:40
NATSJ-JSGCT Joint Symposium
Non-viral Therapeutics

Chairs:
Takanori Yokota/Yasufumi Kaneda
Speakers: Takanori Yokota
Takeshi Wada
Kanjiro Miyata
Yasuyoshi Kimura/Hideki Mochizuki
Tojo Nakayama
(J)

Room 2
3F 301

8:30–10:00
Symposium 5
Oncolytic virus
Chairs: Tomoki Todo/Toshiyoshi Fujiwara
Speakers: Khalid Shah
Chae-Ok Yun
Ryuhei Okuyama
Ken-ichiro Kosai
Hiroshi Tazawa
(E)

10:00–11:30
Symposium 6
Young investigators session: explore a new era of gene therapy research
Chairs: Fuminori Sakurai /Makoto Otsu
Speakers: Keisuke Nimura
Shigeki Yagyu
Yoko Endo-Takahashi
Akitsu Hotta
(J)

12:00–12:50 **Corporate Seminar V**
Chair: Torayuki Okuyama
Speakers: Ryuichi Morishita
Akihiko Murakami
(AnGes, Inc.)

15:00–15:30 **Tea break seminar III** (J)
Speakers: Nobuo Ogita/Kentaro Toriumi
(Thermo Fisher Scientific)

15:30–17:00
Symposium 7
Regulatory Sciences
Chairs: Akihiro Kume/Eriko Uchida
Speakers: Eriko Uchida
Keisuke Okamoto/Mika Sasabuchi
Akira Sakurai
(J)

17:00–18:30
Symposium 8
Neuromuscular Disorders
Chairs:
Shin-ichi Muramatsu/Hideki Mochizuki
Speakers: Kazuhiro Muramatsu
Noriko Miyake
Sandra P. Reyna
Tatsushi Toda
(E or J)

Room 3
3F 304

8:30–9:42
Oral Session V
Genetic Diseases
Chairs: Toru Uchiyama/Naoya Uchida
(E or J)

9:42–11:18
Oral Session VI
Vectors②
Chairs: Hiroyuki Nakai/Naoya Uchida
(E or J)

12:00–12:50 **Corporate Seminar VI**
Chair: Toru Uchiyama
Speak: Yasumichi Hitoshi
(Kiko Tech Co., Ltd.)

15:00–15:30 **Tea break seminar IV** (J)
Chair: Takuro Kasuga
Speaker: Tomoyuki Okuyama
(Cytiva (Global Life Sciences Technologies Japan))

15:30–16:30
Oral Session VII
Cancers③
Chairs: Hideki Kasuya/Hiroaki Ikeda
(E or J)

16:30–17:42
Oral Session VIII
Clinical Trials
Chairs: Yozo Nakazawa/Toshihiko Okazaki
(E or J)

Exhibition Room
3F 305・306

Exhibition

10:30–11:30
Pitch presentation

Exhibition

15:00–15:30
Pitch presentation

Exhibition

※ **19:00–21:00 Welcome Reception (Oriental Hotel Fukuoka Hakata Station 3F YAMAKASA)**

Day 3 July 16 (Sat.)

Room 1
3F 302・303

8:30–11:00
Presidential Special Program 4
CAR-T/TCR/NK and beyond:
Breakthrough technologies for solid tumors

Chairs: Hiroshi Shiku/Yui Harada
Speakers: Hiroaki Ikeda
Hiroshi Fujiwara
Shin Kaneko
Katy Rezvani
Yui Harada

(E or J)

11:00–11:50
Educational Lecture 3
Chair: Yasuhiro Ikeda
Speaker: Masayo Takahashi
(J)

12:00–13:00
Corporate Seminar VII
Chair: Tatsuji Enoki
Speaker: Naoto Hirano
(Takara Bio Inc.)

13:10–14:40
Symposium 11
Regenerative Medicine &
Cardiovascular diseases
Chairs: Katsuto Tamai/Hironori Nakagami
Speakers: Shigeru Miyagawa
Yuichi Oike
Katsuto Tamai
(J)

14:40– **closing Remarks**

Room 2
3F 301

8:30–10:00
Symposium 9
Genetic diseases
Chairs:
Masafumi Onodera/Torayuki Okuyama
Speakers: Toru Uchiyama
Hiroshi Kobayashi
Karin Kojima
Reiko Arakawa
(E or J)

10:00–11:30
Symposium 10
Cancer Gene Therapy2
Chairs: Kazunori Aoki/Takafumi Nakamura
Speakers: Kazunori Aoki
Takafumi Nakamura
Koji Tamada
Tomomi Nakahara
(E)

12:30–
Training Course Reception

13:00–16:30
7th Gene Therapy Clinical Trial Training Course

Room 3
3F 304

8:30–9:42
Oral Session IX
Neurogenic, Ophthalmic,
and Musculo-skeletal Diseases
Chairs: Seiichi Nagano/Akiko Ishii
(E or J)

9:42–10:54
Oral Session X
Vectors ③
Chairs: Yukihiko Hirai/Hiroaki Mizukami
(E or J)

12:00–13:00
Corporate Seminar VIII
Chair: Takanori Yamagata
Speakers: Taku Omata
Takanori Yamagata
(Novartis Pharma K.K.)

13:10～14:34
Oral Session XI
Basic science
Chairs:
Shigeki Yagyu/Hiromi Hayashita-Kinoh
(E or J)

Exhibition Room
3F 305・306

Exhibition

8:00
30
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18:00
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19:00

Program

理事長講演 (J)

7月14日（木） 13:00〜13:30 第1会場

座長：衛藤 義勝（一般財団法人脳神経疾患研究所附属先端医療研究センター＆遺伝病治療研究所／
東京慈恵会医科大学 名誉教授）

遺伝子細胞治療の夜明け
森下 竜一（大阪大学）

会長講演 (J)

7月15日（金） 14:00〜14:40 第1会場

座長：金田 安史（大阪大学）

固形がんを破壊可能な新しい NK 細胞様フェノタイプ GAIA-NK の臨床開発
米満 吉和（九州大学大学院薬学研究院）

理事長特別企画 (E or J)

–COVID-19 Vaccines as gene-based technologies–

7月14日（木） 16:00〜18:30 第1会場

座長：森下 竜一（大阪大学）
米満 吉和（九州大学大学院薬学研究院）

mRNA as Medicine
Melissa Moore（Eleanor Eustis Farrington Chair of Cancer Research/
Professor, RNA Therapeutics Institute/
University of Massachusetts Medical School/
Chief Scientific Officer, Platform Research, Moderna）

コロナ禍が生んだワクチン開発研究のカンブリア紀と進化の行方
石井 健（東京大学 医科学研究所 国際ワクチンデザインセンター）

コミナティの開発
森久保典子（ファイザーR&D合同会社 薬事統括部）

COVID-19 アデノウイルスベクターワクチンの開発と接種プログラムへの導入
田中 倫夫（アストラゼネカ株式会社 研究開発本部）

COVID-19 DNA ワクチン開発
中神 啓徳（大阪大学大学院医学系研究科 健康発達医学）

新型コロナウイルスワクチンの審査今後の在り方について
荒木 康弘（独立行政法人医薬品医療機器総合機構）

会長特別企画1 (E)

ASGCT/ESGCT/JSGCT Joint Symposium

7月14日（木） 14:00〜16:00 第1会場

座長：笠原　典之（University of California, San Francisco (UCSF)）
　　中村　貴史（鳥取大学医学部医学科）

Improving efficacy of Adeno-Associated-Virus (AAV) vectors for in vivo gene therapy
Hildegard Büning（Institute for Experimental Hematology, Hannover Medical School, Hannover, Germany/
REBIRTH – Cluster of Excellence, Hannover Medical School, Hannover, Germany/
German Center for Infection Research (DZIF), partner site Hannover-Braunschweig）

Long-Term Follow-up of the Phase I/II Gene Therapy Trial in Fanconi Anemia-A Patients
Juan A. Bueren（Hematopoietic Innovative Therapies Division,
CIEMAT/CIBERER/Fund. Jiménez Díaz, Madrid, Spain）

Targeting hematopoietic stem cells for ex vivo and in vivo gene therapy
Hans-Peter Kiem（Director, Stem Cell and Gene Therapy Program/
Fred Hutchinson Cancer Research Center）

The next generation of AAV gene therapy
Guangping Gao（Director, Horae Gene Therapy Center and Viral Vector Core,
Co-Director, Li Weibo Institute for Rare Diseases Research,
Professor of Microbiology and Physiological Systems,
Penelope Booth Rockwell Professor in Biomedical Research,
University of Massachusetts Medical School）

Plasmid DNA-based Gene Therapy: From Regenerative Medicine to Vaccine for COVID-19
森下　竜一（大阪大学）

会長特別企画2 (J)

– 再生医療等製品における薬価制度と早期・条件付承認制度を考える –

7月15日（金） 8:30〜11:00 第1会場

座長：植田真一郎（琉球大学大学院医学研究科　臨床薬理学）
　　米満　吉和（九州大学大学院薬学研究院）

イントロダクション：遺伝子治療・再生医療を取り巻く諸問題
米満　吉和（九州大学大学院薬学研究院）

医薬品の価格とアクセスの動向及び課題
白沢　博満（MSD株式会社）

再生医療等製品の条件及び期限付承認制度について
奥平　真一（医薬品医療機器総合機構）

再生医療等製品の現状と多様性に起因する種々の課題
加納　浩之（一般社団法人再生医療イノベーションフォーラム（FIRM）／アステラス製薬株式会社）

パネルディスカッション

会長特別企画3 (J)

–Gene and Cell Therapy: Industrial Views–

7月15日（金） 14:40〜16:40 第1会場

座長：山田　英（アンジェス株式会社）
　　仲尾　功一（タカラバイオ株式会社）

ノバルティスの細胞治療・遺伝子治療開発への取組み
廣瀬　徹（ノバルティス ファーマ株式会社）

希少疾患領域における遺伝子治療の開発戦略
小林美穂子（ファイザーR&D合同会社）

細胞医療・遺伝子治療の産業化に向けたアステラス製薬の取り組み
志鷹　義嗣（アステラス製薬株式会社）

遺伝子治療におけるタカラバイオのポジション
峰野　純一（タカラバイオ株式会社）

プラスミドベクターによる遺伝子治療の可能性
中條　光章（アンジェス株式会社）

会長特別企画4 (E or J)

–CAR-T/TCR/NK and beyond: Breakthrough technologies for solid tumors–

7月16日（土） 8:30〜11:00 第1会場

座長：珠玖　洋（三重大学大学院医学系研究科　個別化がん免疫治療学）
　　原田　結（九州大学大学院薬学研究院）

TCR-T 療法：患者個別性と腫瘍不均一性の克服を目指して
池田　裕明（長崎大学　大学院医歯薬学総合研究科　腫瘍医学分野）

固形がんに対する CAR-T 細胞の戦略的バイオエンジニアリング
藤原　弘（三重大学大学院医学系研究科　個別化がん免疫治療学）

iPS 細胞技術を用いた、がん免疫治療のための CD8 キラー T 細胞、NK 細胞の再生
金子　新（京都大学／筑波大学）

NK cells: next generation cell therapies for cancer
Katy Rezvani（University of Texas, MD Anderson Cancer Center）

新たな NK 様細胞製剤 : GAIA-102 が固形腫瘍を破壊する ～革新的免疫治療の開発～
原田　結（九州大学大学院薬学研究院）

特別講演 (E)

7月14日（木） 13:30〜14:00 第1会場

座長：笠原　典之（University of California, San Francisco (UCSF)）

Publishing Clinical Research at Nature Medicine
Anna Maria Ranzoni（Nature Medicine）

教育講演1 (J)

7月14日（木） 11:00〜11:50 第1会場

座長：谷　憲三朗（東京大学定量生命科学研究所）

痛みの慢性化に関わる脊髄後角細胞
津田　　誠（九州大学）

教育講演2 (J)

7月15日（金） 11:00〜11:50 第1会場

座長：望月　秀樹（大阪大学大学院医学系研究科　神経内科学）

脳神経・筋系疾患の機能再生治療を実現するサイバニクス医療イノベーション
〜そして【サイバニクス×再生医療】による更なる挑戦へ〜
山海　嘉之（筑波大学 システム情報系、サイバニクス研究センター、未来社会工学開発研究センター／
CYBERDYNE株式会社）

教育講演3 (J)

7月16日（土） 11:00〜11:50 第1会場

座長：池田　康博（宮崎大学医学部　眼科学）

網膜再生医療とサステナブルメディスン
髙橋　政代（株式会社ビジョンケア／神戸アイセンター病院 研究センター／立命館大学 RARA）

NATSJ-JSGCTジョイントシンポジウム (J)

–Non-viral Therapeutics–
－核酸医薬を中心としたNon-viral Therapeuticsの最前線－

7月15日（金） 16:40〜18:40 第1会場

座長：横田　隆徳（東京医科歯科大学脳神経病態学分野（脳神経内科））
金田　安史（大阪大学）

血液脳関門通過型ヘテロ2本鎖核酸
横田　隆徳（東京医科歯科大学脳神経病態学分野（脳神経内科））

核酸医薬の有効性と安全性を向上させる新規分子技術
和田　　猛（東京理科大学）

核酸医薬を届ける・留まらせる高分子ナノ医薬
宮田完二郎（東京大学　大学院工学系研究科　マテリアル工学専攻）

核酸医薬による神経難病治療の最前線
木村　康義、望月　秀樹（大阪大学大学院医学系研究科　神経内科学）

核酸医薬を用いた希少神経疾患に対する個別化医療の展望と課題
中山　東城（ハーバード医科大学ボストン小児病院／東京医科歯科大学）

シンポジウム1 (J)

–Ocular gene and cell therapy–

ー眼科領域の細胞治療と遺伝子治療ー

7月14日（木） 9:45〜11:35　第2会場

座長：大橋　十也（東京慈恵会医科大学医学部看護学科健康科学疾病治療学）
　　池田　康博（宮崎大学医学部　眼科学）

S1-1. 角膜上皮の再生医療
大家　義則（大阪大学大学院医学系研究科眼科学教室）

S1-2. ES/iPS 細胞由来網膜組織を用いた網膜色素変性に対する再生医療
万代　道子（神戸市立神戸アイセンター病院）

S1-3. 網膜色素変性に対するゲノム編集遺伝子治療
西口　康二（名古屋大学）

S1-4. オプトジェネティック遺伝子を利用した視覚再建のための遺伝子治療
冨田　浩史（岩手大学）

S1-5. 抗血管新生因子や神経保護因子を用いた網膜遺伝子治療
村上　祐介（九州大学大学院医学研究院　眼科学）

シンポジウム2 (E or J)

–Gene Editing & Mitochondria Manipulation–

ーゲノム編集とミトコンドリアエンジニアリングー

7月14日（木） 13:30〜15:00　第2会場

座長：三谷幸之介（埼玉医科大学）
　　和田　健一（九州大学／理化学研究所）

S2-1. CRISPR-free Base Editor for Precise Editing of Mitochondrial and Nuclear DNA
Beverly Y. Mok（Merkin Institute of Transformative Technologies in Healthcare, Broad Institute of MIT and Harvard, Cambridge, MA, USA/
Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA./
Howard Hughes Medical Institute, Harvard University, Cambridge, MA, USA/
*Current affiliation: Molecular Engineering Lab, Institute of Molecular and Cell Biology, A*STAR, Singapore）

S2-2. ミトコンドリア DDS を基盤とした遺伝子細胞治療への挑戦
山田　勇磨（北海道大学大学院薬学研究院／JST・創発的研究支援事業）

S2-3. マイクロ流体デバイスを用いたミトコンドリアゲノム改変技術
和田　健一（九州大学／理化学研究所）

S2-4. 改変型 Cas を用いたゲノム編集治療の可能性
大森　　司（自治医科大学医学部生化学講座病態生化学部門）

S2-5. Advancing Homologous Recombination based Genome Editing of Hematopoietic Stem Cells
Matthew Porteus（Department of Pediatrics, Stanford University School of Medicine）

シンポジウム3 (E or J)

–Vector Development–

ーベクター開発ー

7月14日（木） 15:30〜17:00 第2会場

座長：岡田　尚巳（東京大学医科学研究所　遺伝子・細胞治療センター）
　　鐘ヶ江裕美（東京慈恵会医科大学・総合医科学研究センター）

S3-1. mRNA 創薬の遺伝子治療への応用
位高　啓史（東京医科歯科大学　生体材料工学研究所）

S3-2. アデノウイルスベクター及びワクシニアウイルスベクターによる COVID-19 病態解析とその制御
小原　道法（東京都医学総合研究所）

S3-3. MSC を活用した低用量 AAV ベクターによる遺伝子治療
喜納　裕美（東京大学医科学研究所　遺伝子・細胞治療センター　分子遺伝医学分野）

S3-4. Robust AAV Purification Platform Tolerant of Changes in Harvest Material
Jessica M. Tate（Thermo Fisher Scientific, Patheon）

シンポジウム4 (E or J)

–Cancer Gene Therapy 1–

ー癌遺伝子治療の臨床に向けた技術的進歩ー

7月14日（木） 17:00〜18:30 第2会場

座長：那須　保友（岡山大学）
　　山本　正人（University of Minnesota）

S4-1. Engineering of the cancer-targeted oncolytic adenovirus for systemic therapy of advanced cancers
Mizuho Sato-Dahlman（University of Minnesota）

S4-2. Preclinical development of oncolytic virus therapies for malignant brain tumors using cancer stem-like cell-based models
Hiroaki Wakimoto（Massachusetts General Hospital, Harvard Medical School）

S4-3. 多発性骨髄腫に対する CAR-T 細胞療法
保仙　直毅（大阪大学大学院医学系研究科）

シンポジウム5 (E)

–Oncolytic virus–

ーウイルス療法ー

7月15日（金） 8:30〜10:00 第2会場

座長：藤堂　具紀（東京大学医科学研究所　先端医療研究センター　先端がん治療分野）
　　藤原　俊義（岡山大学大学院医歯薬学総合研究科　消化器外科学）

S5-1. Gene Edited and Engineered Cell based therapies for Cancer: From Bench to Bedside
Khalid Shah（BWH, Harvard Medical School）

S5-2. Addressing the challenges of conventional oncolytic virotherapy
Chae-Ok Yun（Department of Bioengineering, College of Engineering, Hanyang University, Seoul, Korea/ GeneMedicine Co., Ltd., Seoul, Korea）

S5-3. IL-12 発現型がん治療用ウイルスを用いた悪性黒色腫に対する革新的ウイルス免疫療法開発
奥山　隆平（信州大学　医学部　皮膚科）

S5-4. サバイビン反応性多因子増殖制御型アデノウイルス：基礎研究から第 II 相試験まで
小戝健一郎（鹿児島大学大学院医歯学総合研究科／鹿児島大学病院）

S5-5. テロメラーゼ特異的腫瘍溶解ウイルス療法の臨床開発の現況
田澤　　大（岡山大学大学院医歯薬学総合研究科消化器外科学／岡山大学病院新医療研究開発センター）

シンポジウム6 (J)

–Young investigators session: explore a new era of gene therapy research–

－若手研究者が拓く次世代の遺伝子治療研究－

7月15日（金） 10:00〜11:30 第2会場

座長：櫻井　文教（大阪大学大学院薬学研究科）
　　大津　　真（北里大学医学部　輸血・細胞移植学）

S6-1. 生体内における非増殖性ウイルス HVJ-E による抗腫瘍効果誘導メカニズムの解明
二村　圭祐（大阪大学大学院医学系研究科）

S6-2. 固形腫瘍に対する非ウイルス遺伝子改変 CAR-T 細胞療法の開発
柳生　茂希（信州大学　学術研究・産学官連携推進機構）

S6-3. 超音波応答性ナノバブルの開発と核酸・遺伝子治療への応用
髙橋　葉子（東京薬科大学）

S6-4. ゲノム編集治療実現を目指した独自送達技術開発
堀田　秋津（京都大学 iPS細胞研究所／T-CiRA プログラム）

シンポジウム7 (J)

–Regulatory Sciences–

－遺伝子細胞治療開発レギュラトリーサイエンスの潮流－

7月15日（金） 15:30〜17:00 第2会場

座長：久米　晃啓（自治医科大学　附属病院臨床研究センター）
　　内田恵理子（国立医薬品食品衛生研究所　遺伝子医薬部）

S7-1. 遺伝子細胞治療の開発にかかる規制の最新動向
内田恵理子（国立医薬品食品衛生研究所　遺伝子医薬部）

S7-2. 再生医療等安全性確保法の見直しについて
岡本　圭祐、笹渕　美香（厚生労働省）

S7-3. カルタヘナ法の運用改善について
櫻井　　陽（独立行政法人　医薬品医療機器総合機構）

シンポジウム8 (E or J)

–Neuromuscular Disorders–

－神経・筋疾患－

7月15日（金） 17:00〜18:30 第2会場

座長：村松　慎一（自治医科大学　神経遺伝子治療）
　　望月　秀樹（大阪大学大学院医学系研究科　神経内科学）

S8-1. オートファジー病の遺伝子治療
村松　一洋（自治医科大学　小児科学）

S8-2. 異染性白質ジストロフィーの遺伝子治療
三宅　紀子（日本医科大学　生化学・分子生物学（分子遺伝学））

S8-3. Gene Therapy for Spinal Muscular Atrophy: Clinical and Real-World Experience and Clinical Program Update
Sandra P. Reyna（Novartis Gene Therapies）

S8-4. 福山型筋ジストロフィーの治療戦略
戸田　達史（東京大学大学院医学系研究科神経内科学）

シンポジウム9 (E or J)

–Genetic diseases–

ー遺伝性疾患遺伝子治療の本邦における研究状況ー

7月16日（土） 8:30〜10:00 第2会場

座長：小野寺雅史（国立成育医療研究センター 遺伝子細胞治療推進センター）
奥山 虎之（埼玉医科大学 ゲノム医療科）

S9-1. 原発性免疫不全症に対する遺伝子治療の現状と今後の展望
内山 徹（国立成育医療研究センター研究所 成育遺伝研究部 疾患遺伝子構造研究室）

S9-2. ライソゾーム病に対する遺伝子治療の開発
小林 博司（東京慈恵会医科大学 総合医科学研究センター 遺伝子治療研究部）

S9-3. AADC 欠損症遺伝子治療の長期効果
小島 華林（自治医科大学 小児科学）

S9-4. アデノ随伴ウイルスベクターを用いた脊髄性筋萎縮症に対する遺伝子治療の実際
荒川 玲子（国立国際医療研究センター病院 臨床ゲノム科／国立国際医療研究センター研究所 メディカルゲノムセンター）

シンポジウム10 (E)

–Cancer Gene Therapy2–

ー次世代がん遺伝子細胞治療を目指してー

7月16日（土） 10:00〜11:30 第2会場

座長：青木 一教（国立がん研究センター研究所）
中村 貴史（鳥取大学医学部医学科）

S10-1. 腫瘍免疫微小環境に基づいた、肺がん免疫療法開発の新規標的探索
青木 一教（国立がん研究センター研究所）

S10-2. 細胞融合能を有する腫瘍溶解性ワクシニアウイルスは、腫瘍微小環境を調節することにより全身性の抗腫瘍免疫応答を増強する
中村 貴史（鳥取大学医学部医学科）

S10-3. 固形がんに対する CAR-T 細胞療法の最新技術
玉田 耕治（国立大学法人山口大学 大学院医学系研究科）

S10-4. HPV ゲノムを標的とする超多重ガイド RNA・Cas9 発現一体型アデノウイルスベクターを用いた子宮頸がん・頭頸部がんのゲノム編集治療の開発
中原 知美（国立がん研究センター研究所）

シンポジウム11 (J)

–Regenerative Medicine & Cardiovascular diseases–

ー遺伝子細胞治療のトランスレーショナルリサーチー

7月16日（土） 13:10〜14:40 第1会場

座長：玉井 克人（大阪大学大学院医学系研究科）
中神 啓徳（大阪大学大学院医学系研究科 健康発達医学）

S11-1. 重症心不全に対する心筋組織移植を用いたトランスレーショナルリサーチ
宮川 繁（大阪大学大学院医学系研究科心臓血管外科）

S11-2. ミトコンドリア機能維持を基盤とした心不全治療戦略
尾池 雄一（熊本大学）

S11-3. 栄養障害型表皮水疱症に対する再生誘導医薬開発
玉井 克人（大阪大学大学院医学系研究科）

第7回遺伝子治療臨床試験トレーニングコース
～DNA/RNAワクチン開発に向けて～

主催：日本遺伝子細胞治療学会（JSGCT）

7月16日（土） 13:00 ～16:30　第2会場

Session I.　イントロダクション・品質（13:00 ～ 14:20）

1. ワクチン開発オーバービュー
久米　晃啓（自治医科大学　附属病院臨床研究センター）

2. DNA/RNA ワクチン等の開発における品質要件
三ツ木元章（医薬品医療機器総合機構　ワクチン等審査部）

休憩（14:20 ～ 14:35）

Session II.　非臨床・臨床（14:35 ～ 16:30）

3. DNA/RNA ワクチン等の非臨床評価
真木　一茂（医薬品医療機器総合機構　ワクチン等審査部）

4. DNA/RNA ワクチン等の臨床試験デザイン
岡田真由美（医薬品医療機器総合機構　ワクチン等審査部）

5. 総合討論
久米　晃啓（自治医科大学　附属病院臨床研究センター）

※事前参加登録制
本学術集会とは別に、参加費（10,000円）が必要となります。
※定員：現地100名、Web 500名

Plenary Session (E or J)

7月14日（木） 9:30〜11:00　第1会場

座長：福原　　浩（杏林大学医学部泌尿器科）
　　鐘ヶ江裕美（東京慈恵会医科大学・総合医科学研究センター）

PS-01. A leucine residue in the N terminal degron of AAV AAP is one of the amino acids essential for its degradation
Anusha Sairavi（Molecular And Medical Genetics, Oregon Health And Science University）

PS-02. ゲノム編集 iPS 細胞を用いた悪性神経膠腫に対する遺伝子幹細胞療法の開発
田村　亮太（慶應義塾大学医学部）

PS-03. Antitumor effect of GAIA-102 on refractory tumors and its underlying mechanism
鄭　　思拓（九州大学大学院薬学研究院　バイオ医薬創成学）

PS-04. CD117 抗体 - 薬物複合体の単回投与が遺伝子改変 CD34+ 細胞の効率的な生着を可能とすることを、レンチウイルス遺伝子治療の非ヒト霊長類モデルにより示した
内田　直也（米国国立衛生研究所　心肺血液部門　細胞分子治療分野／
東京大学医科学研究所　遺伝子・細胞治療センター　分子遺伝医学分野）

PS-05. アデノ随伴ウイルスベクターの分析と品質管理の現状と展望
内山　　進（大阪大学大学院工学研究科／次世代バイオ医薬品製造技術研究組合）

PS-06. HER2 陽性の再発・進行骨・軟部肉腫及び婦人科悪性腫瘍を対象とする非ウイルス遺伝子改変 HER2 CAR-T 細胞の臨床第 I 相医師主導治験
平林　耕一（信州大学医学部小児医学教室）

Oral Session I (E or J)

Cell Therapy

7月14日（木） 9:45〜11:09　第3会場

座長：花園　　豊（自治医科大学）
　　喜納　宏昭（ナノ医療イノベーションセンター　片岡/喜納ラボ）

OR-07. EPH ファミリーたんぱくに多重特異性を有するリガンド型 CAR-T 細胞療法の開発
盛田このみ（信州大学医学部　小児医学）

OR-08. RetroNectin® 誘導 T リンパ球を用いた NK 拡大培養法の CAR-NK への応用と機能解析
西江　敏和（タカラバイオ株式会社　基盤技術開発センター）

OR-09. Expression of CAR targets on solid tumors by armed oncolytic virus has synergetic effect on CAR-T cell therapy
Mona Alhussein Aboalela（Cancer Immune Therapy Research Center, Graduate School of Medicine, Nagoya University, Nagoya, Japan / Department of Surgery II, Graduate School of Medicine, Nagoya University, Nagoya, Japan / Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt）

OR-10. 羊膜間葉系幹細胞による筋ジストロフィーモデルを用いた細胞治療の有効性
笠原　優子（東京大学医科学研究所　遺伝子・細胞治療センター　分子遺伝医学分野）

OR-11. 脳虚血再灌流障害における羊膜由来間葉系幹細胞移植による脳保護効果の検討
高橋　史郎（日本医科大学大学院　医学研究科　神経内科学分野）

OR-12. A cryopreservation method to maintain the viability and cytotoxicity of highly activated natural killer-like cells
忻　　雨夫（九州大学大学院薬学研究院　バイオ医療創成学）

OR-13. iPS 細胞由来ミクログリアを用いたアルツハイマー型認知症治療法の開発
加藤　智朗（公益財団法人京都大学 iPS 細胞研究財団）

Oral Session II (E or J)

Cancer ①

7月14日（木） 13:30〜14:42 第3会場

座長：小戝健一郎（鹿児島大学大学院医歯学総合研究科）
田澤　大（岡山大学病院　新医療研究開発センター）

OR-14. 患者由来肉腫細胞におけるがん治療用ヘルペスウイルスの効果の検討
田口　慧（東京大学医学部　泌尿器科）

OR-15. 35型腫瘍溶解性アデノウイルスによる抗腫瘍効果誘導メカニズムの解明
小野　良輔（大阪大学大学院　薬学研究科　分子生物学分野）

OR-16. p53感作樹状細胞ワクチンは大腸癌に対するp53搭載腫瘍融解ウイルスの抗腫瘍効果を増強する
山田　元彦（岡山大学大学院医歯薬学総合研究科消化器外科学）

OR-17. 増殖型レトロウイルスベクターを用いたイヌ悪性腫瘍に対するがん自殺遺伝子療法
園田絵観子（兵庫医科大学・先端医学研究所・分子遺伝治療学部門）

OR-18. 肺腺癌に対する非ウイルス遺伝子改変リガンド型IGF1R CART細胞の開発
三島　修治（信州大学医学部　外科学教室　呼吸器外科学分野）

OR-19. 2'3'-cGAMPはC-REVによる腫瘍免疫治療効果を促進する
Patricia Angela Alvero Sibal（名古屋大学理学部／名古屋大学医学系研究科・癌免疫治療研究室）

Oral Session III (E or J)

Cancer ②

7月14日（木） 15:30〜16:42 第3会場

座長：久保　秀司（兵庫医科大学　先端医学研究所　分子遺伝治療学部門）
黒崎　創（鳥取大学医学部医学科　ゲノム再生医学講座　分子医学分野）

OR-20. レオウイルス感染腫瘍細胞由来細胞外小胞による抗腫瘍効果に関する検討
種昴なお実（大阪大学大学院薬学研究科）

OR-21. Gliomaに対する第2世代REIC/Dkk-3遺伝子発現アデノウイルスとbevacizumabの併用療法
井本　良二（岡山大学大学院　脳神経外科）

OR-22. ミトコンドリア阻害剤による代謝リプログラミングは非解糖系膵臓癌における腫瘍融解アデノウイルスの治療感受性を増強する
庄司　良平（岡山大学大学院医歯薬学総合科学研究科　消化器外科学）

OR-23. Novel armed oncolytic HSV exhibits strong antitumor effects that lead to complete tumor regression
Mohamed Abdelmoneim（Cancer Immune Therapy Research Center, Graduate School of Medicine, Nagoya University, Nagoya, Japan / Department of Surgery II, Graduate School of Medicine, Nagoya University, Nagoya, Japan / Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt）

OR-24. 肺癌に対する増殖型レトロウイルスベクターを用いたプロドラッグ活性化遺伝子治療
平岡　圭（北海道大学大学院医学研究院　消化器外科学教室Ⅱ／国立病院機構函館病院　臨床研究部）

OR-25. 増殖型レトロウイルスベクターを用いたプロドラッグ活性化遺伝子治療により誘導された抗腫瘍免疫応答の膵癌モデルに対する治療効果
丹羽　弘貴（北海道大学大学院医学研究院消化器外科学教室Ⅱ）

Oral Session IV (E or J)

Vectors ①

7月14日（木） 16:42〜18:18 第3会場

座長：卜部 匡司（自治医科大学 遺伝子治療研究部）
喜納 裕美（東京大学医科学研究所 遺伝子・細胞治療センター 分子遺伝医学分野）

OR-26. スナネズミ海馬におけるアデノ随伴ウイルスベクターの神経幹細胞への指向性
瀬原 吉英（自治医科大学分子病態治療研究センター遺伝子治療研究部）

OR-27. AAV ベクターを用いた Fabry 病への遺伝子治療研究
林 夢夏（自治医科大学分子病態治療研究センター遺伝子治療研究部）

OR-28. マウス脳内神経細胞及び血管内皮細胞に効率よく遺伝子導入が可能な新規 AAV2 キャプシド CereAAV の開発
田中 佳典（タカラバイオ株式会社）

OR-29. リゾリン脂質アシル転移酵素 LPCAT4/LPLAT10 の肝臓特異的な高発現による、2 型糖尿病モデルマウスに対する治療効果の検討
清水かほり（大阪大谷大学薬学部）

OR-30. Successful Re-administration of Adeno-associated Virus Vectors to Change the Serotypes in Mice
ネメフバヤル バータルツォグト（Department of Biochemistry, Jichi Medical University School of Medicine, Tochigi, Japan）

OR-31. アデノ随伴ウイルス受容体 AAVR を利用した温和な AAV 精製法の開発
牧野友理子（東ソー株式会社）

OR-32. アデノ随伴ウイルス（AAV）ベクター粒子の自動判別システムの開発
松坂 恭成（東京大学医科学研究所遺伝子・細胞治療センター分子遺伝医学分野）

OR-33. 遺伝子治療用レンチウイルスベクターの構造最適化
槇 いづみ（タカラバイオ株式会社 基盤技術開発センター）

Oral Session V (E or J)

Genetic Diseases

7月15日（金） 8:30〜9:42 第3会場

座長：内山 徹（国立成育医療研究センター研究所 成育遺伝研究部 疾患遺伝子構造研究室）
内田 直也（東京大学医科学研究所 遺伝子・細胞治療センター 分子遺伝医学分野）

OR-34. 中枢神経移行型酵素を搭載した AAV による肝臓を標的としたムコ多糖症 II 型への遺伝子治療
嶋田 洋太（東京慈恵会医科大学 総合医科学研究センター 遺伝子治療研究部）

OR-35. Preclinical Safety and Efficacy Validation of CD4LVFOXP3 Cells as an Innovative Treg-like Cell-based Gene Therapy for IPEX Syndrome
Yohei Sato（Stanford University）

OR-36. アデノウイルスベクターを用いたⅡ型くる病モデルラットの遺伝子治療およびゲノム編集治療
木瀬 智子（富山県立大学）

OR-37. 中枢神経移行型酵素を搭載した AAV による GM1 ガングリオシドーシスへの遺伝子治療
松島 小貴（東京慈恵会医科大学 総合医科学研究センター 遺伝子治療研究部）

OR-38. 低侵襲かつ高効率な栄養障害型表皮水疱症遺伝子治療法開発
小林 亮介（大阪大学大学院 医学系研究科 幹細胞遺伝子治療学共同研究講座／株式会社ステムリム）

OR-39. 低ホスファターゼ症に対する遺伝子治療薬（ARU-2801）の有効性及び安全性の検討
趙 東威（日本医科大学）

Oral Session VI (E or J)

Vectors ②

7月15日（金） 9:42〜11:18 第3会場

座長：中井 浩之（オレゴン健康科学大学）
内田 直也（東京大学医科学研究所 遺伝子・細胞治療センター 分子遺伝医学分野）

OR-40. 腫瘍溶解性単純ヘルペスウイルスの精製および保存方法が収量および機能性に及ぼす影響について
黒田 誠司（日本医科大学大学院 生体制御再生医学領域 分子遺伝医学）

OR-41. Inter-subunit associations of AAV icosahedron inform AAP requirement for capsid assembly
Swapna Kollu（Department of Molecular and Medical Genetics, Oregon Health Sciences University）

OR-42. Identification and characterization of an AAV capsid that efficiently transduces the pancreas with limited off-target consequences in non-human primates following retrograde pancreatic duct injection
Kei Adachi（Department of Molecular & Medical Genetics, Oregon Health & Science University）

OR-43. AAVKP1 は局所投与により肝臓への非特異的感染を回避し、効率的な対象臓器への遺伝子導入が可能である
古荘 泰佑（Oregon Health & Science University）

OR-44. 効率的な AAV ベクター生産のためのヒト不死化細胞株のスクリーニング
宮川世志幸（日本医科大学 生化学・分子生物学（分子遺伝学））

OR-45. 接着性 HEK293EB 細胞を用いた固定床バイオリアクターによる AAV9 ベクター生産法の検討
平井 幸彦（東京大学 医科学研究所 遺伝子・細胞治療センター 分子遺伝医学分野）

OR-46. アデノ随伴ウイルス（AAV）ベクターの抽出法開発
坂本 修平（タカラバイオ株式会社）

OR-47. mRNA 医薬開発用合成試薬の開発
松本 裕之（タカラバイオ株式会社）

Oral Session VII (E or J)

Cancer ③

7月15日（金） 15:30〜16:30 第3会場

座長：粕谷 英樹（名古屋大学大学院医学系研究科 癌免疫治療研究室）
池田 裕明（長崎大学 医歯薬学総合研究科 腫瘍医学分野）

OR-48. 固形腫瘍に対する非ウイルス遺伝子改変 IGF1R CAR-T 細胞
大野龍之助（信州大学医学部／クイーンズ大学健康科学部）

OR-49. Wilms' tumor 1 を表層発現する遺伝子組換えビフィズス菌と免疫チェックポイント阻害薬の併用による腎細胞癌治療効果の検討
北川 孝一（神戸大学大学院科学技術イノベーション研究科）

OR-50. p53 を搭載したテロメラーゼ特異的腫瘍融解アデノウイルスの抗腫瘍効果を予測するバイオマーカーの検討
杉本 龍馬（岡山大学院医歯薬総合研究科消化器外科学）

OR-51. 融合型腫瘍溶解性ワクシニアウイルスと HDAC 阻害剤との併用は細胞融合能を強化することで相乗的に抗腫瘍効果を誘導する
中武 大夢（鳥取大学医学部医学科 分子医学）

OR-52. 第二世代遺伝子改変 miRNA 標的配列搭載コクサッキーウイルス B 群 3 型の非臨床毒性試験
宮本 将平（東京大学 定量生命科学研究所 ALA 先端医療学社会連携部門）

Oral Session VIII (E or J)

Clinical Trials

7月15日（金） 16:30〜17:42 第3会場

座長：中沢 洋三（信州大学医学部小児医学教室）
岡崎 利彦（大阪大学医学部附属病院 未来医療開発部 未来医療センター）

OR-53. SaCas9 によるゲノム編集の安全性評価のために考慮すべきオフターゲット変異候補配列に関する検討
山下 拓真（国立医薬品食品衛生研究所）

OR-54. ファブリー病への Ex vivo Gene & Cell Therapy, Macroencapsulated Spheroid with Scaffold (MESS) Transplantation
上 大介（京都府立医科大学 人工臓器・心臓移植再生医学講座）

OR-55. 閉鎖系自動細胞調製システムを用いた RetroNectin® による遺伝子導入細胞の新製法
小川 彩空（タカラバイオ株式会社）

OR-56. キメラ抗原受容体を樹状細胞 (dendritic cell: DC) に組み込んだ新規細胞療法 CAR-DC
直江 吉則（名古屋大学）

OR-57. 非小細胞性肺癌脳転移に対する HSV-TK 発現脱落乳歯歯髄幹細胞 (SHED) を用いた自殺遺伝子療法の有効性
大石 知也（浜松医科大学 脳神経外科学講座）

OR-58. 陰イオン交換 -UHPLC によるアデノ随伴ウイルスベクターの中空粒子評価法に関する研究
山本 武範（国立医薬品食品衛生研究所 遺伝子医薬部）

Oral Session IX (E or J)

Neurogenic, Ophthalmic, and Musculo-skeletal Diseases

7月16日（土） 8:30〜9:42 第3会場

座長：長野 清一（大阪大学大学院医学系研究科神経難病認知症探索治療学寄附講座）
石井亜紀子（筑波大学医学医療系神経内科学）

OR-59. 細胞内結晶化抑制型 NEU1 と CTSA の二重搭載 AAV5 ベクターを用いたミオクローヌスモデルマウスの遺伝子治療
三好 瑞希（徳島大学大学院薬科学教育部）

OR-60. 細胞内非結晶性 NEU1 及び CTSA 遺伝子同時搭載 AAVPHP.eB を用いたガラクトシアリドーシスモデルマウスに対する遺伝子治療
福池 凜（徳島大学大学院医歯薬研究部（薬学域））

OR-61. 組換えウイルスベクターを用いたアルツハイマー病の能動免疫療法
田平 武（順天堂大学大学院医学研究科認知症診断・予防・治療学講座）

OR-62. PET による非ヒト霊長類での脳室内または大槽内投与後の AAV ベクターの脳脊髄液内早期分布の観察
熊谷 真一（自治医科大学脳神経外科）

OR-63. ニーマンピック病 C 型における免疫系による神経変性制御
安田 徹（国立成育医療研究センター）

OR-64. 筋萎縮性側索硬化症における蛋白質翻訳機能障害と治療への応用
長野 清一（大阪大学大学院医学系研究科神経難病認知症探索治療学／大阪大学大学院医学系研究科神経内科学）

Oral Session X (E or J)

Vectors ③

7月16日（土） 9:42〜10:54 第3会場

座長：平井 幸彦（東京大学医科学研究所 遺伝子・細胞治療センター 分子遺伝医学分野）
水上 浩明（自治医科大学 分子病態治療研究センター 遺伝子治療研究部）

OR-65. プラズマ遺伝子導入法のゲノムリアレンジメントフリー性
田村 亮太（愛媛大学大学院理工学研究科）

OR-66. Mdmx plays a crucial role in neuronal damage after ischemic stroke
Haomin Yan（大阪大学大学院医学系研究科 神経内科学）

OR-67. rAAV9 三元系ナノシステムは、HIFU を用いることによって肝障害を回避し、脳への遺伝子導入を飛躍的に向上させる
喜納 宏昭（ナノ医療ノベーションセンター 片岡・喜納ラボ）

OR-68. レトロウイルスベクター産生ヒト羊膜間葉系細胞 (VP-hMSCs) 作製プロトコルの最適化
山崎 吉之（日本医科大学 大学院医学研究科 分子遺伝医学分野）

OR-69. 大規模製造に向けたゾーナル超遠心による短時間高精度組換えアデノ随伴ウイルスベクター精製法の開発
和田美加子（東京大学 医科学研究所 遺伝子・細胞治療センター 分子遺伝医学分野）

OR-70. 日本人血友病患者の抗 AAV 中和抗体保有率
柏倉 裕志（自治医科大学 医学部 生化学講座）

Oral Session XI (E or J)

Basic science

7月16日（土） 13:10〜14:34 第3会場

座長：柳生 茂希（信州大学 学術研究・産学官連携推進機構）
喜納 裕美（東京大学医科学研究所 遺伝子・細胞治療センター 分子遺伝医学分野）

OR-71. M1 ミクログリア標的化アポトーシスペプチドによる新生児低酸素性虚血性脳症への新規治療法開発
全 梨花（滋賀医科大学 生化学分子生物学講座 再生修復医学部門／滋賀医科大学 産科学婦人科学講座）

OR-72. 膵臓選択的ハイドロダナミック遺伝子導入法による効率的な膵癌モデル動物の確立
田中 裕登（新潟大学大学院医歯学総合研究科 消化器病学分野）

OR-73. 腫瘍溶解性ウイルスであるレオウイルスによる肝線維化抑制効果〜レオウイルス作用による肝星細胞の脱活性化〜
石神 育歩（大阪大学大学院・薬学研究科）

OR-74. 次世代 CAR-T（CD19-JAK/STAT CAR-T, TBI-2001）製造技術移管後の同等性評価
田中 真哉（タカラバイオ株式会社）

OR-75. A Microfluidic 3D Endothelium-on-a-Chip Model to Study Transendothelial Migration of T Cells in diseases
Luc Zhang（MIMETAS BV / MIMETAS Japan K.K.）

OR-76. Targeted genome repair in selectively expanded mouse long-term hematopoietic stem cells
Suvd Byambaa（Division of Regenerative Medicine, Jichi Medical University, Tochigi, Japan）

OR-77. CRISPR-Cas3 を利用した次世代 CAR-T 細胞療法の開発
藤井 智明（東京大学医科学研究所 実験動物研究施設 先進動物ゲノム研究分野）

コーポレートセミナー

コーポレートセミナーⅠ

遺伝子細胞治療のこれまでとこれから
（ミルテニーバイオテク株式会社）

7月14日（木） 12:00〜12:50 第1会場

座長：米満　吉和（九州大学大学院薬学研究院　革新的バイオ医薬創成学）

アカデミアの CAR 輸注療法開発 ―細胞調整の Centralization と Decentralization―
珠玖　洋（三重大学大学院医学系研究科　個別化がん免疫治療学）

コーポレートセミナーⅡ

AAV 製品のクオリティ・バイ・デザイン (QbD)
（日本ポール株式会社）

7月14日（木） 12:00〜12:50 第2会場

座長：針金谷尚人（日本ポール株式会社）

AAV 製品のクオリティ・バイ・デザイン (QbD)
Peiqing Zhang（Pall Corporation）

遺伝子治療アプリケーションへの Allegro™ STR シングルユースバイオリアクターの活用
堀内　賢一（日本ポール株式会社）

コーポレートセミナーⅢ

CAR-T 細胞療法の現状と展望
（株式会社スクラム）

7月14日（木） 12:00〜12:50 第3会場

座長：多田　誠一（株式会社スクラム）

CAR-T 細胞療法の現状と展望
保仙　直毅（大阪大学大学院医学研究科 血液・腫瘍内科学）

コーポレートセミナーⅣ

合成生物学がもたらす遺伝子治療領域の技術革新とウイルスベクターの製造技術と品質管理の最新動向
（株式会社シンプロジェン）

7月15日（金） 12:00〜12:50 第1会場

座長：山田　尚之（株式会社シンプロジェン）

合成生物学の世界動向と細胞・遺伝子治療領域への展開
近藤　昭彦（神戸大学）

遺伝子治療用ウイルスベクターの製造技術と品質管理のトレンド（レギュレーション含む）
内田　和久（神戸大学大学院　科学技術イノベーション研究科）

コーポレートセミナーⅤ

遺伝子治療の新時代
（アンジェス株式会社）

7月15日（金） 12:00〜12:50 第2会場

座長：奥山　虎之（埼玉医科大学　ゲノム医療科）

遺伝子治療のプラットフォーム・モダリティとしてのプラスミド DNA
森下　竜一（大阪大学大学院医学系研究科　臨床遺伝子治療学）

EmendoBio におけるゲノム編集治療の開発
村上　晶彦（アンジェス株式会社）

コーポレートセミナーVI

非ウイルス性遺伝子導入による遺伝子・細胞治療開発
（キコーテック株式会社）

7月15日（金） 12:00〜12:50 第3会場

座長：内山　徹（国立成育医療研究センター研究所　成育遺伝研究部　疾患遺伝子構造研究室）

iPS 細胞由来免疫細胞を用いた癌治療剤の開発
等　泰道（サイアス株式会社）

コーポレートセミナーVII

遺伝子改変 T 細胞療法の最新動向
（タカラバイオ株式会社）

7月16日（土） 12:00〜13:00 第1会場

座長：榎　竜嗣（タカラバイオ株式会社）

遺伝子改変 T 細胞療法の最新動向
平野　直人（Princess Margaret Cancer Centre, University of Toronto）

コーポレートセミナーVIII

AAV ベクターを用いた遺伝子治療の現状と展望
（ノバルティス ファーマ株式会社）

7月16日（土） 12:00〜13:00 第3会場

座長：山形　崇倫（自治医科大学　小児科学）

SMA に対する遺伝子治療の実臨床の経験
小俣　卓（千葉県こども病院　神経科）

AAV 遺伝子治療の課題と今後の展望
山形　崇倫（自治医科大学　小児科学）

ティーブレイクセミナー

ティーブレイクセミナー Ⅰ

デジタル PCR の効果的な運用方法について
（株式会社キアゲン）

7月14日（木） 15:00〜15:30 第2会場

ナノプレート方式のデジタル PCR による AAV ベクターの正確な定量と効果的な運用方法
四柳 雄一（マーケティング部 ビジネスディベロップメント マネージャー）

ティーブレイクセミナー Ⅱ

幹細胞治療の臨床応用に向けた取り組み
（株式会社フコク）

7月14日（木） 15:00〜15:30 第3会場

座長：原田 結（九州大学）

SphereRing を用いて作製された臨床向け脂肪由来幹細胞スフェロイドの遺伝子解析
石垣 靖人（金沢医科大学）

ティーブレイクセミナー Ⅲ

細胞治療・遺伝子治療における新しい製造ソリューション
（サーモフィッシャーサイエンティフィック）

7月15日（金） 15:00〜15:30 第2会場

細胞処理と遺伝子導入を閉鎖系で自動化する新しい細胞製造ソリューション
荻田 伸夫（サーモフィッシャーサイエンティフィック ライフテクノロジーズジャパン株式会社）

AAV ベクターの開発・製造に提案するプラットフォーム技術製品
鳥海健太郎（サーモフィッシャーサイエンティフィック ライフテクノロジーズジャパン株式会社）

ティーブレイクセミナー Ⅳ

アデノ随伴ウイルスの精製工程におけるスケーラブルな精製プロセスのご提案
（Cytiva（グローバルライフサイエンステクノロジーズジャパン株式会社））

7月15日（金） 15:00〜15:30 第3会場

座長：春日 卓郎（Cytiva（グローバルライフサイエンステクノロジーズジャパン株式会社））

スケーラブルなアデノ随伴ウイルス精製法
奥山 知之（Cytiva（グローバルライフサイエンステクノロジーズジャパン株式会社））

JSGCT Chairman's Lecture (J)

Date: July 14, 13:00–13:30, Room 1

Chairperson: Yoshikatsu Eto (*Advanced Clinical Research Center, Southern Tohoku Research Center for Neuroscience/ Prof.* (*Emeritus*)*, Tokyo Jikei University School of Medicine*)

Great Journey of Gene & Cell Therapy in Japan
Ryuichi Morishita (*Osaka Univeristy*)

President's Lecture (J)

Date: July 15, 14:00–14:40, Room 1

Chairperson: Yasufumi Kaneda (*Osaka University*)

GAIA-NK: Off-the-shelf 'NK-like' cell product that can eliminate solid tumors
Yoshikazu Yonemitsu (*Graduate School of Pharmaceutical Sciences, Kyushu University*)

JSGCT Special Program (E or J)

–COVID-19 Vaccines as gene-based technologies–

Date: July 14, 16:00–18:30, Room 1

Chairpersons: Ryuichi Morishita (*Osaka Univeristy*)
Yoshikazu Yonemitsu (*Graduate School of Pharmaceutical Sciences, Kyushu University*)

mRNA as Medicine
Melissa Moore (*Eleanor Eustis Farrington Chair of Cancer Research/ Professor, RNA Therapeutics Institute/ University of Massachusetts Medical School/ Chief Scientific Officer, Platform Research, Moderna*)

Destructive innovation and evolution of vaccine research and development
Ken J Ishii (*International Vaccine Design Center, The Institute of Medical Science, The University of Tokyo*)

Overview of Comirnaty Development
Noriko Morikubo (*Regulatory Affairs, Pfizer R&D*)

COVID-19 Adenovirus Vector Vaccine; Development and Introduction to Inoculation Program
Michio Tanaka (*Research & Development, AstraZeneca*)

DNA vaccine for COVID-19
Hironori Nakagami (*Department of Health Development and Medicine, Osaka University Graduate School of Medicine*)

Current PMDA's Review of COVID-19 Vaccines in Japan and in the Future
Yasuhiro Araki (*Pharmaceuticals and Medical Devices Agency*)

Presidential Special Program 1 (E)

–ASGCT/ESGCT/JSGCT Joint Symposium–

Date: July 14, 14:00–16:00, Room 1

Chairpersons: Noriyuki Kasahara (*University of California, San Francisco* (*UCSF*))
Takafumi Nakamura (*Tottori University Faculty of Medicine*)

Improving efficacy of Adeno-Associated-Virus (AAV) vectors for *in vivo* gene therapy
Hildegard Büning *(Institute for Experimental Hematology, Hannover Medical School, Hannover, Germany/ REBIRTH – Cluster of Excellence, Hannover Medical School, Hannover, Germany/ German Center for Infection Research* (*DZIF*)*, partner site Hannover-Braunschweig*)

Long-Term Follow-up of the Phase I/II Gene Therapy Trial in Fanconi Anemia-A Patients
Juan A. Bueren (*Hematopoietic Innovative Therapies Division, CIEMAT/CIBERER/Fund. Jiménez Díaz, Madrid, Spain*)

Targeting hematopoietic stem cells for ex vivo and in vivo gene therapy
Hans-Peter Kiem (*Director, Stem Cell and Gene Therapy Program/ Fred Hutchinson Cancer Research Center*)

The next generation of AAV gene therapy
Guangping Gao (*Director, Horae Gene Therapy Center and Viral Vector Core, Co-Director, Li Weibo Institute for Rare Diseases Research, Professor of Microbiology and Physiological Systems, Penelope Booth Rockwell Professor in Biomedical Research, University of Massachusetts Medical School*)

Plasmid DNA-based Gene Therapy: From Regenerative Medicine to Vaccine for COVID-19
Ryuichi Morishita (*Osaka Univeristy*)

Presidential Special Program 2 (J)

Date: July 15, 8:30–11:00, Room 1

Chairpersons: Shinichiro Ueda (*Department of Clinical Pharmacology & Therapeutics, University of the Ryukyus*)
Yoshikazu Yonemitsu (*Graduate School of Pharmaceutical Sciences, Kyushu University*)

Introduction: Arising practical issues related to Gene and Cell Therapies in Japan
Yoshikazu Yonemitsu (*Graduate School of Pharmaceutical Sciences, Kyushu University*)

Trend and issue of pharmaceutical price and access
Hiromichi Shirasawa (*MSDKK*)

Conditional Time-limited Approval of Regenerative Products
Shinichi Okudaira (*Pharmaceuticals and Medical Devices Agency*)

Current status and various issues caused by diversity of regenerative medical products
Hiroyuki Kanoh (*Forum for Innovative Regenerative Medicine* (*FIRM*) */ Astellas.Pharma Inc.*)

Panel discussion

Presidential Special Program 3 (J)

–Gene and Cell Therapy: Industrial Views–

Date: July 15, 14:40–16:40, Room 1

Chairpersons: Ei Yamada (*AnGes, Inc.*)
Koichi Nakao (*Takara Bio Inc.*)

Developments of Cell and Gene therapies in Novartis
Tohru Hirose (*Novartis Pharma K.K.*)

Development Strategy for Gene Therapy in Rare Disease
Mihoko Kobayashi (*Pfizer R&D Japan*)

Astellas' Approach to Industrialize Cell and Gene Therapy
Yoshitsugu Shitaka (*Astellas Pharma Inc.*)

Position of Takara Bio in Gene Therapy
Junichi Mineno (*Takara Bio Inc.*)

Potentiality of Plasmid Vector for Gene Therapy
Mitsuaki Chujo (*AnGes, Inc.*)

Presidential Special Program 4 (E or J)

–CAR-T/TCR/NK and beyond: Breakthrough technologies for solid tumors–

Date: July 16, 8:30–11:00, Room 1

Chairpersons: Hiroshi Shiku (*Department of Personalized cancer Immunotherapy, Mie University Graduate School of Medicine*)
Yui Harada (*Graduate School of Pharmaceutical Sciences, Kyushu University*)

Harnessing TCR-T therapy to overcome personalization and tumor heterogeneity
Hiroaki Ikeda (*Department of Oncology, Nagasaki University Graduate School of Biomedical Sciences*)

Strategical bioengineering for CAR-T cell against solid cancers
Hiroshi Fujiwara (*Department of Personalized Cancer Immunotherapy, Mie University Graduate School of Medicine*)

CD8 killer T cells and NK cells from iPSC for cancer immunotherapy
Shin Kaneko (*CiRA, Kyoto University / TMRC, University of Tsukuba*)

NK cells: next generation cell therapies for cancer
Katy Rezvani (*University of Texas, MD Anderson Cancer Center*)

Off-the-shelf NK-like cell product: GAIA-102 ~A breakthrough in the immunotherapy against various solid tumors~
Yui Harada (*Graduate School of Pharmaceutical Sciences, Kyushu University*)

Special Lecture (E)

Date: July 14, 13:30–14:00, Room 1

Chairperson: Noriyuki Kasahara (*University of California, San Francisco* (*UCSF*))

Publishing Clinical Research at Nature Medicine
Anna Maria Ranzoni (*Nature Medicine*)

Educational Lecture 1 (J)

Date: July 14, 11:00–11:50, Room 1

Chairperson: Kenzaburo Tani (*Institute for Quantitative Biosciences, The University of Tokyo*)

Spinal cells involved in chronic pain
Makoto Tsuda (*Kyushu University*)

Educational Lecture 2 (J)

Date: July 15, 11:00–11:50, Room 1

Chairperson: Hideki Mochizuki (*Department of Neurology, Osaka University Graduate School of Medicine*)

Cybernics Medical Innovation realizing functional regeneration for Neurological and Neuromuscular Diseases
— Towards further challenges with "Cybernics×Regenerative Medicine" —
Yoshiyuki Sankai (*University of Tsukuba, Faculty of Engineering, Information and Systems, Center for Cybernics Research, R&D Center for Frontiers of MIRAI in Policy and Technology / CYBERDYNE Inc.*)

Educational Lecture 3 (J)

Date: July 16, 11:00–11:50, Room 1

Chairperson: Yasuhiro Ikeda (*Department of Ophthalmology, Faculty of Medicine, University of Miyazaki*)

Retinal cell therapy and sustainable medicine
Masayo Takahashi (*Vision Care Inc. / Research Center, Kobe City Eye Hospital / Ritsumeican Advanced Research Academy, Ritsumeican University*)

NATSJ-JSGCT Joint Symposium (J)

–Non-viral Therapeutics–

Date: July 15, 16:40–18:40, Room 1

Chairpersons: Takanori Yokota (*Department of Neurology and Neurological Science, Tokyo Medical and Dental University*)
Yasufumi Kaneda (*Osaka Univeristy*)

Blood-brain-barrier (BBB) crossing heteroduplex oligonucleotide
Takanori Yokota (*Department of Neurology and Neurological Science, Tokyo Medical and Dental University*)

New molecular technologies to improve efficacy and safety of oligonucleotide therapeutics
Takeshi Wada (*Tokyo University of Science*)

Polymer-based nanomedicines for nucleic acid delivery and retention
Kanjiro Miyata (*Department of Materials Engineering, Graduate School of Engineering, The University of Tokyo*)

Oligonucleotide therapeutics for intractable neurological disorders: Recent Advances
Yasuyoshi Kimura, Hideki Mochizuki (*Department of Neurology, Osaka University Graduate School of Medicine*)

Individualized medicine with antisense oligonucleotides for rare neurological disorders
Tojo Nakayama (*Harvard Medical School, Boston Children's Hospital, MA, USA / Tokyo Medical and Dental University, Tokyo, Japan*)

Symposium 1 (J)

–Ocular gene and cell therapy–

Date: July 14, 9:45–11:35, Room 2

Chairpersons: Toya Ohashi (*The Jikei University School of Nursing, Department of Human Health Science and Therapeutics*)
Yasuhiro Ikeda (*Department of Ophthalmology, Faculty of Medicine, University of Miyazaki*)

S1-1. Regenerative medicine for corneal epithelium
Yoshinori Oie (*Osaka University*)

S1-2. Regenerative therapy using ESC/iPSC-derived retinas for retinal degeneration
Michiko Mandai (*Kobe City Eye Hospital*)

S1-3. Genome editing gene therapy for retinitis pigmentosa
Koji Nishiguchi (*Nagoya University*)

S1-4. Gene therapy using an optogenetic gene for restoring vision
Hiroshi Tomita (*Iwate University*)

S1-5. Retinal gene therapy using anti-angiogenic and neuroprotective factors
Yusuke Murakami (*Department of Ophthalmology, Graduate Shool of Medical Sciences, Kyushu University*)

Symposium 2 (E or J)

–Gene Editing & Mitochondrial Manipulation–

Date: July 14, 13:30–15:00, Room 2

Chairpersons: Kohnosuke Mitani (*Saitama Mediccal University*)
Ken-Ichi Wada (*kyushu University / RIKEN*)

S2-1. CRISPR-free Base Editor for Precise Editing of Mitochondrial and Nuclear DNA
Beverly Y. Mok (*Merkin Institute of Transformative Technologies in Healthcare, Broad Institute of MIT and Harvard, Cambridge, MA, USA/*
Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA, USA./
Howard Hughes Medical Institute, Harvard University, Cambridge, MA, USA/
**Current affiliation: Molecular Engineering Lab, Institute of Molecular and Cell Biology, A*STAR, Singapore*)

S2-2. Challenge to gene-cell therapy based on mitochondrial DDS
Yuma Yamada (*Faculty of Pharmaceutical Sciences, Hokkaido University /*
FOREST Program, Japan Science and Technology Agency)

S2-3. Mitochondrial genome manipulation using a microfluidic device
Ken-Ichi Wada (*kyushu University / RIKEN*)

S2-4. The Potential of Genome Editing Treatment with an Engineered Cas
Tsukasa Ohmori (*Department of Biochemistry, Jichi Medical University School of Medicine*)

S2-5. Advancing Homologous Recombination based Genome Editing of Hematopoietic Stem Cells
Matthew Porteus (*Department of Pediatrics, Stanford University School of Medicine*)

Symposium 3 (E or J)

–Vector Development–

Date: July 14, 15:30–17:00, Room 2

Chairpersons: Takashi Okada (*Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo*)
Yumi Kanegae (*Research Center for Medical Science, Jikei University School of Medicine*)

S3-1. mRNA as a new modality for gene therapy
Keiji Itaka (*Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University* (*TMDU*))

S3-2. COVID-19 pathological analysis and control using mice transduced with an adenoviral vector expressing human ACE2 and attenuated vaccinia vaccine encoding the SARS-CoV-2 spike protein
Michinori Kohara (*Tokyo Metropolitan Institute of Medical Science*)

S3-3. Development of dosing protocol to reduce the required dose of rAAV using adult stem cells
Hiromi Hayashita-Kinoh (*Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo*)

S3-4. Robust AAV Purification Platform Tolerant of Changes in Harvest Material
Jessica M. Tate (*Thermo Fisher Scientific, Patheon*)

Symposium 4 (E or J)

–Cancer Gene Therapy 1–

Date: July 14, 17:00–18:30, Room 2

Chairpersons: Yasutomo Nasu (*Okayama University*)
Masato Yamamoto (*University of Minnesota*)

S4-1. Engineering of the cancer-targeted oncolytic adenovirus for systemic therapy of advanced cancers
Mizuho Sato-Dahlman (*University of Minnesota*)

S4-2. Preclinical development of oncolytic virus therapies for malignant brain tumors using cancer stem-like cell-based models
Hiroaki Wakimoto (*Massachusetts General Hospital, Harvard Medical School*)

S4-3. CAR-T cell therapy for multiple myeloma
Naoki Hosen (*Osaka University Graduate School of Medicine*)

Symposium 5 (E)

–Oncolytic virus–

Date: July 15, 8:30–10:00, Room 2

Chairpersons: Tomoki Todo (*Division of Innovative Cancer Therapy, The Institute of Medical Science, The University of Tokyo*)
Toshiyoshi Fujiwara (*Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences*)

S5-1. Gene Edited and Engineered Cell based therapies for Cancer: From Bench to Bedside
Khalid Shah (*BWH, Harvard Medical School*)

S5-2. Addressing the challenges of conventional oncolytic virotherapy
Chae-Ok Yun (*Department of Bioengineering, College of Engineering, Hanyang University, Seoul, Korea/ GeneMedicine Co., Ltd., Seoul, Korea*)

S5-3. An investigator-initiated clinical trial of third generation oncolytic virus armed with IL-12 against malignant melanoma
Ryuhei Okuyama (*Department of Dermatology, School of Medicine, Shinshu University*)

S5-4. Survivin-Responsive Conditionally Replicating Adenovirus Regulated with Multiple Factors (Surv.m-CRA): From Basic Research to Phase II Clinical Trials
Ken-ichiro Kosai (*Kagoshima University Graduate School of Medical and Dental Sciences / Kagoshima University Hospital*)

S5-5. Recent progress in clinical application of telomerase-specific oncolytic virotherapy
Hiroshi Tazawa (*Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences / Center for Innovative Clinical Medicine, Okayama University Hospital*)

Symposium 6 (J)

–Young investigators session: explore a new era of gene therapy research–

Date: July 15, 10:00–11:30, Room 2

Chairpersons: Fuminori Sakurai (*Graduate School of Pharmaceutical Sciences, Osaka University*)
Makoto Otsu (*Department of Transfusion and Cell Transplantation, Kitasato University School of Medicine*)

S6-1. Elucidating a mechanism for HVJ-E-induced anti-tumor effects in vivo
Keisuke Nimura (*Osaka University School of Medicine*)

S6-2. Non-viral CAR-T cells in the treatment of solid tumors
Shigeki Yagyu (*Shinshu University Innovative Research & Liaison Organization*)

S6-3. Development of ultrasound-responsive nanobubbles and its application to gene and nucleic acid-based therapy
Yoko Endo-Takahashi (*Tokyo University of Pharmacy and Life Sciences*)

S6-4. Development of delivery technologies for genome editing therapy
Akitsu Hotta (*CiRA, Kyoto University / T-CiRA Joint Program*)

Symposium 7 (J)

–Regulatory Sciences–

Date: July 15, 15:30–17:00, Room 2

Chairpersons: Akihiro Kume (*Center for Clinical Investigation, Jichi Medical University Hospital*)
Eriko Uchida (*Division of Molecular Target and Gene Therapy Products, National Institute of Health Sciences*)

S7-1. Regulatory Update on the Development of Gene and Cell Therapies
Eriko Uchida (*Division of Molecular Target and Gene Therapy Products, National Institute of Health Sciences*)

S7-2. Act on the Safety of Regenerative Medicine and the Direction in the Amendment
Keisuke Okamoto, Mika Sasabuchi (*Ministry of Health, Labour and Welfare*)

S7-3. Improvement of the oparation of the Cartagena Act
Akira Sakurai (*Pharmaceuticals and Medical Devices Agency*)

Symposium 8 (E or J)

–Neuromuscular Disorders–

Date: July 15, 17:00–18:30, Room 2

Chairpersons: Shin-ichi Muramatsu (*Neurological Gene Therapy, Jichi Medical University*)
Hideki Mochizuki (*Department of Neurology, Osaka University Graduate School of Medicine*)

S8-1. Gene therapy for autophagy disease
Kazuhiro Muramatsu (*Jichi Medical University, Pediatrics*)

S8-2. Gene Therapy of Metachromatic Leukodystrophy
Noriko Miyake (*Department of Biochemistry and Molecular Biology, Nippon Medical School*)

S8-3. Gene Therapy for Spinal Muscular Atrophy: Clinical and Real-World Experience and Clinical Program Update
Sandra P. Reyna (*Novartis Gene Therapies*)

S8-4. Treatment Strategies for Fukuyama Muscular Dystrophy
Tatsushi Toda (*Department of Neurology, Graduate School of Medicine, The University of Tokyo*)

Symposium 9 (E or J)

–Genetic diseases–

Date: July 16, 8:30–10:00, Room 2

Chairpersons: Masafumi Onodera (*Gene & Cell Therapy Promotion Center, National Center for Child Health and Development*)
Torayuki Okuyama (*Department of Clinical Genomics, Saitama Mediccal University*)

S9-1. Progress of gene therapy for inborn errors of immunity
Toru Uchiyama (*National Center for Child Health and Development*)

S9-2. Research and Development of Gene and Cell therapy for lysosomal storage diseases
Hiroshi Kobayashi (*Division of Gene Therapy, Research Center for Medical Sciences, The Jikei University School of Medicine*)

S9-3. Long-term efficacy of gene therapy for AADC deficiency using AAV2-AADC vector
Karin Kojima (*Department of Pediatrics, Jichi Medical University*)

S9-4. Implementation of gene therapy for spinal muscular atrophy using an adeno-associated virus vector
Reiko Arakawa (*Department of Genomic Medicine, National Center for Global Health and Medicine / Medical Genomics Center, National Center for Global Health and Medicine*)

Symposium 10 (E)

–Cancer Gene Therapy 2–

Date: July 16, 10:00–11:30, Room 2

Chairpersons: Kazunori Aoki (*National Cancer Center Research Institute*)
Takafumi Nakamura (*Tottori University Faculty of Medicine*)

S10-1. Exploration of novel targets for the development of lung cancer immunotherapy based on the immune tumor microenvironment
Kazunori Aoki (*National Cancer Center Research Institute*)

S10-2. Fusogenic oncolytic vaccinia virus enhances systemic antitumor immune response by modulating the tumor microenvironment
Takafumi Nakamura (*Tottori University Faculty of Medicine*)

S10-3. Novel technologies of CAR-T cell therapy for solid cancers
Koji Tamada (*Yamaguchi University Graduate School of Medicine*)

S10-4. Development of HPV genome-targeted gene therapy for HPV induced cancers by an all-in-one adenovirus vector expressing multicopy guide RNAs and Cas9 nickase
Tomomi Nakahara (*National Cancer Center Research Institute*)

Symposium 11 (J)

–Regenerative Medicine & Cardiovascular diseases–

Date: July 16, 13:10–14:40, Room 1

Chairpersons: Katsuto Tamai (*Osaka University Graduate School of Medicine*)
Hironori Nakagami (*Department of Health Development and Medicine, Osaka University Graduate School of Medicine*)

S11-1. Translational research using myocardial tissue transplantation for severe heart failure
Shigeru Miyagawa (*The Department of Cardiovascular Surgery, Osaka University Graduate School of Medicine*)

S11-2. Mitochondria and DNA damage: therapeutic targets against heart failure
Yuichi Oike (*Kumamoto University*)

S11-3. Development of regeneration-inducing medicine for dystrophic epidermolysis bullosa
Katsuto Tamai (*Osaka University Graduate School of Medicine*)

Plenary Session (E or J)

Date: July 14, 9:30–11:00, Room 1

Chairpersons: Hiroshi Fukuhara (*Department of Urology, Kyorin University School of Medicine*)
Yumi Kanegae (*Research Center for Medical Science, Jikei University School of Medicine*)

PS-01. A leucine residue in the N terminal degron of AAV AAP is one of the amino acids essential for its degradation
Anusha Sairavi (*Molecular and Medical Genetics, Oregon Health and Science University*)

PS-02. Gene therapy using genome-edited iPS cells for malignant glioma
Ryota Tamura (*Keio University School of Medicine*)

PS-03. Antitumor effect of GAIA-102 on refractory tumors and its underlying mechanism
Situo Zheng (*R&D Laboratory for Innovative Biotherapeutics Science Graduate School of Pharmaceutical Sciences, Kyushu University*)

PS-04. A single-dose CD117 antibody-drug conjugate allows for efficient engraftment of gene-modified CD34+ cells in a non-human primate model for lentiviral gene therapy
Naoya Uchida (*Cellular and Molecular Therapeutics Branch, NHLBI/NIDDK, National Institutes of Health / Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo*)

PS-05. Current status and perspectives of characterization and quality control of adeno-associated virus vectors
Susumu Uchiyama (*Graduate School of Engineering, Osaka University / Manufacturing Techonology Association Biologics*)

PS-06. A phase I investigator-initiated clinical trial of non-viral gene-modified HER2 CAR-T cells in HER2-positive relapsed / advanced bone / soft tissue sarcoma and gynecologic malignancies
Koichi Hirabayashi (*Department of Pediatrics, Shinshu University School of Medicine*)

Oral Session I (E or J)

Cell Therapy

Date: July 14, 9:45–11:09, Room 3

Chairpersons: Yutaka Hanazono (*Jichi Medical University*)
Hiroaki Kinoh (*Innovation Center of NanoMedicine, Kataoka-Kinoh Lab*)

OR-07. Ligand-based bispecific chimeric antigen receptor T cells redirected to EPH-family protein for solid tumors
Konomi Morita (*Shinshu University School of Medicine, Department of Pedicatrics*)

OR-08. Characterization of CAR-NK produced by expansion method using RetroNectin® induced T-cells
Toshikazu Nishie (*Technology Development Center, Takara Bio Inc.*)

OR-09. Expression of CAR targets on solid tumors by armed oncolytic virus has synergetic effect on CAR-T cell therapy
Mona Alhussein Aboalela (*Cancer Immune Therapy Research Center, Graduate School of Medicine, Nagoya University, Nagoya, Japan / Department of Surgery II, Graduate School of Medicine, Nagoya University, Nagoya, Japan / Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt*)

OR-10. Amnion mesenchymal stem cells can maintain the muscle function as an inflammatory regulator in animal models of Duchenne muscular dystrophy
Yuko Nitahara Kasahara (*Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo*)

OR-11. Neuroprotective effect of amnion-derived mesenchymal stem cell transplantation in cerebral ischemia-reperfusion injury
Shiro Takahashi (*Department of Neurological Science, Graduate School of Medicine, Nippon Medical School*)

OR-12. A cryopreservation method to maintain the viability and cytotoxicity of highly activated natural killer-like cells
Yufu Xin (*R&D Laboratory for Innovative Biotherapeutics Science Graduate School of Pharmaceutical Sciences, Kyushu University*)

OR-13. Development of the treatment for Alzheimer's disease by iPS cell-derived microglia
Tomoaki M Kato (*CiRA Foundation*)

Oral Session II (E or J)

Cancer①

Date: July 14, 13:30–14:42, Room 3

Chairpersons: Ken-ichiro Kosai (*Kagoshima University Graduate School of Medical and Dental Sciences*)
Hiroshi Tazawa (*Center for Innovative Clinical Medicine, Okayama University Hospital*)

OR-14. Efficacy of a third-generation oncolytic herpes simplex virus type 1 in patient-derived sarcoma cells
Satoru Taguchi (*Department of Urology, Graduate School of Medicine, The University of Tokyo*)

OR-15. The mechanism of novel oncolytic adenovirus serotype 35-mediated anti-tumor effects
Ryosuke Ono (*Laboratory of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences, Osaka University*)

OR-16. Immunization with adenovirus-p53-transduced dendritic cell vaccine enhances the antitumor efficacy of p53-armed oncolytic virotherapy in colorectal cancer
Motohiko Yamada (*Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan*)

OR-17. Suicide gene therapy for canine malignancies by using retroviral replicating vectors
Emiko Sonoda (*Laboratory of Molecular and Genetic Therapeutics, Advanced Medical Science, Hyogo Medical University*)

OR-18. Development of a novel CAR-T cell therapy targeting insulin-like growth factor I receptor for lung adenocarcinoma
Shuji Mishima (*Division of General Thoracic Surgery, Department of Surgery, Shinshu University school of medicine*)

OR-19. STING Activator 2'3'-cGAMP Enhances the Anti-tumor Efficacy of C-REV, an HSV1 based Oncolytic Virus
Patricia Angela Alvero Sibal (*Nagoya University School of Science / Nagoya University Graduate School of Medicine Cancer Immune Therapy Research Center*)

Oral Session III (E or J)

Cancer②

Date: July 14, 15:30–16:42, Room 3

Chairpersons: Shuji Kubo (*Laboratory of Molecular and Genetic Therapeutics, Institute for Advanced Medical Sciences, Hyogo College of Medicine*)
Hajime Kurosaki (*Division of Molecular Medicine, Department of Genomic Medicine and Regenerative Therapeutics, School of Medicine, Tottori University Faculty of Medicine*)

OR-20. Evaluation of antitumor effects of extracellular vesicles derived from reovirus-infected tumor cells
Naomi Shuwari (*Graduated School of Pharmaceutical Sciences, Osaka University*)

OR-21. The combination treatment with the adenovirus-mediated REIC/Dkk-3 gene and bevacizumab
Ryoji Imoto (*Department of Neurological Surgery, Okayama University Graduate School*)

OR-22. Metabolic reprogramming by anti-mitochondrial agent promotes sensitivity to oncolytic adenoviruses in non-glycolytic pancreatic cancer
Ryohei Shoji (*Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan*)

OR-23. Novel armed oncolytic HSV exhibits strong antitumor effects that lead to complete tumor regression
Mohamed Abdelmoneim (*Cancer Immune Therapy Research Center, Graduate School of Medicine, Nagoya University, Nagoya, Japan / Department of Surgery II, Graduate School of Medicine, Nagoya University, Nagoya, Japan / Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt*)

OR-24. Retroviral replicating vector-mediated prodrug activator gene therapy for lung cancer
Kei Hiraoka *(Department of Gastroenterological Surgery II, Hokkaido University Graduate School of Medicine / Department of Clinical Research, National Hospital Organization* (*NHO*) *Hakodate National Hospital*)

OR-25. Retroviral replicating vector-mediated gene therapy activates anti-tumor immune responses in an immunocompetent murine pancreatic cancer model
Hiroki Niwa (*Department of Gastroenterological Surgery II, Hokkaido University Graduate School of Medicine*)

Oral Session IV (E or J)

Vectors①

Date: July 14, 16:42–18:18, Room 3

Chairpersons: Masashi Urabe (*Division of Genetic Therapeutics, Jichi Medcal University*)
Hiromi Hayashita-Kinoh (*Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo*)

OR-26. Tropism of Adeno-Associated Virus toward Neural Stem Cells in Gerbil Hippocampus
Yoshihide Sehara (*Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical University*)

OR-27. Systemic therapy for Fabry disease using AAV vector
Yuka Hayashi (*Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical University*)

OR-28. A novel engineered AAV2 capsid variant CereAAV for efficient in vivo gene transduction into mouse brain neurons and microvascular endothelial cells
Yoshinori Tanaka (*Takarabio Inc.*)

OR-29. Therapeutic effects of liver-specific overexpression of lysophosphatidylcholine acyltransferase 4 (LPCAT4)/ lysophospholipid acyltransferase 10 (LPLAT10) on type 2 diabetes mellitus
Kahori Shimizu (*Pharm., Osaka Ohtani Univ.*)

OR-30. Successful Re-administration of Adeno-associated Virus Vectors to Change the Serotypes in Mice
Nemekhbayar Baatartsogt (*Department of Biochemistry, Jichi Medical University School of Medicine, Tochigi, Japan*)

OR-31. Development of mild AAV purification method with the affinity chromatography column using Adeno-Associated Virus Receptor (AAVR)
Yuriko Makino (*Tosoh corp.*)

OR-32. Development of automatic detection system of Adeno-associated virus (AAV) vector particles
Yasunari Matsuzaka (*Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo*)

OR-33. Lentiviral vector optimization for gene therapy
Izumi Maki (*Technology Development Center, Takara Bio Inc.*)

Oral Session V (E or J)

Genetic Diseases

Date: July 15, 8:30–9:42, Room 3

Chairpersons: Toru Uchiyama (*National Center for Child Health and Development*)
Naoya Uchida (*Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo*)

OR-34. Liver-specific gene therapy for mucopolysaccharidosis type II using AAV vector carrying blood-brain barrier-penetrable enzyme
Yohta Shimada (*The Jikei University School of Medicine*)

OR-35. Preclinical Safety and Efficacy Validation of CD4LVFOXP3 Cells as an Innovative Treg-like Cell-based Gene Therapy for IPEX Syndrome
Yohei Sato (*Stanford University*)

OR-36. Gene therapy and genome editing therapy for rickets type II model rats using adenoviral vector
Satoko Kise (*Toyama Prefectural University*)

OR-37. Gene therapy for GM1 gangliosidosis mediated by AAV vector carrying BBB-penetrable enzyme
Saki Matsushima (*Division of Gene Therapy, Research Center for Medical Science, The Jikei University*)

OR-38. Development of a minimally invasive and highly efficient gene therapy for Dystrophic Epidermolysis Bullosa
Ryosuke Kobayashi (*Department of Stem Cell Gene Therapy Science, Graduate School of Medicine, Osaka University / StemRIM Inc.*)

OR-39. Validity and safety preclinical in vivo study of gene therapy for hypophosphatasia using ARU-2801 (AAV8 vector expressing TNALP-D10)
Dongwei Zhao (*Nippon Medical School*)

Oral Session VI (E or J)

Vectors②

Date: July 15, 9:42–11:18, Room 3

Chairpersons: Hiroyuki Nakai (*Oregon Health & Science University*)
Naoya Uchida (*Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo*)

OR-40. Impacts of purification and storage methods on the yield and functionality of oncolytic herpes simplex virus
Seiji Kuroda (*Department of Biochemistry and Molecular Biology, Nippon Medical School, Tokyo, Japan*)

OR-41. Inter-subunit associations of AAV icosahedron inform AAP requirement for capsid assembly
Swapna Kollu (*Department of Molecular and Medical Genetics, Oregon Health Sciences University*)

OR-42. Identification and characterization of an AAV capsid that efficiently transduces the pancreas with limited off-target consequences in non-human primates following retrograde pancreatic duct injection
Kei Adachi (*Department of Molecular & Medical Genetics, Oregon Health & Science University*)

OR-43. AAVKP1 Shows a Peculiar Biological Phenotype Effective for Localized In Vivo Gene Delivery While Detargeting the Liver
Taisuke Furusho (*Oregon Health & Science University*)

OR-44. Screening human immortalized cell lines for efficient production of recombinant adeno-associated virus vector
Yoshitaka Miyagawa (*Department of Biochemistry and Molecular Biology, Nippon Medical School, Tokyo, Japan*)

OR-45. Optimization of AAV9 vectors Production by Transient Transfection of Adherent HEK293EB Cells in Serum-Free Media using Fixed-Bed Bioreactor
Yukihiko Hirai (*Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, the Institute of Medical Science, the University of Tokyo,*)

OR-46. Development of extraction method for adeno-associated virus (AAV) vector
Shuhei Sakamoto (*Takara Bio Inc.*)

OR-47. Development of mRNA synthesis reagents for mRNA medicine development
Hiroyuki Matsumoto (*Takara Bio Inc.*)

Oral Session VII (E or J)

Cancer③

Date: July 15, 15:30–16:30, Room 3

Chairpersons: Hideki Kasuya (*Nagoya University Graduate School of Medicine, Cancer Immune Therapy Center*)
Hiroaki Ikeda (*Department of Oncology, Nagasaki University Graduate School of Biomedical Sciences*)

OR-48. Non-viral gene-modified CAR-T cells targeting IGF1 receptors against solid tumors
Ryunosuke Ohno (*Shinshu University School of Medicine / Faculty of Health Sciences, Queens University*)

OR-49. Anti-tumor effect of recombinant Bifidobacterium displaying Wilms' tumor 1 combined with anti-PD-1 therapy against renal cell carcinoma in mice
Koichi Kitagawa (*Kobe University Graduate School of Science, Technology and Innovation*)

OR-50. Biomarkers predicting the antitumor effect of p53-armed telomerase specific oncolytic adenovirus
Ryoma Sugimoto (*Department of Gastroenterological Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan*)

OR-51. Combination of fusogenic oncolytic vaccinia virus and HDAC inhibitor treatment synergistically induces anti-cancer effect through enhancing viral cell-cell fusion
Motomu Nakatake (*Department of Molecular Medicine, Graduate School of Medical Sciences, Tottori University*)

OR-52. Preclinical study of second-generation microRNA-targeted coxsackievirus B3
Shohei Miyamoto (*Laboratory of ALA Advanced Medical Research, Institute for Quantitative Biosciences, The University of Tokyo*)

Oral Session VIII (E or J)

Clinical Trials

Date: July 15, 16:30–17:42, Room 3

Chairpersons: Yozo Nakazawa (*Department of Pediatrics, Shinshu University School of Medicine*)
Toshihiko Okazaki (*Osaka University Hospital, Dept. of Medical Innovation, Medical Center for Translational and Clinical Research*)

OR-53. Analysis of candidate off-target mutation sequences to be considered for safety assessment of genome editing with SaCas9
Takuma Yamashita (*National Institute of Health Sciences*)

OR-54. Ex vivo Gene & Cell Therapy for Fabry disease, Macroencapsulated Spheroid with Scaffold (MESS) Transplantation
Daisuke Kami (*Kyoto Prefectural University of Medicine*)

OR-55. The novel method of manufacturing for gene modified cells with RetroNectin® using a closed system automated cell preparation system
Sara Ogawa (*Takara Bio Inc.*)

OR-56. Development of new cell therapy of chimeric antigen receptors into dendritic cells
Yoshinori Naoe (*Nagoya University*)

OR-57. Efficacy of HSV-TK/GCV system suicide gene therapy using stem cells from human exfoliated deciduous teeth (SHED) expressing modified HSV-TK against brain metastasis of non-small cell lung cancer
Tomoya Oishi (*Department of Neurosurgery, Hamamatsu University School of Medicine*)

OR-58. Evaluation of full/empty capsid ratio of adeno-associated viral vectors by anion-exchange ultra high performance liquid chromatography
Takenori Yamamoto (*Devision of Molecular Target and Gene Therapy Products, National Institute of Health Sciences*)

Oral Session IX (E or J)

Neurogenic, Ophthalmic, and Musculo-skeletal Diseases

Date: July 16, 8:30–9:42, Room 3

Chairpersons: Seiichi Nagano (*Department of Neurotherapeutics, Osaka University Graduate School of Medicine*)
Akiko Ishii (*Department of Neurology, Faculty of Medicine, University of Tsukuba*)

OR-59. Gene therapy for mice model of myoclonus using AAV5 carrying the human CTSA and modified intracellular crystallization-suppressed NEU1
Mizuki Miyoshi (*Graduate School of Pharmaceutical Sciences, Tokushima University*)

OR-60. Gene therapy for galactosialidosis model mice using AAVPHP.eB carrying the human CTSA and modified NEU1
Rin Fukuike (*Graduate School of Pharmaceutical Sciences, Tokushima University*)

OR-61. Active immunization therapy for Alzheimer's disease using recombinant viral vectors
Takeshi Tabira (*Department of Diagnosis, Prevention and Treatment of Dementia, Graduate School of Medicine, Juntendo University*)

OR-62. Early distribution of 18F-labeled AAV9 vectors in the cerebrospinal fluid after intraventricular or intracisternal infusion in non-human primates
Shinichi Kumagai (*Department of Neurosurgery, Jichi Medical University*)

OR-63. Modulation of neurodegeneration by peripheral immune system in Niemann-Pick disease type C
Toru Yasuda (*National Center for Child Health and Development*)

OR-64. Protein translation dysfunction in amyotrophic lateral sclerosis and its therapeutic application
Seiichi Nagano (*Department of Neurotherapeutics, Osaka University Graduate School of Medicine / Department of Neurology, Osaka University Graduate School of Medicine*)

Oral Session X (E or J)

Vectors③

Date: July 16, 9:42–10:54, Room 3

Chairpersons: Yukihiko Hirai (*Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo*)
Hiroaki Mizukami (*Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical University*)

OR-65. Is the plasma gene transfer method free from genome rearrangement?
Ryota Tamura (*Ehime University Graduate School of Science and Engineering*)

OR-66. Mdmx plays a crucial role in neuronal damage after ischemic stroke
Haomin Yan (*Department of Neurology, Graduate School of Medicine, Osaka University*)

OR-67. Targeted Gene Delivery to the Brain with Smartly-Coated AAV9 Assisted by High Intensity Focused Ultrasound Enhances Safety and Efficacy
Hiroaki Kinoh (*Innovation Center of NanoMedicine, Kataoka-Kinoh Lab*)

OR-68. Protocol optimization for generation of retroviral vector-producing human mesenchymal stem cells (VP-hMSCs)
Yoshiyuki Yamazaki (*Department of Biochemistry and Molecular Biology, Nippon Medical School*)

OR-69. Large-scale purification of functional recombinant adeno-associated virus with short-term ultracentrifugation in a zonal rotor
Mikako Wada (*Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan*)

OR-70. The seroprevalence of neutralizing antibodies against AAV capsids in Japanese hemophilia patients
Yuji Kashiwakura (*Department of Biochemistry, Jichi Medical University School of Medicine*)

Oral Session XI (E or J)

Basic science

Date: July 16, 13:10–14:34, Room 3

Chairpersons: Shigeki Yagyu (*Shinshu University Innovative Research & Liaison Organization*)
Hiromi Hayashita-Kinoh (*Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo*)

OR-71. Development of new therapy for Hypoxic Ischemic Encephalopathy by M1 microglia-depletion using M1 microglia-targeting apoptotic peptide
Rika Zen (*Department of Stem Cell Biology and Regenerative Medicine, Shiga University of Medical Science / Department of Obstetrics and Gynecology, Shiga University of Medical Science*)

OR-72. Establishment of a Pancreatic Cancer Rat Model using the Pancreas-Targeted Hydrodynamic Gene Delivery
Yuto Tanaka (*Division of Gastroenterology and Hepatology, Graduate School of Medical and Dental Sciences, Niigata University*)

OR-73. Reovirus-mediated anti-fibrotic effects: functional restoration of hepatic stellate cells following reovirus treatment
Ikuho Ishigami (*Graduate School of Pharmaceutical Sciences, Osaka University*)

OR-74. Comparability assessment after transfer of the manufacturing process for a next generation CAR-T (CD19-JAK/STAT CAR-T, TBI-2001)
Shinya Tanaka (*Takara Bio Inc.*)

OR-75. A Microfluidic 3D Endothelium-on-a-Chip Model to Study Transendothelial Migration of T Cells in diseases
Luc Zhang (*MIMETAS BV / MIMETAS Japan K.K.*)

OR-76. Targeted genome repair in selectively expanded mouse long-term hematopoietic stem cells
Suvd Byambaa (*Division of Regenerative Medicine, Jichi Medical University, Tochigi, Japan*)

OR-77. Development of next generation CAR-T cell therapies with CRISPR-Cas3
Tomoaki Fujii (*Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo*)

Corporate Seminar I

Future Development of Gene and Cell Therapy
(Miltenyi Biotec K.K.)

Date: July 14, 12:00–12:50, Room1

Chairperson: Yoshikazu Yonemitsu (*R&D Laboratory for Innovative Biotherapeutics Science, Graduate School of Pharmaceutical Sciences, Kyushu University*)

Development of CAR-T cell therapy —Centralization and Decentralization of cellular preparation—
Hiroshi Shiku (*Personalized Cancer Immunotherapy, Mie University Graduate School of Medicine*)

Corporate Seminar II

Quality by Design of AAV Products
(Pall Corporation)

Date: July 14, 12:00–12:50, Room2

Chairperson: Naohito Hariganeya (*Pall Corporation*)

Quality by Design of AAV Products
Peiqing Zhang (*Pall Corporation*)

Allegro™ STR Single-Use Bioreactor Platform for Gene Therapy Applications
Kenichi Horiuchi (*Pall Corporation*)

Corporate Seminar III

Current Status and Perspective of CAR-T cell Therapy
(Scrum Inc)

Date: July 14, 12:00–12:50, Room3

Chairperson: Seiichi Tada (*Scrum Inc*)

Current Status and Perspective of CAR-T Cell Therapy
Naoki Hosen (*Department of Hematology and Oncology, Osaka University Graduate School of Medicine.*)

Corporate Seminar IV

The latest innovations in the field of gene therapy brought about by synthetic biology and the latest trends in manufacturing technology and quality control of viral vectors
(Synplogen Co.,Ltd.)

Date: July 15, 12:00–12:50, Room1

Chairperson: Naoyuki Yamada (*Synplogen Co.,Ltd.*)

Global Trends in Synthetic Biology and its Application to Cell and Gene Therapy
Akihiko Kondo (*Kobe University*)

Trends in manufacturing technology and quality control of viral vectors for gene therapy
Kazuhisa Uchida (*Graduate School of Science,Technology and Innovation Kobe University*)

Corporate Seminar V

New Era in Gene Therapy
(AnGes, Inc.)

Date: July 15, 12:00–12:50, Room2

Chairperson: Torayuki Okuyama (*Department of Clinical Genomics, Saitama Medical University*)

Plasmid DNA Gene Therapy as Platform Modality
Ryuichi Morishita (*Department of Clinical Gene Therapy, School of Medicine, Osaka University*)

Development of Genome Editing Therapeutics at EmendoBio
Akihiko Murakami (*AnGes, Inc.*)

Corporate Seminar VI

Gene and cell therapy development by non-viral gene delivery
(Kiko Tech Co., Ltd.)

Date: July 15, 12:00–12:50, Room3

Chairperson: Toru Uchiyama (*Division of Molecular Pathogenesis, Department of Human Genetics, National Center for Child Health and Development*)

Development of iPSC-derived immune cells for the treatment of cancers
Yasumichi Hitoshi (*Thyas Co., Ltd.*)

Corporate Seminar VII

Updates on T cell gene therapies
(Takara Bio Inc.)

Date: July 16, 12:00–13:00, Room1

Chairperson: Tatsuji Enoki (*Takara Bio Inc.*)

Updates on T cell gene therapies
Naoto Hirano (*Princess Margaret Cancer Centre, University of Toronto*)

Corporate Seminar VIII

Clinical practice and Future prospects of AAV based gene therapy
(Novartis Pharma K.K.)

Date: July 16, 12:00–13:00, Room3

Chairperson: Takanori Yamagata (*Department of Pediatrics, Jichi Medical University*)

Practical experience with gene therapy for SMA
Taku Omata (*Division of Child Neurology, Chiba Children's Hospital*)

Clinical considerations and future prospects of AAV gene therapy
Takanori Yamagata (*Department of Pediatrics, Jichi Medical University*)

Tea break seminar I

Effective operation of Digital PCR
(QIAGEN K.K.)

Date: July 14, 15:00–15:30, Room2

Accurate quantification and effective operation method of AAV vector by nanoplate digital PCR
Yuichi Yotsuyanagi (*Business Development Manager, Marketing Dept.*)

Tea break seminar II

Development of stem cell culture methods for clinical application
(FUKOKU Co., Ltd.)

Date: July 14, 15:00–15:30, Room3

Chairperson: Yui Harada (*Kyushu University*)

Evaluation of the Usefulness of Human Adipose-Derived Stem Cell Spheroids Formed Using SphereRing®
Yasuhito Ishigaki (*Kanazawa Medical University*)

Tea break seminar III

New manufacturing solution for cell and gene therapy products
(Thermo Fisher Scientific)

Date: July 15, 15:00–15:30, Room2

New automated and closed systems for cell processing and gene transfer
Nobuo Ogita (*Thermo Fisher Scientific Life Technologies Japan Ltd.*)

Manufacturing platform for AAV vectors
Kentaro Toriumi (*Thermo Fisher Scientific Life Technologies Japan Ltd.*)

Tea break seminar IV

Different downstream step conditions were compared to give load capacity, recovery, reduction of empty capsids, and purity. From these findings, we propose a scalable purification process
(Cytiva (Global Life Sciences Technologies Japan))

Date: July 15, 15:00–15:30, Room3

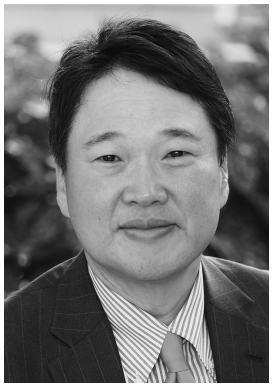
Chairperson: Takuro Kasuga (*Cytiva* (*Global Life Sciences Technologies Japan*))

Development of a scalable adeno-associated virus purification process
Tomoyuki Okuyama (*Cytiva* (*Global Life Sciences Technologies Japan*))

JSGCT Chairman's Lecture

Abstract & Curriculum Vitae

略 歴



名前 森下　竜一

所属 大阪大学

研究分野 gene therapy, cardiovascular disease, plasmid DNA, angiogenesis

学歴

昭和62年大阪大学医学部卒業。米国スタンフォード大学循環器科研究員・客員講師、大阪大学助教授を経て、平成15年より大阪大学大学院医学系研究科臨床遺伝子治療学寄付講座教授（現職）

職歴

内閣府 健康・医療戦略室戦略参与、日本遺伝子細胞治療学会理事長、日本脳血管認知症学会理事長、日本抗加齢医学会副理事長、2025年日本国際博覧会大阪パビリオン推進委員会総合プロデューサーなどを務める。

最近の関連出版物・論文

Yoshida S, Nakagami H, Hayashi H, Ikeda Y, Sun J, Tenma A, Tomioka H, Kawano T, Shimamura M, Morishita R, Rakugi H. The CD153 vaccine is a senotherapeutic option for preventing the accumulation of senescent T cells in mice. Nature Communicaton 2020 May 18;11(1):2482

Fukami H, Morinaga J, Nakagami H, Hayashi H, Okadome Y, Matsunaga E, Kadomatsu T, Horiguchi H, Sato M, Sugizaki T, Kuwabara T, Miyata K, Mukoyama M, Morishita R, Oike Y. Vaccine targeting ANGPTL3 ameliorates dyslipidemia and associated diseases in mouse models of obese dyslipidemia and familial hypercholesterolemia. Cell Reports Medcine 2021;2:100446, http://creativecommons.org/licenses/by-nc-nd/4.0/

Hayashi H, Sun J, Yanagida Y, Otera T, Kubota-Koketsu R, Shioda T, Ono C, Matsuura Y, Arase H, Yoshida S, Nakamura R, Ju N, Ide R, Tenma A, Kawabata S, Ehara T, Sakaguchi M, Tomioka H, Shimamura M, Okamoto S, Amaishi Y, Chono H, Mineno J, Komatsuno T, Saito Y, Rakugi H, Morishita R, Nakagami H. Preclinical study of a DNA vaccine targeting SARS-CoV-2. Current Research in Translational Medicine (in press)

Nakagami H, Hayashi H, Ishihama T, Daikyoji Y, Sasakura C, Mikami T, Katsumoto T, Saito Y, Suzuki K, Murakami A, Sato N, Yamada E, Rakugi H, Morishita R. Study protocol for a randomized, open-label, non-controlled Phase I/II Study to assess safety and immunogenicity of twice or three times dosing of intramuscular COVID-19 DNA vaccine in healthy adults. Translational and Regulatory Sciences (in press)

Nakagami H, Ishihama T, Daikyouji Y, Sasakura C, Yamada E, Morishita R. Brief report on a phase I/IIa study to assess the safety, tolerability, and immune response of AGMG0201 in patients with essential hypertension. Hypertension Research (in press)

遺伝子細胞治療の夜明け

森下　竜一

大阪大学

遺伝子細胞治療は、新型コロナウイルスに対するワクチン開発に、アデノウイルスベクター、mRNA技術、プラスミドDNA技術が使用され、急速に実用化が進んできた。海外のみならず、国内においても、従来の創薬技術を超えたこれらの新規モダリティの重要性が認識され、第二期健康医療戦略にその重要性が指摘され、政府全体で研究開発の支援の取り組みが行われている。しかし、これら新規モダリティを実用化するためのハードルは高く、1）アカデミアでの基礎研究、2）基礎研究から臨床への実用化の橋渡し、3）実用化を担うバイオベンチャーや製薬企業、など多くのプレーヤーが必要にもかかわらず、国内での状況は極めて立ち遅れている。社団法人化された日本遺伝子細胞治療学会では、遺伝子細胞治療技術を国民のために普及させていくことを目的として、今後基礎研究の充実やトランスレーショナルリサーチ・レギュラトリーサイエンスの発展などに貢献していくことを理事長として目指したい。本講演では、現状を紹介しながら、学会や政府の取り組みなどを紹介したい。

Adenovirus vectors, mRNA technology, and plasmid DNA technology have been used to develop new types of vaccines against covid-19, and practical application has rapidly progressed. Not only overseas but also in Japan, the importance of these new modalities beyond conventional drug discovery technologies has been recognized, and the importance of the second health and medical strategy in Japan Government has been pointed out, and the government as a whole is working to support R&D. However, the hurdles to practical application of these new modalities are high, and despite the need for many players such as 1) basic research in academia, 2) bridging practical application from basic research to clinical practice, and 3) bio-ventures and pharmaceutical companies responsible for practical application, the situation in Japan is extremely lagging behind. As a president, I would like to contribute to the enhancement of basic research and the development of translational research and regulatory science in the future with the aim of spreading gene cell therapy technology for the public. In this lecture, I would like to introduce the current situation and the efforts of academic societies and the government.

President's Lecture

Abstract & Curriculum Vitae

CURRICULUM VITAE

Name Yoshikazu Yonemitsu

Affiliation Graduate School of Pharmaceutical Sciences,
Kyushu University

Field of Research Gene and cell therapy
Cancer immunology and immunotherapy

Education

1996 Ph.D., Dr. of Medical Science, Kyushu University
1990 M.D., Faculty of Medicine, Kyushu University

Professional Experience

2020.10 - present Special Advisor (Academia-Industry Relations), to the President of Kyushu University
2009.10 - present Professor, R&D Laboratory for Innovative Biotherapeutics,
Graduate School of Pharmaceutical Sciences, Kyushu University
4 - 2009. 9 Professor, Department of Gene Therapy, Graduate School of Medical Science, Chiba University
10 - 2006. 3 Associate Professor, Department of Pathology, Graduate School of Medical Sciences,
Kyushu University
6 - 2004. 10 Senior Assistant Professor and Labo Director, Graduate School of Medical Sciences,
Kyushu University
5 - 2003. 5. Assistant Professor, Department of Pathology, Kyushu University Hospital
11 - 1999.4 Research Associate, Department of Gene Therapy, Imperial College School of Medicine at the
National Heart and Lung Institute, London, UK (The Wellcome Trust Fellow)
4 - 1997.10 Stuff surgeon in vascular surgery unit, Department of Surgery II, Kyushu University Hospital
4 - 1992. 3 Stuff surgeon in General Surgery, Kyushu Central Hospital
4 - 1991. 3 Resident in Department of Surgery II, and Emergency and Critical Care Medicine,
Kyushu University Hospital
1990.4. Passed the Examination of National Board

GAIA-NK: Off-the-shelf 'NK-like' cell product that can eliminate solid tumors

Yoshikazu Yonemitsu

Graduate School of Pharmaceutical Sciences, Kyushu University

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GAIA-102 is distinct from primary NK cells in view of surface markers as well as functions, therefore, GAIA BioMedicine has Name d it a "GAIA-NK-like cell platform". GAIA-102 can be produced by their original culture method using a mixture of multiple donors PBMCs met with defined HLA/KIR types without any genetic modification.
The outstanding features of GAIA-102 are as follows;
1) It accumulates and kills solid tumors efficiently and rapidly.
2) It induces efficient mass reduction and protective acquired antitumor immunity in vivo in the use of host CTL by 'me too' chemokine signaling.

Interestingly, GAIA-102 holds memory-like NK cell (ML-NK)-like properties; however, genome-wide assessment of epigenetic status and single-cell RNA-seq suggested that these two products are fundamentally different.
The first Phase I/II clinical study of GAIA-102 for non-small cell lung cancer has been opened (ClinicalTrials.gov Identifier: NCT05207371), and those for peritoneal dissemination of gastrointestinal malignancies and for pediatric solid tumors would be started in 2022.

JSGCT Special Program

Abstract & Curriculum Vitae

COVID-19 Vaccines as gene-based technologies

CURRICULUM VITAE

Name Prof. Melissa Moore

Affiliation Eleanor Eustis Farrington Chair of Cancer Research
Professor, RNA Therapeutics Institute
University of Massachusetts Medical School
Chief Scientific Officer, Platform Research, Moderna

Professional Experience

Dr. Melissa J. Moore currently serves as Chief Scientific Officer, Scientific Affairs, at Moderna. She joined Moderna in 2016 from the University of Massachusetts Medical School (UMMS), where she served as Professor of Biochemistry & Molecular Pharmacology, Eleanor Eustis Farrington Chair in Cancer Research, and a long-time Investigator at the Howard Hughes Medical Institute (HHMI). Dr. Moore was also a founding Co-Director of the UMMS RNA Therapeutics Institute (RTI) and was instrumental in creating the Massachusetts Therapeutic and Entrepreneurship Realization initiative (MassTERi), a faculty-led program intended to facilitate the translation of UMMS discoveries into drugs, products, technologies and companies. Dr. Moore is an elected member of the National Academy of Sciences (2017), a Fellow of the American Academy of Arts and Sciences (2019), and recipient of the RNA Society Lifetime Achievement Award (2021). She currently sits on the Board of Directors of Tessera Therapeutics and multiple Scientific Advisory Boards and has co-founded two companies with UMMS colleagues.

Dr. Moore holds a B.S. in Chemistry and Biology from the College of William and Mary, and a Ph.D. in Biological Chemistry from MIT, where she specialized in enzymology under Prof. Christopher T. Walsh. She began working on RNA metabolism during her postdoctoral training with Phillip A. Sharp at MIT. During her 23 years as faculty member, first at Brandeis University and then at UMMS, her research encompassed a broad array of topics related to the roles of RNA and RNA-protein (RNP) complexes in gene expression, and touched on many human diseases including cancer, neurodegeneration and preeclampsia.

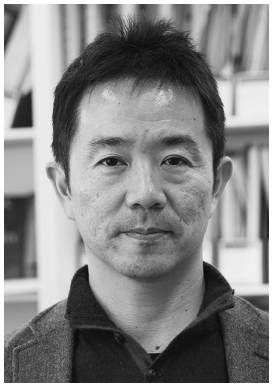
mRNA as Medicine

Prof. Melissa Moore

Eleanor Eustis Farrington Chair of Cancer Research
Professor, RNA Therapeutics Institute
University of Massachusetts Medical School
Chief Scientific Officer, Platform Research, Moderna

With synthetic mRNA now fully validated as a platform for the rapid creation and distribution of highly effective vaccines, the age of mRNA medicines is upon us. Because mRNAs can program the body to produce any desired protein (e.g., cytoplasmic, intraorganelle, membrane-bound, secreted) or set of proteins (e.g., multiprotein complexes) in their native state, possible applications are nearly infinite. In addition to a plethora of new vaccines (both prophylactic and therapeutic), experimental mRNA medicines already in the clinic include pro-inflammatory cytokines as anticancer agents, an angiogenic promoting blood vessel regrowth in damaged heart muscle, and protein replacement therapies for inborn metabolic diseases. I will discuss Moderna's overall process for production of mRNA medicines and our new "mRNA Access" initiative designed get Moderna's formulated mRNAs into the hands of academics interested in helping create the mRNA medicines of the future.

略 歴



名前　　石井　健

所属　　東京大学　医科学研究所　国際ワクチンデザインセンター

研究分野　　ワクチン学、免疫学、レギュラトリーサイエンス

学歴

1993年　横浜市立大学 医学部 卒業
2003年　横浜市立大学 医学部　大学院　学位取得

職歴

1996年　同上大学院在学中に米国政府食品医薬品局（FDA）留学、研究員、臨床試験審査官
2003年　大阪大学微生物病研究所　ERATO審良自然免疫プロジェクトグループリーダー
2006年　大阪大学微生物病研究所准教授
2010年　医薬基盤研究所アジュバント開発プロジェクトリーダー
2010年　大阪大学免疫学フロンティア研究センターワクチン科学分野特任教授
2017年　医薬基盤健康栄養研究所ワクチンアジュバント研究センター長
2019年　東京大学医科学研究所ワクチン科学分野教授
2022年　東京大学医科学研究所国際ワクチンデザインセンターセンター長

最近の関連出版物・論文

Desmet C and Ishii KJ ***Nature Reviews Immunology*** 12, 479–491（2012）
Kobiyama K and Ishii KJ ***Nature Immunology*** in press（2022）
石井健　ワクチン設計のサイエンス　医学のあゆみ　Vol 279 No.10（2021）
石井健　DNAワクチンの先の基礎研究～核酸と生体防御のメカニズムを解き明かす　実験医学40 No.4（2022）

コロナ禍が生んだワクチン開発研究のカンブリア紀と進化の行方

石井　健

東京大学　医科学研究所　国際ワクチンデザインセンター

新型コロナウイルスのパンデミックは世界を一変させ、科学、医療、行政、そして外交や経済にまで大きく影響を与えました。特に、2020年はワクチン開発研究の革命が２つ起きた年として歴史に刻まれるでしょう。一つはｍRNAという新たなワクチンの登場、２つ目はワクチンの臨床試験方法の革命です。30年前に登場した核酸ワクチンの歴史とサイエンス[1,2]、これから近未来に起こるワクチンに関する話題を基礎研究から臨床試験までお伝えします[3,4]。

今回ほどワクチンが世界の人々にとって「自分事」になったことは今までなかったことですし、感染症や免疫だけでなく、サイエンス全体も巻き込む基礎研究、臨床研究分野にも新しい潮流が生まれてきており、異分野融合が進むことが期待されます。一方、世界を見渡すと、ワクチン忌避や、ワクチン接種が進んでいない国も多くある現実があり、日本はもっと安全で良く効くワクチンを世界に提供しGlobal health coverageに貢献することが期待されています。本講義では「100 Days Mission to Respond to Future Pandemic Threats」やポストコロナのワクチン開発研究の新展開を議論できれば幸いです。

The pandemic of the new coronavirus has transformed the world and has had a profound impact on science, medicine, government, and even diplomacy and economics. In particular, the year 2020 will go down in history as the year of two revolutions in vaccine development research. The first is the emergence of a new vaccine called mRNA, and the second is a revolution in the way vaccines are tested in clinical trials.

Never before have vaccines become a “personal matter” for people around the world as they have this time, and a new trend is emerging in the fields of basic and clinical research involving not only infectious diseases and immunology but also science as a whole, and it is expected that the fusion of different fields will continue. On the other hand, looking around the world, there are many countries where people avoid vaccines or vaccinations have not progressed, and Japan is expected to contribute to global health coverage by providing safer and more effective vaccines to the world. In this lecture, we hope to discuss “100 Days Mission to Respond to Future Pandemic Threats” and new developments in post-coronary vaccine development research.

略 歴

名前　　森久保　典子

所属　　ファイザーR&D合同会社　薬事統括部

職歴
2005年　ファイザー株式会社入社
2019年より、開発薬事において内科・感染症およびワクチン領域の責任者を務め、現在に至る。

コミナティの開発

森久保　典子

ファイザーR&D合同会社　薬事統括部

通常，ワクチンの開発期間は10-15年と言われるが，コミナティ筋注の開発は非臨床試験開始からEUA承認までで約10ヵ月であった。COVID-19パンデミックはワクチン開発を私たちの想定を大きく超える程に加速させた。本セッションでは，コミナティの開発経験に基づき，開発を加速できた要因を検討し，今後の臨床開発にどのように生かせるかについても議論したい。

Development period for Comirnaty from the start of non-clinical trials to EUA approval was approximately 10 months, although making a vaccine can take up to 10-15 years under normal circumstances because of the complexity of vaccine development. The acute state of the COVID-19 pandemic prompted an unprecedented level of acceleration of vaccine development including regulatory processes and interactions. In my presentation, based on the experience in Comirnaty development, factors that are allowed for fast development and will remain relevant for future efforts will be discussed.

略 歴

名前　　田中　倫夫

所属　　アストラゼネカ株式会社　研究開発本部

学歴

1988　京都大学薬学部卒業

職歴

1988-1996　マリオン・メレル・ダウ社（現サノフィ株式会社）
1997-　アストラゼネカ株式会社　研究開発本部
（2009年から約3年間 英国アストラゼネカ 研究開発拠点にて従事）

COVID-19アデノウイルスベクターワクチンの開発と接種プログラムへの導入

田中　倫夫

アストラゼネカ株式会社　研究開発本部

After the first report of COVID-19, AstraZeneca, in cooperation with the University of Oxford, immediately started the development of preventive vaccine (adenovirus vector vaccine) without profit-making purpose in the pandemic, and in only 2 months, we initiated an early phase clinical trial in March 2020. In parallel, AstraZeneca Japan started preparation of clinical trials of the vaccine, and has successfully collected clinical data from Japanese participants which was required for the approval application in Japan. This was achieved when the data became available from the overseas clinical trials, and the vaccine was granted exceptional approval in Japan in May 2021. The vaccine rollout started in UK and other European countries in January 2021, preceding other regions. In March 2021, however, an adverse reaction of thrombosis with thrombocytopenia syndrome (TTS) was reported. Investigations including those led by UK reported the rate of the event and fatal outcome. Given this finding, eligible candidate for this vaccine was limited to those with relatively low risk in Japan, before initiation of domestic rollout of the vaccine. As of May 2022, a total of approximately 120,000 doses have been administered. In the meantime, we also started to establish a manufacturing system in Japan to assure stable supply, because vaccines may be seized by some countries or regions in the pandemic. Despite that establishment of manufacturing system usually requires more than several years, our first shipment was achieved in only 10 months or so from the start of the preparation, thanks to the great cooperation by the authorities and our partner companies.

We are witnessing greater mobility of human in a global scale, and new variants are frequently emerging. Although COVID-19 may be suppressed successfully in one region, it is unlikely that we can expect end of pandemic, unless the infection is suppressed also in the other regions. International cooperation is therefore essential to make this disease under control. COVAX, an international framework of multilateral supply of vaccine, on top of direct bilateral supply, has been established for this purpose. Our vaccine manufactured in Japan has already been delivered extensively to overseas countries in this framework. As of May 2022, more than 42 million doses have been delivered to foreign countries or regions including Asian nations.

略 歴

名前　　中神　啓徳

所属　　大阪大学大学院医学系研究科　健康発達医学

研究分野　　老年医学、ワクチン

学歴
1994年　奈良県立医科大学卒業
2003年　医学博士（大阪大学老年科）

職歴
1994年－1997年　自治医科大学内科レジデント・循環器内科医員
1997年－2000年　大阪大学医学部老年病医学（第4内科）研究生
2000年－2001年　愛媛大学医学部　助手（医化学第一講座）
2001年－2003年　米国Harvard大学医学部Brigham and Women's病院研究員
2003年　大阪大学医学部付属病院未来医療センター特別研究員
2003年－2010年　大阪大学大学院　助手（医学系研究科　遺伝子治療学）
2010年－2015年　大阪大学大学院　連合小児発達学研究科　健康発達医学寄附講座教授
2015年ー 現在　大阪大学大学院　医学系研究科　健康発達医学寄附講座教授

最近の関連出版物・論文

Hayashi H, Sun J, Yanagida Y, Otera T, Kubota-Koketsu R, Shioda T, Ono C, Matsuura Y, Arase H, Yoshida S, Nakamaru R, Ju N, Ide R, Tenma A, Kawabata S, Ehara T, Sakaguchi M, Tomioka H, Shimamura M, Okamoto S, Amaishi Y, Chono H, Mineno J, Komatsuno T, Saito Y, Rakugi H, Morishita R, Nakagami H. Preclinical study of a DNA vaccine targeting SARS-CoV-2. Curr Res Transl Med. 2022 Apr 20;70(4):103348.

Yoshida S, Nakagami H, (Corresponding author) et al. The CD153 vaccine with CpG adjuvant is a novel senotherapeutic option for preventing the accumulation of senescence T cells from visceral adipose tissues in mice. ***Nat Commun*** 2020 May 18;11(1):2482.

Suda M, Nakagami H,, et al. Senolytic vaccination improves normal and pathological age-related phenotypes and increases lifespan in progeroid mice ***Nat Aging*** 2021 Dec, 1, 1117-1126.

Liu Y, Nakagami H, et al. An infectivity-enhancing site on the SARS-CoV-2 spike protein targeted by COVID-19 patient antibodies. ***Cell***, 2021 May 24;184(13):3452-3466.e18.

Nakagami H. Development of COVID-19 vaccines utilizing gene therapy technology. Int Immunol.2021 Sep 25;33(10):521-527.

COVID-19 DNA ワクチン開発

中神　啓徳

大阪大学大学院医学系研究科　健康発達医学

新型コロナウイルス感染症（COVID-19）の世界での感染拡大に対し、迅速に新型コロナウイルス（SARS-CoV-2）のゲノム配列が解明・公開され、SARSやMERSに対する先行研究と遺伝子配列情報からウイルスベクターあるいは核酸医薬技術（RNAワクチン・DNAワクチン）などの遺伝子治療技術を用いたワクチン開発が多く実施された。その中で、開発から1年以内でファイザー/ビオンテック、モデルナが開発したmRNAワクチンはともにフェーズ3の検証試験において、90%以上の発症予防効果を証明することに成功した。

我々は産学連携体制でDNAワクチンを設計・GMP合成を行い、動物実験での有効性・安全性の検証を開始した。投与経路として、従来の筋肉内投与に加え、新規投与デバイスを用いた皮内投与での免疫応答の検証を行い、抗体価誘導を確認し、感染防御実験として、ハムスターあるいはマウスを用いた感染防御試験での有効性評価を行った。また、並行して感染者血清検体を用い、ワクチン評価に必要な測定法の確立を実施した。このように可能な範囲で、迅速に臨床試験への準備を進めることが出来た一方で、ヒト臨床試験へのトランスレーショナルリサーチにおけるDNAワクチンの課題も改めて明らかとなった。本シンポジウムでは、ワクチン開発を実際に遂行する上で感じた課題と今後の取り組みについてご紹介出来ればと思います。

In response to the global spread of novel coronavirus infection (COVID-19), the genome sequence of the novel coronavirus (SARS-CoV-2) was rapidly elucidated and disclosed, and many vaccines were developed using viral vectors or nucleic acid medicine technology (RNA vaccine, DNA vaccine) based on previous research on SARS and MERS and genetic sequence information. Among them, it was a great surprise that the mRNA vaccines developed by Pfizer/Biontec and Moderna both proved to be more than 90% effective in preventing the onset of disease in Phase 3 validation studies within one year of their development.We have designed and synthesized a DNA vaccine under an industry-academia collaboration, and have started to verify the antibody titer in mice and other animals. In addition to the conventional intramuscular route of administration, we verified the immune response by intradermal administration using a novel administration device, confirmed the induction of antibody titer, and evaluated the efficacy of the vaccine in infection prevention tests using hamsters or mice. In parallel, we used serum samples from infected individuals to establish the measurement methods necessary for vaccine evaluation. While we were able to rapidly advance preparations for clinical trials to the extent possible, the challenges of DNA vaccines in translational research into human clinical trials became clear once again. In this symposium, we hope to introduce the challenges through our vaccine development and our future efforts.

略 歴

名前　荒木　康弘

所属　独立行政法人医薬品医療機器総合機構

研究分野　薬学、レギュラトリーサイエンス

学歴

1999年　東京大学大学院薬学系研究科修士課程修了
2022年　昭和大学薬学研究科博士課程修了

職歴

2015年8月　厚生労働省医薬・生活衛生局安全対策課課長補佐
2017年2月　厚生労働省医薬・生活衛生局医薬品審査管理課課長補佐
2020年8月　医薬品医療機器総合機構ワクチン等審査部長（現在に至る）

最近の関連出版物・論文

Araki Y. (2022). これからの新型コロナウイルスワクチンの開発・評価について. YAKUGAKU ZASSHI 142. in print

新型コロナウイルスワクチンの審査今後の在り方について

荒木　康弘

独立行政法人医薬品医療機器総合機構

新型コロナウイルス感染症は2019年末に最初の患者が報告されて以降、全世界にパンデミックが広がり、甚大な影響を与えた一方、最初のワクチンは最初の患者の報告から1年以内に世界に供給されるなど、極めて速やかに進められた。

本講演では、新型コロナウイルスワクチンの開発と並行して進められた、ワクチンの評価の考え方の変遷について俯瞰すると共に、今後の評価のあり方について述べる。

After the first case of COVID-19 in the world was reported at the end of 2019, COVID-19 had spread worldwide as a pandemic and given enormous international impacts. On the other hand, the development of vaccines for COVID-19 progressed exceptionally quickly. Within a year from the first case, the first COVID-19 vaccine was supplied in the UK, and after that, it promptly became available internationally.
In this presentation, I will show the current concepts of COVID-19 vaccine evaluation, which proceeded in parallel with the development of COVID-19 vaccines, and also show future consideration of the evaluation of COVID-19 vaccines.

Presidential Special Program 1

Abstract & Curriculum Vitae

ASGCT/ESGCT/JSGCT Joint Symposium

CURRICULUM VITAE

©MHH

Name Hildegard Büning

Affiliation Institute for Experimental Hematology, Hannover Medical School, Hannover, Germany.
REBIRTH – Cluster of Excellence, Hannover Medical School, Hannover, Germany.
German Center for Infection Research (DZIF), partner site Hannover-Braunschweig.

Field of Research Prof. Dr. Büning has a long-standing expertise in the area of viral vector development and infection research. Key competences include capsid engineering as well as cell entry (transductional), transcriptional and genomic targeting of Adeno-Associated Viral (AAV) vectors, vaccine development, and characterization of the AAV-host interaction including immune recognition. Dr. Büning has published 139 articles (91 original papers and 48 reviews/editorials/commentaries) in peer reviewed journals and 12 book chapters.
Dr. Büning is a strong believer in cell and gene therapy and has dedicated her entire scientific career to this endeavor. To help moving the field forward, Dr. Büning is active in gene therapy societies. Dr. Büning is President of the European Society of Gene and Cell Therapy (ESGCT) since 2018, is part of the leadership of the German Society for Gene Therapy (DG-GT) since 2008 and has started in June 2021 her term as Member of the Board of Directors of the American Society of Gene & Cell Therapy (ASGCT).

Education

1988-1993	Study of Biology, University of Münster and University of Munich, Germany
1993	Diploma in Biology, University of Munich, Germany
1997	PhD (Dr. rer. nat.), University of Munich, Germany
2008	Lecture Qualification (Habilitation) in Molecular Medicine, University of Cologne, Germany

Professional Experience

1997-2003	Post-doctoral Fellow, Gene Center of the University of Munich, Germany
2004-2015	Research Group Leader, University of Cologne, Germany
since 2012	Member, German Center for Infection Research (DZIF)
since 2015	Research Group Leader, Hannover Medical School, Hannover, Germany
since 2015	Professor, Hannover Medical School, Hannover, Germany

Recent Related Publications (5 Papers)

Pavlou M.[1], C. Schön[1], L. M. Occelli, A. Rossi, N. Meumann, R. F. Boyd, J. T. Bartoe, J. Siedlecki, M. J. Gerhardt, S. Babutzka, J. Bogedein, J. E. Wagner, S. G Priglinger, M. Biel, S. M. Petersen-Jones, H. Büning[2], S. Michalakis[2]. Novel AAV capsids for intravitreal gene therapy of photoreceptor disorders. EMBO Mol. Med. (2021): 13(4): e13392. ([1]equal contribution; [2]co-senior authors)

Hösel M., A. Huber, S. Bohlen, J. Lucifora, G. Ronzitti, F. Puzzo, F. Boisgerault, U.T. Hacker, W.J. Kwanten, N. Klöting, M. Blüher, A. Gluschko, M. Schramm, O. Utermöhlen, W. Bloch, F. Mingozzi[1], O. Krut[1], H. Büning[1]. Autophagy determines efficiency of liver-directed gene therapy with adeno-associated viral vectors. Hepatology. (2017): 66 :252-265. ([1]equal contribution)

Münch R.C.[1], A. Muth[1], T. Friedel, J. Schmatz, B. Dreier, A. Trkola, A. Plückthun, H. Büning[2], C. Buchholz[2]. Off-target-free gene delivery by affinity-purified receptor-targeted viral vectors. Nat. Commun. (2015): 6: 6246 ([1]equal contribution; [2]equal contribution)

Hösel M.[1], J. Lucifora[1], T. Michler, G. Holz, M. Gruffaz, S. Stahnke, F. Zoulim, D. Durantel, M. Heikenwalder, D. Nierhoff, R. Millet, A. Salvetti, U. Protzer[2] and H. Büning[2]. Hepatitis B Virus infection enhances susceptibility towards Adeno-Associated Viral vector transduction *in vitro* and *in vivo*. Hepatology (2014): 59: 2110-20. ([1]equal contribution; [2]co-senior authors)

Hösel M., M. Broxtermann, H. Janicki, K. Esser, S. Arzberger, P. Hartmann, S. Gillen, J. Kleeff, D. Stabenow, M. Odenthal, P. Knolle, M. Hallek, U. Protzer, H. Büning. TLR2-mediated innate immune response in human non-parenchymal liver cells towards adeno-associated viral (AAV) vectors. Hepatology. (2012): 55: 287-297.

Improving efficacy of Adeno-Associated-Virus (AAV) vectors for *in vivo* gene therapy

Hildegard Büning[1,2,3]

[1] Institute for Experimental Hematology, Hannover Medical School, Hannover, Germany.
[2] REBIRTH – Cluster of Excellence, Hannover Medical School, Hannover, Germany.
[3] German Center for Infection Research (DZIF), partner site Hannover-Braunschweig.

AAV vectors are currently the most widely used delivery system for *in vivo* gene therapy with so far three AAV vector-based gene therapies that have received market authorization by the European Medicines Agency (EMA). Despite this success, we must acknowledge that AAV vectors need to be optimized to unlock their full potential. Major challenges are the prevalence of pre-existing neutralizing anti-AAV antibodies as well as the need for high vector doses to overcome pre- and post-entry barriers in target cell transduction and to counterbalance the loss of vector particles in off-target tissues.
In response to these challenges, we and others are developing and applying capsid engineering strategies. These are either based on rational design approaches or use high-throughput *ex vivo* or *in vivo* screenings of AAV capsid libraries. Thereby, AAV capsid variants with target cell selective tropism and improved *in vivo* transduction efficiency are developed. Vector efficacy can also be improved by the in-depth characterization of the vector-target cell interaction and targeted manipulation of key limiting steps in cell transduction. Besides improving efficacy of AAV vectors in gene therapy, the gain in knowledge on the vector-host interaction combined with capsid-engineering strategies is also paving the way for novel applications such as developing AAV vectors as vaccines in cancer immunotherapy.

CURRICULUM VITAE

Name Juan A. Bueren

Affiliation Hematopoietic Innovative Therapies Division, CIEMAT/CIBERER/Fund. Jiménez Díaz, Madrid, Spain

Field of Research Prof Juan Bueren is Director of the Biomedical Innovation Unit (CIEMAT and Biomedical Network Centre for Research on Rare Diseases, CIBERER) and Co-ordinator of the Advanced Therapies Unit (IIS. Fundación Jiménez Díaz/CIEMAT). Dr Bueren serves as Vice-President of the European Society for Gene and Cell Therapy, is Member of the International Committee of the American Society for Gene and Cell Therapy, and is Consultant for Rocket Pharmaceuticals. Dr Bueren is Scientific Director of two gene therapy programs, one of them for the treatment of Fanconi anemia patients, and has participated in the development of therapeutic lentiviral vectors designed as Orphan Drugs by the European Medicines Agency and by the FDA for the treatment of Fanconi anemia and other monogenic diseases. Dr Bueren has published more than 160 papers in the field of stem cells and gene therapy, and has been awarded by the FARF for commitment with FA research and dedication.

Education
1975-1979: Graduated, University Complutense of Madrid
1982: Ph.D University Complutense of Madrid

Professional Experience
1978-1982: Junior Scientist. Junta Energía Nuclear, Spain
1982-1992: Associated researcher CIEMAT
2002-2014: Head Division Hematopoietic Innovative Therapies. CIEMAT
2014-: Coordinator Advanced Therapies Unit Fundación Jiménez Díaz/CIEMAT
2017- Director of the Hematological, Innumological and Dermatological cancer Unit (U710). CIBER Rare Diseases
2021-: Head of the Biomedical Innovation Department. CIEMAT

Recent Related Publications (5 Papers)

Successful Engraftment of Gene Corrected Hematopoietic Stem Cells in Non-conditioned Fanconi Anemia Patients. Paula Rio, Susana Navarro, Wei Wang, Rebeca Sánchez-Domínguez, Roser M. Pujol, José C. Segovia, Massimo Bogliolo, Eva Merino, Ning Wu, Rocío Salgado, María L. Lamana, Rosa M. Yañez, José A. Casado, Yari Giménez, Francisco J. Román-Rodríguez, Lara Álvarez, Omaira Alberquilla, Anna Raimbault, Guillermo Guenechea, M. Luz Lozano, Laura Cerrato, Miriam Hernando, Eva Gálvez, Raquel Hladun, Irina Giralt, Jordi Barquinero, Anne Galy, Nagore García de Andoín, Ricardo López, Albert Catalá, Jonathan D. Schwartz, Jordi Surralles, Jean Soulier, Manfred Schmidt, Cristina Díaz de Heredia, Julián Sevilla, Juan A. Bueren. **Nat Med. 2019 Sep;25(9):1396-1401.**

NHEJ-mediated repair of CRISPR-Cas9-induced DNA breaks can efficiently correct mutations in HSPCs from patients with Fanconi Anemia. Francisco Jose Roman-Rodriguez, Laura Ugalde, Lara Alvarez, Begoña Diez, Maria Jose Ramirez, Cristina Risueño, Marta Corton, Massimo Bogliolo, Sara Bernal, Francesca March, Carmen Ayuso, Helmut Hanenberg, Julian Sevilla, Sandra Rodriguez-Perales, Raul Torres-Ruiz, Jordi Surralles, Juan A. Bueren and Paula Rio. **Cell Stem Cell. 2019 Nov 7;25(5):607-621.e7.**

Targeted gene therapy and cell reprogramming in Fanconi anemia. Rio P, Baños R, Lombardo A, Quintana-Bustamante O, Alvarez L, Garate Z, Genovese P, Almarza E, Valeri A, Díez B, Navarro S, Torres Y, Trujillo JP, Murillas R, Segovia JC, Samper E, Surralles J, Gregory PD, Holmes MC, Naldini L, Bueren JA. **EMBO Mol Med. 2014 Jun;6(6):835-48.**

Engraftment and in vivo proliferation advantage of gene-corrected mobilized CD34+ cells from Fanconi anemia patients. Río P, Navarro S, Guenechea G, Sánchez-Domínguez R, Lamana ML, Yañez R, Casado JA, Mehta PA, Pujol MR, Surrallés J, Charrier S, Galy A, Segovia JC, Díaz de Heredia C, Sevilla J, Bueren JA. **Blood. 2017 Sep 28;130(13):1535-1542.**

Disease-corrected haematopoietic progenitors from Fanconi anaemia induced pluripotent stem cells. Raya A, Rodríguez-Pizà I, Guenechea G, Vassena R, Navarro S, Barrero MJ, Consiglio A, Castellà M, Río P, Sleep E, González F, Tiscornia G, Garreta E, Aasen T, Veiga A, Verma IM, Surrallés J, Bueren JA, Izpisúa Belmonte JC. **Nature. 2009 Jul 2;460(7251):53-9.**

Long-Term Follow-up of the Phase I/II Gene Therapy Trial in Fanconi Anemia-A Patients

Juan A. Bueren[1], Paula Río[1], Julián Sevilla[2], Susana Navarro[1], Josune Zubicaray[2], Eileen Nicoletti[3], Rebeca Sánchez-Domínguez[1], Wei Wang[4], Roser M. Pujol[5], Michael Rothe[6], José C. Segovia[1], Yari Giménez[1], Omaira Alberquilla[1], Elena Almarza[3], Jordi Barquinero[7], Albert Catalá[10], Francois Lefrere[11], Marina Cavazzana[11], Axel Schambach[6], Gayatri Rao[3], Jordi Surrallés[5], Jean Soulier[12], Cristina Díaz de Heredia[7], Jonathan D. Schwartz[3]

[1] Hematopoietic Innovative Therapies Division, CIEMAT/CIBERER/Fund. Jiménez Díaz, Madrid, Spain,
[2] Servicio Hemato-Oncología Pediátrica, Fundacion Biomédica Hospital Niño Jesús, Madrid, Spain, [3] Rocket Pharma, New York, NY,
[4] GeneWerk GmbH, Heidelberg, Germany, [5] Hospital Sant Pau/Autonoma University of Barcelona/CIBERER Barcelona, Spain,
[6] Hannover Medical School, Hannover, Germany, [7] Hospital Val d'Hebron, Barcelona, Spain,
[8] Hospital Universitario de Donostia, San Sebastián, Spain, [9] Hospital de Cruces, Bilbao, Spain,
[10] Hospital San Joan de Deu, Barcelona, Spain, [11] Hopital Necker-Enfants Malades, Paris, France,
[12] Hôpital Saint-Louis and University Paris Diderot., Paris, France

Fanconi anemia (FA) is a monogenic inherited disorder mainly characterized by congenital abnormalities, childhood bone marrow failure (BMF) and cancer predisposition. Herein, we report the results obtained 2-6 years after lentiviral-mediated gene therapy of patients with Fanconi anemia, subtype A (FA-A). Data corresponds to the FANCOLEN-I phase I/II and subsequent long-term follow-up clinical trial in which patients participate after 3 years of follow-up in the FANCOLEN-I study. Patients were infused with mobilized CD34+-enriched cells after transduction with the PGK-FANCA.Wpre* lentiviral vector without any pre-conditioning regimen. The number of CD34+-enriched cells infused in the patients ranged from 7.3x104 to 1.9x106 CD34+ cells/kg. Vector copy numbers (VCN) in colonies derived from the manufacturing products ranged from 0.2 to 0.9 VCN/cell. Progressive engraftment of gene-corrected cells was observed in six of the eight evaluable patients, with observed values of 10% to 70% in either BM or PB at 2-6 years post-gene therapy. No evidence of HSC exhaustion was observed during long-term follow up of these patients. In all instances a progressive rise in the proportion of corrected cells was associated with increases in MMC-resistance in BM progenitors, and also with a reduction in the chromosomal instability in PB T cells exposed to diepoxybutane. Four of the eight evaluable patients who were treated at advanced stages of BMF, and/or infused with very low numbers of corrected CD34+ cells, showed progressive evolution of BMF, requiring alternative treatments including transfusions and/or allogenic transplantation. Nonetheless, two of the patients who were among those receiving higher numbers of corrected CD34+ cells had more rapid and robust engraftment levels and showed stabilized and subsequently improved PB cell counts. The results obtained in these phase I/II and long-term clinical trials show the importance of performing FA gene therapy in early stages of the disease, and reveal the potential efficacy of this therapeutic approach when significant numbers of corrected CD34+ cells are infused prior to BMF progression. Based on these results, global phase II trials are currently ongoing under the sponsorship of Rocket Pharmaceuticals Inc., with the goal of treating patients in early stages of the disease with high numbers of gene-corrected CD34+ cells and averting BMF.

CURRICULUM VITAE

Name Hans-Peter Kiem

Affiliation Director, Stem Cell and Gene Therapy Program
Fred Hutchinson Cancer Research Center

Field of Research Dr. Hans-Peter Kiem is a world-renowned pioneer in stem cell and gene therapy. His focus has been the development of improved hematopoietic stem cell (HSC) gene therapy and genome editing approaches for the treatment of patients with genetic and infectious diseases or cancer. His lab has developed gene therapy trials for Fanconi anemia, glioblastoma, and HIV. More recently his lab has described a refined HSC population for improved HSC engineering and targeting. A major recent focus has also been the development of in vivo gene therapy using various delivery platforms including viral vectors and nanoformulations to make HSC gene therapy and gene editing more portable and more widely accessible also in low- and middle-income countries and setting where HIV and hemoglobinopathies are most prevalent. He has had continuous NIH funding for the past 25 years and has published more than 300 manuscripts, mostly in the area of HSC biology/transplantation and gene therapy. Dr. Kiem was the inaugural recipient of the José Carreras/E. Donnall Thomas Endowed Chair for Cancer Research at the Fred Hutch from 2009 to 2014 and now holds the Stephanus Family Endowed Chair for Cell and Gene Therapy and is Director of the Stem Cell and Gene therapy Program at Fred Hutch. He served on the Recombinant DNA Advisory Committee (RAC) 2011 to 2016 and chaired the committee during the last year. He served on the American Society for Gene and Cell Therapy (ASGCT) and American Society of Hematology (ASH) Stem Cell committees, and for both societies also as Chair in 2016/17. In May of 2020 was elected vice president of ASGCT and became president-elect in 2021 and will serve as president in 2022.

Education

1987/88	MD,PhD, University of Ulm, Germany
1990	Research Fellow, Stanford University, Stanford, CA
1992	Residency: Internal Medicine, Vanderbilt University, Nashville, TN

Professional Experience

1992-1995	Senior Fellow in Oncology, FHCRC and University of Washington (UW), Seattle, WA
1995-1997	Research Associate/Associate in Clinical Research Division, FHCRC
1995-present	Attending Physician, FHCRC
1996-1998	Acting Instructor, Department of Medicine, UW
1997-2002	Assistant Member, Clinical Research Division, FHCRC
1998-2003	Assistant Professor, Department of Medicine, UW
2002-2007	Associate Member, Clinical Research Division, FHCRC
2003-2008	Associate Professor, Department of Medicine, UW
2003-present	Faculty Member, Molecular and Cellular Biology Graduate Program, UW
2004-2008	Adjunct Associate Professor, Department of Pathology, UW
2004-present	Chair, Institutional Biosafety Committee, Seattle Cancer Care Alliance, Seattle, WA
2007-present	Full Member/Professor, Clinical Research Division, FHCRC
2008-present	Adjunct Professor, Department of Pathology, UW
2008-present	Professor, Department of Medicine, UW
2009-2014	José Carreras/E. Donnall Thomas Endowed Chair for Cancer Research
2012-present	Associate Program Head, Transplantation Biology, FHCRC
2013-present	Associate Head, Heme Malignancy Program, UW/FHCRC Cancer Consortium
2015-2018	Endowed Chair for Cell and Gene Therapy, FHCRC
2015-present	Director/Head, Stem Cell and Gene Therapy Program, FHCRC
2017-present	Full Member/Professor, Vaccine and Infectious Disease Division, FHCRC
2018-present	Stephanus Family Endowed Chair for Cell and Gene Therapy
2020-2021	Vice President, American Society of Gene & Cell Therapy
2021-present	President-elect, American Society of Gene & Cell Therapy

Targeting hematopoietic stem cells for ex vivo and in vivo gene therapy

Hans-Peter Kiem[1,2]

[1] Director, Stem Cell and Gene Therapy Program
[2] Fred Hutchinson Cancer Research Center

Despite tremendous progress in the hematopoietic stem cell (HSC) gene therapy and gene editing field, there are a number of significant limitations to the availability and accessibility of these therapies. In my presentation, I will discuss how we can better target a more refined HSC population to significantly reduce the amounts of lentiviral vectors or gene editing reagents needed for the genetic engineering of HSCs. I will also discuss how we can make these therapies more portable and accessible by developing in vivo gene therapy approaches that would not require rare and highly sophisticated cell manufacturing facilities and thus could be more accessible and also available for patients in low and middle-income settings.

Recent Related Publications (5 Papers)

Efficient polymer nanoparticle-mediated delivery of gene editing reagents into human hematopoietic stem and progenitor cells.
El-Kharrag R, Berckmueller KE, Madhu R, Cui M, Campoy G, Mack HM, Wolf CB, Perez AM, Humbert O, **Kiem HP**, Radtke S.Mol Ther. 2022 Feb 28:S1525-0016(22)00154-X. doi: 10.1016/j.ymthe.2022.02.026. Online ahead of print.PMID: 35240320

Bringing gene therapy to where it's needed.
Radtke S, **Kiem HP**.Trends Mol Med. 2022 Mar;28(3):171-172. doi: 10.1016/j.molmed.2022.01.005. Epub 2022 Jan 24.PMID: 35086771

In Vivo Hematopoietic Stem Cell Gene Therapy for SARS-CoV2 Infection Using a Decoy Receptor.
Wang H, Li C, Obadan AO, Frizzell H, Hsiang TY, Gil S, Germond A, Fountain C, Baldessari A, Roffler S, **Kiem HP**, Fuller DH, Lieber A.Hum Gene Ther. 2022 Apr;33(7-8):389-403. doi:

Safe and efficient *in vivo* hematopoietic stem cell transduction in nonhuman primates using HDAd5/35++ vectors.
Li C, Wang H, Gil S, Germond A, Fountain C, Baldessari A, Kim J, Liu Z, Georgakopoulou A, Radtke S, Raskó T, Pande A, Chiang C, Chin E, Yannaki E, Izsvák Z, Papayannopoulou T, **Kiem HP**, Lieber A.Mol Ther Methods Clin Dev. 2021 Dec 6;24:127-141. doi: 10.1016/j.omtm.2021.12.003. eCollection 2022 Mar 10. 10.1089/hum.2021.295.PMID: 35057635

Therapeutically relevant engraftment of a CRISPR-Cas9-edited HSC-enriched population with HbF reactivation in nonhuman primates.
Humbert O, Radtke S, Samuelson C, Carrillo RR, Perez AM, Reddy SS, Lux C, Pattabhi S, Schefter LE, Negre O, Lee CM, Bao G, Adair JE, Peterson CW, Rawlings DJ, Scharenberg AM, **Kiem HP**. Sci Transl Med. 2019 Jul 31;11(503):eaaw3768. doi: 10.1126/scitranslmed.aaw3768.PMID: 31366580 Free PMC article.

CURRICULUM VITAE

Name Guangping Gao

Affiliation Director, Horae Gene Therapy Center and Viral Vector Core,
Co-Director, Li Weibo Institute for Rare Diseases Research,
Professor of Microbiology and Physiological Systems,
Penelope Booth Rockwell Professor in Biomedical Research,
University of Massachusetts Medical School

Speaker Short Bio

Guangping Gao, PhD is the Director, Horae Gene Therapy Center and Viral Vector Core, Co-Director, Li Weibo Institute for Rare Diseases Research, Professor of Microbiology and Physiological Systems, Penelope Booth Rockwell Professor in Biomedical Research, University of Massachusetts Medical School; Elected fellows, both the US National Academy of Inventors (NAI) and American Academy of Microbiology; Past president, American Society of Gene and Cell Therapy.

Dr. Gao is an internationally well recognized gene therapy researcher who has played a key role in the discovery and characterization of new family of adeno-associated virus (AAV) serotypes, which was instrumental in reviving the gene therapy field, hugely impacting many currently untreatable human diseases. For 30+ years of his scientific research career, Dr. Gao has primarily focused on molecular genetics and viral vector gene therapy of genetic diseases, Dr. Gao has published 330+ research papers, 6 book chapters, and 5 edited books. Dr. Gao holds 212 patents with 429 more patent applications pending. Dr. Gao has been ranked as the World Top 20 Translational Researchers for several years in a row by Nature Biotechnology.

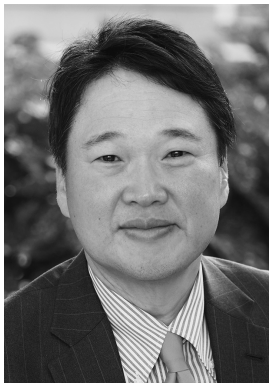
The next generation of AAV gene therapy

Guangping Gao

Director, Horae Gene Therapy Center and Viral Vector Core,
Co-Director, Li Weibo Institute for Rare Diseases Research,
Professor of Microbiology and Physiological Systems,
Penelope Booth Rockwell Professor in Biomedical Research,
University of Massachusetts Medical School

This presentation will discuss some major challenges in AAV gene therapy and showcase strategies to mitigate those challenges by developing the next generation of gene therapy vectors with reduced transgene immunity, improved safety profiles, enhanced therapeutic efficacy and the capability to achieve effective readthrough therapy of genetic diseases caused by premature termination codon mutations.

CURRICULUM VITAE



Name Ryuichi Morishita

Affiliation Osaka Univeristy

Field of Research gene therapy, cardiovascular disease, angiogenesis, plasmid DNA

Education

Ryuichi Morishita, M.D., Ph.D., graduated Osaka University Medical School in 1987, and received Ph. D. from Osaka University in 1991. After following postdoctoral Fellow at Stanford University School of Medicine, he served as an Assistant Professor, Department of Geriatric Medicine, Osaka University Medical School from 1994 to 1998. Then, from 1998 to 2003, he was Associate Professor, Division of Gene Therapy Science, Osaka University Medical School. Then, he became Professor & Chairman of Division of Clinical Gene Therapy, Graduate School of Medicine, Osaka University Medical School from 2003.

Professional Experience

Professor Morishita received over 20 awards from various academic societies including Harry Goldbratt Award in Council of High Blood Pressure, American Heart Association, Award in Japanese of Japan Medical Society, Sato Award in 27th annual meeting of the Japanese Circulation Society and Invitrogen-Nature-Biotechnology Award. Especially, his developed Gene Therapy Drug (Collategene; HGF gene therapy drug to stimulate therapeutic angiogenesis to treat ischemic ulcer in the patients with peripheral arterial disease) was launched in Japan market from 2019 as a first gene therapy drug in Japan, and a first gene therapy drug based on plasmid DNA in the world. Professor Morishita is currently on the editorial board of over 30 scientific journals including Gene Therapy, ATVB, Circulation and Hypertension. He has published over 400 original articles and review papers, primarily on gene therapy and molecular biology in cardiovascular disease. In addition, Professor Morishita is a Chairman of many medical organizations, including Japanese Society of Gene & Cell Therapy.

He is currently Strategic Advisor of Headquarter for HealthCare Strategy (Chief is Prime Minister of Japan), and Special Advisor of Osaka Prefecture and Osaka City. He also worked as a member of Intellectual Property Committee of Koizumi & Abe Cabinet, and Committee for Regulation Reform (Advisor Committee of Prime Minister of Japan).

Recent Related Publications (5 Papers)

Yoshida S, Nakagami H, Hayashi H, Ikeda Y, Sun J, Tenma A, Tomioka H, Kawano T, Shimamura M, Morishita R, Rakugi H. The CD153 vaccine is a senotherapeutic option for preventing the accumulation of senescent T cells in mice. Nature Communicaton 2020 May 18;11(1):2482

Fukami H, Morinaga J, Nakagami H, Hayashi H, Okadome Y, Matsunaga E, Kadomatsu T, Horiguchi H, Sato M, Sugizaki T, Kuwabara T, Miyata K, Mukoyama M, Morishita R, Oike Y. Vaccine targeting ANGPTL3 ameliorates dyslipidemia and associated diseases in mouse models of obese dyslipidemia and familial hypercholesterolemia. Cell Reports Medcine 2021;2:100446, http://creativecommons.org/licenses/by-nc-nd/4.0/

Hayashi H, Sun J, Yanagida Y, Otera T, Kubota-Koketsu R, Shioda T, Ono C, Matsuura Y, Arase H, Yoshida S, Nakamura R, Ju N, Ide R, Tenma A, Kawabata S, Ehara T, Sakaguchi M, Tomioka H, Shimamura M, Okamoto S, Amaishi Y, Chono H, Mineno J, Komatsuno T, Saito Y, Rakugi H, Morishita R, Nakagami H. Preclinical study of a DNA vaccine targeting SARS-CoV-2. Current Research in Translational Medicine (in press)

Nakagami H, Hayashi H, Ishihama T, Daikyoji Y, Sasakura C, Mikami T, Katsumoto T, Saito Y, Suzuki K, Murakami A, Sato N, Yamada E, Rakugi H, Morishita R. Study protocol for a randomized, open-label, non-controlled Phase I/II Study to assess safety and immunogenicity of twice or three times dosing of intramuscular COVID-19 DNA vaccine in healthy adults. Translational and Regulatory Sciences (in press)

Nakagami H, Ishihama T, Daikyouji Y, Sasakura C, Yamada E, Morishita R. Brief report on a phase I/IIa study to assess the safety, tolerability, and immune response of AGMG0201 in patients with essential hypertension. Hypertension Research (in press)

Plasmid DNA-based Gene Therapy: From Regenerative Medicine to Vaccine for COVID-19

Ryuichi Morishita

Osaka Univeristy

Gene therapy has emerged as a novel therapy to promote angiogenesis in patients with critical limb ischemia (CLI) caused by peripheral artery disease. We focused on hepatocyte growth factor (HGF) as pro-angiogenic factors. In phase III clinical trial, naked plasmid DNA encoding HGF showed the safety and their potential for symptomatic improvement in CLI patients. Based on phase III data, HGF gene therapy drug, Collategene, has been approved by PMDA in Japan. Collategene was launched in Japan market as the first gene therapy drug at 2019. In this session, we would like to discuss about future application of HGF gene therapy.

In addition, we recently focused on the therapeutic vaccination which has extended its scope from infectious diseases to chronic diseases. We reported that angiotensin (Ang) II vaccine for hypertension successfully attenuated the high blood pressure in animal models (PLoS One 2013, Sci Rep 2017, Stroke 2017). Increasing the effectiveness of drug adherence interventions may have a great impact on the health of the population, because approximately 50% may not take medications. This poor adherence to medication leads to increased morbidity and death. As a result, the vaccine-induced anti-Ang II antibodies can efficiently ameliorate Ang II-induced high blood pressure and perivascular fibrosis in mice. Phase I/II clinical trial demonstrated good safety profile and the production of antibody against Ang II. In next step, we will start phase IIb study to test the anti-hypertensive efficacy.

Based on plasmid DNA platform technology, we have applied to develop DNA vaccine against COVID-19. Successfully, we have developed DNA vaccine against SARS-Cov2. Now, phase II/III clinical trial using our DNA vaccine was already started, from 4Q on 2020. In addition, AnGes has started another phase 1/2 study to increase the efficacy. As the safety profile of DNA vaccine was very well, in this lecture, I would like to discuss about DNA vaccine against COVID-19.

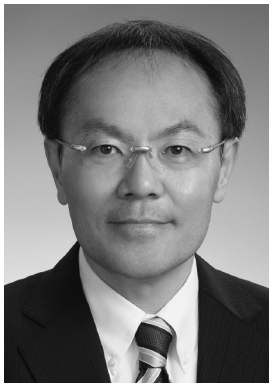
Finally, from our experience, JSCGT would like to proceed to enhance basic research including vector technology & DDS, traslational research and regulatory science. In this symposium, I would like to mention how JSCGT will stimulate the international corroboration among ASCGT & ESCGT.

Presidential Special Program 2

Abstract & Curriculum Vitae

再生医療等製品における薬価制度と
早期・条件付承認制度を考える

略 歴



名前　　白沢　博満

所属　　MSD株式会社

研究分野　　医薬品の研究開発

学歴
1995年　慶應義塾大学医学部卒業

職歴
1995年　河北総合病院　内科
1999年　ファイザー株式会社　研究開発部門
2012年　MSD株式会社　研究開発部門

医薬品の価格とアクセスの動向及び課題

白沢　博満

MSD株式会社

私は製薬会社の研究開発部門で20年以上にわたり医薬品開発や経営に従事しており、そのような立場で個人としての考えを述べる。

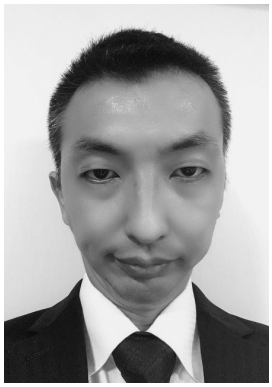
医薬品が患者さんの生活を改善し命を救い、医療現場に影響を与えていくためには2つの重要な要素がある。1つめはその薬剤もしくは適応症の研究開発に成功して薬事承認されることである。2つめはその薬剤の薬価そしてアクセス（保険償還）の要素である。

後者に関しては日本ではこれまであまり語られてこなかったが、海外では薬事承認と同等の大きなハードルとして論点となってきた。また、社内の投資判断もしくはVC等に依存した会社での外部投資家の投資判断においても重要な影響を与える。

日本ではかつてドラッグラグと呼ばれる海外との新薬や新適応の承認や臨床導入のギャップが大きな問題であった。この問題に対応するための施策として、2007年のPMDAによる「国際共同治験に関する基本的考え方」の通知に基づく開発・薬事に関する新たな方向性、そして、2010年の新薬創出加算による新たな薬価の仕組みという２つによりドラッグラグは解消する方向に向かった。しかしながら2015年以降、薬価制度は新薬開発を阻害する方向に変化し、アクセスに関してもその可能性があり、日本では再び過去の状況に戻る可能性の高い状況となってきている。

再生医療等製品においてもこれらの経験や論点は参考となると思われる。

略 歴



名前　　奥平　真一

所属　　医薬品医療機器総合機構

学歴

2000-2004　東京大学薬学部
2004-2009　東京大学大学院薬学系研究科

職歴

2009-2013　東北大学大学院薬学研究科
2013-　医薬品医療機器総合機構

再生医療等製品の条件及び期限付承認制度について

奥平　真一

医薬品医療機器総合機構

再生医療を迅速かつ安全に実施するための法的枠組みとして、「医薬品、医療機器等の品質、有効性及び安全性の確保等に関する法律」（薬機法）及び「再生医療等の安全性の確保等に関する法律」（再生医療等安全性確保法）が2013年の国会で成立して2014年11月に施行された。薬機法では医薬品・医療機器と並んで再生医療等製品が定義されるとともに、再生医療の早期実用化に対応するため条件及び期限付承認制度等ができた。条件及び期限付承認制度は、均質でない再生医療等製品について、有効性が推定され、安全性が確認されれば、条件及び期限付きで特別に早期に承認できる仕組みであり、当該制度で承認された場合、承認後に有効性及び安全性を改めて検証することとなる。導入以来、これまでに複数の品目が条件及び期限付承認制度により承認されている。本発表では条件及び期限付承認制度の適用、課題等について紹介する。

略 歴

名前　加納　浩之

所属　一般社団法人再生医療イノベーションフォーラム（FIRM）
アステラス製薬株式会社

研究分野　骨・軟骨代謝疾患
腎臓疾患
再生医療

学歴

1985-1989　名古屋市立大学 薬学部 製薬学科
1989-1991　名古屋市立大学大学院 薬学研究科

職歴

1991-2002　山之内製薬（現アステラス製薬）研究本部
2002-2016　同　開発本部
2016-2018　同　渉外部
2018-2021　FIRM事務局長（アステラス製薬より出向）
2021-現在　FIRM運営委員長（アステラス製薬より出向）

再生医療等製品の現状と多様性に起因する種々の課題

加納　浩之[1,2]

[1]一般社団法人再生医療イノベーションフォーラム（FIRM）
[2]アステラス製薬株式会社

1999年に『再生医療』という言葉が世に初めて登場してから20年が経ち、現在までに16品目の再生医療等製品が承認されている。その間、2014年の薬機法施行に伴い『再生医療等製品』が新設され、従来の医薬品や医療機器とは異なる特徴について考える端緒を開くと共に、「条件及び期限付承認制度」や、「再生医療等の安全性の確保等に関する法律」が施行され、再生医療を患者さんに届ける仕組みが整備されてきた。また昨年来「新しい資本主義実現会議」が開催され、その中で再生・細胞医療・遺伝子治療に関し、患者さん向けの治療法の開発や創薬など実用化開発を進める旨の言及がなされたことから、産官学一丸で更なる施策の推進が期待される。

再生医療は従来の医薬品等と比較すると多様かつ複雑な側面を持つ。まず「再生医療等製品」には、組織再生、細胞治療、遺伝子治療という複数のモダリティーが包含され、さらに例えば移植手術を伴う製品では、企業が提供する「モノ」としての特性と、医療機関が実施する「医療技術」としての特性を有するなど、課題を包括的に論じることが困難である。現在、再生医療等製品の保険収載価格は、医薬品、医療機器の何れかの例により算定されるが、「モノ」としても「医療技術」としても異なる特徴を有するため、それらをベースに価格を算定することには困難が付きまとう。

また、既承認の16製品の内、「条件及び期限付承認」であるのは4製品である（22年5月時点）。条件及び期限付承認制度は、海外から様々な指摘を受けたが、再生医療を「モノ」と「医療技術」の視点からみると、現実に即した制度とも言える。ただどのような承認が得られるかは治験の結果に基づき申請後の審査の中で定まるため、開発企業としては予見性が乏しい。

当日は製品価格や条件及び期限付承認制度を含め、再生医療等製品を取り巻く現状と課題を概説すると共に、今後の在り方について言及する。

Presidential Special Program 3

Abstract & Curriculum Vitae

Gene and Cell Therapy: Industrial Views

略 歴

名前　　廣瀬　徹

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学歴

1982年　金沢大学薬学部製薬化学科卒業（薬学士）
1984年　金沢大学大学院修士課程薬学研究科修了（薬学修士）
1990年　金沢大学大学院自然科学研究科博士後期課程修了（学術博士）

職歴

1984－2001年　北陸製薬株式会社　研究開発本部
2002－2004年　シュワルツ・ファーマ・ジャパン株式会社 代表
2004年－　ノバルティス ファーマ株式会社　開発本部
2021年8月－　常務取締役 グローバル医薬品開発本部長（現職）

ノバルティスの細胞治療・遺伝子治療開発への取組み

廣瀬　徹

ノバルティス ファーマ株式会社

ノバルティスでは、企業パーパスである「Reimagining Medicine（医薬の未来を描く）」を実現するべく、革新的な医薬品にフォーカスして研究開発を進めており、低分子、高分子（抗体など）医薬品に加えて、第三のプラットフォームとして「細胞・遺伝子療法」の構築に積極的に取り組んでいる。

患者さんの細胞に遺伝子を導入する方法には、患者さんから取り出した細胞に遺伝子を導入した後に患者さんの体に戻すEx-vivo法と、ベクターに組み込んだ遺伝子を患者さんに直接投与するIn vivo法がある。

我々は、Ex-vivoの遺伝子治療として自家細胞を用いるキメラ抗原受容体T細胞（CAR-T）療法による白血病の治療と、In vivoの遺伝子治療としてアデノ随伴ウイルスベクター9（AAV9）を用いる脊髄性筋萎縮症（SMA）の治療で、それぞれ2019年と2020年に国内で再生医療等製品の製造販売承認を得た。

CAR-T療法については、University of Pennsylvaniaから開発販売の権利を取得した後、主にノバルティスにおいて治験薬の製造や臨床試験を実施するなど、申請に必要な情報を集積して製造販売承認を得た。一方AAV9ベクターを用いるSMAの遺伝子治療については、Nationwide Children's Hospitalとベンチャー企業であるAveXis社が実施した臨床試験などのデータを中心として、必要な追加情報をノバルティスが収集して製造販売承認を得た。

アカデミア発の細胞・遺伝子治療から再生医療等製品として申請・承認を得るには、審査で求められる情報の量と質を把握し、開発の初期段階から企業と綿密に協働することが求められる。

本演題では、二つの細胞・遺伝子治療の開発の概略とアカデミアと企業の役割分担について報告する。

略 歴

名前　　小林　美穂子

所属　　ファイザーR&D合同会社

学歴
1997年　岡山大学薬学部　卒業
1999年　岡山大学大学院薬学研究科　修了

職歴
1999-2001年　　帝人株式会社　臨床開発部
2002-2015年　　ファイザー株式会社　デベロップメント・オペレーション統括部
2015年-現在　　ファイザーR&D合同会社　クリニカル・リサーチ統括部（2018年12月分社化により社名変更）

最近の関連出版物・論文
Takeuchi M, Nemoto S, Suzuki Y, Takahashi N, Takenaka N, Takata A, Kobayashi M., Age-Specific Dose Regimens of Dexmedetomidine for Pediatric Patients in Intensive Care Following Elective Surgery: A Phase 3, Multicenter, Open-Label Clinical Trial in Japan. Pediatr Crit Care Med. 2021 Apr 2.

希少疾患領域における遺伝子治療の開発戦略

小林　美穂子

ファイザーR&D合同会社

希少疾患は，これまでに7,000種類以上知られており，全世界で約4億人が罹患し，80%は遺伝子が原因であり，50%は小児であると言われている。希少疾患のうち，1つ以上の治療法が承認されている疾患は5%に過ぎず，アンメットメディカルニーズが非常に高い疾患領域の一つである。

ファイザーは，30年以上にわたり希少疾患に対する治療薬の開発を行っており，現在は，希少疾患のうち，血液疾患，内分泌・代謝疾患，神経疾患，循環器疾患の4つをフォーカスエリアとしている。希少疾患の多くは遺伝子が原因であるため，当社では遺伝子治療の開発にも積極的に取り組んでおり，特にアデノ随伴ウイルス（AAV）を用いた遺伝子治療に注力している。現在，血友病A，血友病Bおよびデュシェンヌ型筋ジストロフィーに対するAAVを用いた遺伝子治療が国内外でフェーズ3の段階であり，これらの製品はいずれも海外のバイオベンチャー企業から導入したものである。また，フォーカスエリアの疾患を中心に，遺伝子治療の豊富なパイプラインを有している。

遺伝子治療は，1回の投与で一生涯に渡る効果が得られる可能性をもつ治療である一方，キャプシドに対する免疫応答など課題もある。当社ではこれらの課題に取り組むため，従来のAAVよりも低用量でより特異的に目的の細胞や組織を標的とし，標的外リスクを低減する新規AAVキャプシドを評価中である。さらに，COVID-19ワクチンにも用いられた脂質ナノ粒子(LNP) とmRNAを利用した塩基編集プログラムに関する共同研究にも取り組んでいる。

本発表では，ファイザーの希少疾患領域における遺伝子治療の開発戦略について概説する。

略 歴

名前　　志鷹　義嗣

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学歴
1996年3月　東京大学大学院薬学系研究科博士課程修了

職歴
1996年　山之内製薬（現:アステラス製薬）に研究職として入社
2012年　薬理研究所 疾患フロンティア研究室長
2015年　製品戦略部 科学戦略グループ長
2016年　Astellas Institute for Regenerative Medicine（AIRM）社長
2021年　現職

細胞医療・遺伝子治療の産業化に向けたアステラス製薬の取り組み

志鷹　義嗣

アステラス製薬株式会社

アンメットメディカルニーズの高い疾患に新たな治療手段を提供する技術として、細胞医療や遺伝子治療への期待が高い。しかし、それらの実用化にはまだ多くの課題がある。アステラス製薬は社外のイノベーションを自社（グループ各社）の技術と融合させ、パートナーとともに深化させることにより課題を克服し、いち早く実用化することを目指している。

細胞医療では、他家多能性幹細胞由来の分化細胞を用いた「オフザシェルフ」細胞医療製品の開発に注力している。他家細胞を用いる際の課題の一つは拒絶反応である。私たちは自社が有する遺伝子改変技術を幹細胞に応用する方法でこの課題の解決に取り組んでいる。さらに、独自の技術プラットフォームACCEL (Advanced Cellular Control through Engineered Ligands)との組み合わせにより、多標的のキメラ抗原受容体（CAR）細胞医療の開発を目指すなど、より多くの疾患への応用にも取り組んでいる。

遺伝子治療では、筋・眼科・中枢疾患領域を中心に、主にアデノ随伴ウイルス（AAV）によるin vivo遺伝子治療の実用化を目指している。AAV医薬品創製においては生産効率の向上および大規模化、臓器指向性の向上等が課題となる。このため積極的な先行投資を行い、製造能力の整備および技術強化を進めている。また、臓器指向性の向上については、機械学習を応用した新規AAVカプシド創製技術の共同研究を開始した。

本講演では実用化へ向けたこれらの取り組みや今後の展望を紹介する。

略 歴

名前　峰野　純一

所属　タカラバイオ株式会社

研究分野　ウイルスベクター開発、GMP 製造、がん免疫遺伝子治療

学歴

1984　京都大学農学部食品工学科（酵素化学研究室）修了
2007　鹿児島大学大学院連合農学研究科　学位取得

職歴

1984 - 1993　寶酒造株式会社（現タカラバイオ株式会社）入社, 楠工場勤務、バイオプロダクツ開発センター勤務
1993 - 1998　同社 企画開発部勤務、営業企画部勤務
1998 - 2003　同社 DNA 機能解析センター勤務
2003 - 2011　同社 細胞・遺伝子治療センター勤務
2011 - 2014　同社 執行役員
2014 -　同社 取締役

最近の関連出版物・論文

Hayashi H, Sun J, Yanagida Y, Otera T, Kubota-Koketsu R, Shioda T, Ono C, Matsuura Y, Arase H, Yoshida S, Nakamaru R, Ju N, Ide R, Tenma A, Kawabata S, Ehara T, Sakaguchi M, Tomioka H, Shimamura M, Okamoto S, Amaishi Y, Chono H, Mineno J, Komatsuno T, Saito Y, Rakugi H, Morishita R, Nakagami H., Preclinical study of a DNA vaccine targeting SARS-CoV-2, Curr Res Transl Med. 2022 Apr 20;70(4):103348. doi: 10.1016/j.retram.2022.103348.

Sato Y, Wada I, Odaira K, Hosoi A, Kobayashi Y, Nagaoka K, Karasaki T, Matsushita H, Yagi K, Yamashita H, Fujita M, Watanabe S, Kamatani T, Miya F, Mineno J, Nakagawa H, Tsunoda T, Takahashi S, Seto Y, Kakimi K., Integrative immunogenomic analysis of gastric cancer dictates novel immunological classification and the functional status of tumor-infiltrating cells, Clin Transl Immunology. 2020 Oct 17;9(10):e1194. doi: 10.1002/cti2.1194. eCollection 2020.

Kawamura K, Tanaka Y, Nakasone H, Ishihara Y, Kako S, Kobayashi S, Tanaka Y, Ohmori T, Uchimaru K, Okamoto S, Mineno J, Shiku H, Nishimura S, Kanda Y., Development of a Unique T Cell Receptor Gene-Transferred Tax-Redirected T Cell Immunotherapy for Adult T Cell Leukemia, Biol Blood Marrow Transplant. 2020 Aug;26(8):1377-1385. doi: 10.1016/j.bbmt.2020.04.006. Epub 2020 Apr 18.

Tomono T, Hirai Y, Chono H, Mineno J, Ishii A, Onodera M, Tamaoka A, Okada T., Infectivity Assessment of Recombinant Adeno-Associated Virus and Wild-Type Adeno-Associated Virus Exposed to Various Diluents and Environmental Conditions., Hum Gene Ther Methods. 2019 Aug;30(4):137- doi: 10.1089/hgtb.2019.082.

Okamoto S, Amaishi Y, Maki I, Enoki T, Mineno J., Highly efficient genome editing for single-base substitutions using optimized ssODNs with Cas9-RNPs., Sci Rep. 2019 Mar 18;9(1):4811. doi: 10.1038/s41598-019-41121-4.

遺伝子治療におけるタカラバイオのポジション

峰野　純一

タカラバイオ株式会社

タカラバイオは日本で初めて制限酵素の国産化に成功して以来、PCRをはじめとする遺伝子工学研究用試薬・細胞工学研究試薬の開発・製品化、機器製品化、受託サービスを進めており、日本最大級のゲノム解析センターを持つ。遺伝子治療との関わりは、1995年のレトロネクチン®（RN）の開発から、まずは創薬支援分野から始まった。その後GMP製造施設の設計・建設を実施し、自社臨床プロジェクトの開始、CDMサービスの開始、と進めており、創薬支援・自社臨床開発・CDMOの3つのカテゴリーで遺伝子治療の領域を推進しており、当社の基本戦略となっている。3つのカテゴリーはそれぞれの技術とノウハウを応用し合うことで一層発展する。例えば臨床開発でex vivo遺伝子治療のHSV-TK, TCR-T, CAR-Tを実施してきたが、それを支える支援技術としてRNをはじめ培地や抗CD3抗体といった開発製品が用いられ、ウイルスベクター製造技術と細胞加工技術はCDMOサービスに応用展開されている。AAVに関しては創薬支援として研究用試薬を開発・製品化し、それらの技術を応用したCDMOサービス、更に最近基礎研究にて効率よく脳に遺伝子導入ができ肝臓への遺伝子導入が低いAAV（CereAAV™）を開発、ライセンスアウトも念頭に今後の臨床開発を考えている。COVID-19で話題となったmRNAワクチンはnon-viral遺伝子治療の一部と考えているが、mRNA合成酵素やキャッピング酵素を遺伝子工学試薬として開発・製品化するのと並行して、RNのGMP製造のノウハウを利用し、これら酵素のGMP製造も計画している。このように、創薬支援・臨床開発・CDMOの三角形の中を行き来しながら、遺伝子治療領域における当社のポジショニングを大きくし、日本の、更には世界の遺伝子治療の発展に貢献していきたいと考えている。

略 歴

名前　　中條　光章

所属　　アンジェス株式会社

学歴

1978-1980　新潟大学大学院理学研究科
1991-1996　東海大学医学部研究員

職歴

1980-2003　中外製薬株式会社
2004-2013　ノバルティスファーマ株式会社
2014-現在　アンジェス株式会社

最近の関連出版物・論文

Morishita R, Shimamura M, Takeya Y, Nakagami H, Chujo M, Ishihama T, Yamada E, Rakugi H. Combined Analysis of Clinical Data on HGF Gene Therapy to Treat Critical Limb Ischemia in Japan. Curr Gene Ther. 2020;20:25-35.

プラスミドベクターによる遺伝子治療の可能性

中條　光章

アンジェス株式会社

遺伝子治療は、目的遺伝子を標的臓器あるいは細胞に導入し疾病を治療することであり、遺伝子導入の手段としてウイルスベクター、プラスミドベクターなどが50年前から検討されている。1980年にプラスミドベクターをラット下肢筋肉に投与することで、目的とするタンパク質が発現することが報告され、プラスミドベクターの臨床応用の可能性が示された。重症下肢虚血は末梢動脈疾患の最も進行した病態で、下肢切断のリスクとともに生活の質の低下や生命予後の不良などが治療課題となっている。肝細胞成長因子（HGF）は、肝細胞再生増殖以外に血管新生促進、炎症調節、線維化抑制、組織再生促進などの多彩な生理作用を有するサイトカインである。コラテジェンはHGFのcDNAを搭載したプラスミドベクターで、下肢筋肉に投与することで筋肉細胞に取り込まれる、取り込まれたコラテジェンからHGFが産生され、HGFによる血管新生により重症下肢虚血の血行改善が期待できる。また、閉塞性動脈硬化症患者の血管壁ではHGF産生量は低下しているので、コラテジェンによるHGF補充は理にかなっていると考えられる。コラテジェンの臨床検討は2001年に日本で開始された。2004年に閉塞性動脈硬化症を対象とした二重盲検比較試験が実施された。同時に米国でも臨床検討が実施された。これらの成績をもとに、2019年に治療抵抗性の重症下肢虚血における潰瘍治療として、条件及び期限付で承認され、臨床応用が可能となった。コラテジェンの開発経緯をたどることにより、遺伝子治療用製品としてのプラスミドベクター開発の課題を考えてみたい。

Presidential Special Program 4

Abstract & Curriculum Vitae

CAR-T/TCR/NK and beyond:
Breakthrough technologies for solid tumors

CURRICULUM VITAE

Name Hiroaki Ikeda

Affiliation Department of Oncology,
Nagasaki University Graduate School of Biomedical Sciences

Field of Research Tumor Immunology

Education

1990 M.D. Nagasaki University School of Medicine
1996 Ph.D. Graduate School of Nagasaki University School of Medicine

Professional Experience

1990-1992	Internist: 2nd Department of Internal Medicine, Nagasaki University
1992-1996	Graduate Student: Graduate School of Nagasaki University School of Medicine
1996-1999	Medical Staff: 2nd Department of Internal Medicine, Mie University Hospital
1999-2004	Research Associate: Department of Pathology and Immunology, Washington University School of Medicine in St. Louis
2004-2006	Associate Professor: Division of Immunoregulation, Hokkaido University Institute for Genetic Medicine
2006-2016	Associate Professor-Professor: Department of Cancer Vaccine-Department of Immuno-Gene Therapy, Mie University
2016-	Present Professor: Department of Oncology, Nagasaki University

Recent Related Publications (5 Papers)

Chemical augmentation of mitochondrial electron transport chains tunes T cell activation threshold in tumors. Yosuke Dotsu et al. Journal for ImmunoTherapy of Cancer. 10(2), e003958-e003958, 2022.

Prognostic significance of NY-ESO-1 antigen and PIGR expression in esophageal tumors of CHP-NY-ESO-1-vaccinated patients as adjuvant therapy. Yasuhiro Nagata et al. Cancer Immunology, Immunotherapy. In press, 2022.

CD4+ T cells support polyfunctionality of cytotoxic CD8+ T cells with memory potential in immunological control of tumor. Naoko Imai et al. Cancer Science. 111(6):1958-1968, 2020.

Antigen delivery targeted to tumor-associated macrophages overcomes tumor immune resistance. Daisuke Muraoka et al. The Journal of Clinical Investigation. 129(3):1278-1294, 2019.

Antitumor activity of CAR-T cells targeting the intracellular oncoprotein WT1 can be enhanced by vaccination. Yasushi Akahori eat al. Blood. 13;132(11):1134-1145, 2018.

Harnessing TCR-T therapy to overcome personalization and tumor heterogeneity

Hiroaki Ikeda

Department of Oncology, Nagasaki University Graduate School of Biomedical Sciences

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Because of few ideal cell surface antigens, development of T-cell receptor (TCR)- engineered T cells (TCR-T) that target intracellular antigens is a promising approach for patients with solid tumors. We performed a clinical trial of adoptive transfer of autologous PBMC transduced with NY-ESO-1-specific high affinity TCR genes in patients with solid tumors and observed significant tumor response with manageable cytokine release syndrome in patients with synovial cell sarcoma. To overcome personalization and expand the application as well as the efficacy of TCR- T therapy, we are in process to create allogeneic T cell therapy, as an off-the-shelf cell therapy, utilizing siRNA and CRISPER/Cas9 that reduce GVHD and rejection of transferred cells. In addition, we are aiming to develop “Immune cell death inducer (IDI)” therapy that combine T cell therapy and compounds that induce tumor cell death at immune activated microenvironment to overcome tumor heterogeneity.

CURRICULUM VITAE

Name Hiroshi Fujiwara

Affiliation Department of Personalized Cancer Immunotherapy,
Mie University Graduate School of Medicine

Field of Research Hematology/Oncology,
Allogeneic hematopoietic stem cell transplantation,
Tumor Immunology,
Trasnplantation Immunology,
Gene and cellular immunotherapy

Education

1980-1986; Department of Medical Science, Faculty of Medicine, University of Occupational and Environmental Health, Japan

Professional Experience

1986-1991,1992-2001; First department of Internal Medicine, Faculty of Medicine, Kagoshima University
1991-1992; Second department of Internal Medicine, Hyogo Medical College
2001-2004; Hematology Branch, National Heart, Lung and Blood Institute/ National Institute of Health, USA
2005-2007; Department of Hematology and Immunology, Kagoshima University Hospital
2007-2018; Department of hematology, immunology and infectious disease, Ehime University Hospital
c/o 2009-2013; Department of Cell growth and Cancer regulation, Ehime Proteo-Medicine Research Center
2018- present; Department of Personalized Cancer Immunotherapy, Mie University Graduate School of Medicine

Recent Related Publications (5 Papers)

1. Fujiwara H. Efforts to maximize the potential of CAR-T therapy for cancer, from T-bodies to CAR-immune cells (review). Int J Hematol. 2021 Nov;114(5):529-531. doi: 10.1007/s12185-021-03213-8.
2. Ochi T, Maruta M, Tanimoto K, Kondo F, Yamamoto T, Kurata M, Fujiwara H, Masumoto J, Takenaka K, Yasukawa M. A single-chain antibody generation system yielding CAR-T cells with superior antitumor function. Commun Biol. 2021 Mar 2;4(1):273. doi: 10.1038/s42003-021-01791-1.
3. Casey NP, Kyte JA, Fujiwara H. Use of RNA Interference with TCR Transfer to Enhance Safety and Efficiency. Methods Mol Biol. 2020; 2115:327-349.doi: 10.1007/978-1-0716-0290-4_18.
4. Maruta M, Ochi T, Tanimoto K, Asai H, Saitou T, Fujiwara H, Imamura T, Takenaka K, Yasukawa M. Direct comparison of target-reactivity and cross-reactivity induced by CAR- and BiTE-redirected T cells for the development of antibody-based T-cell therapy. Sci Rep. 2019 Sep 16;9(1):13293.doi: 10.1038/s41598-019-49834-2.
5. Ohta R, Demachi-Okamura A, Akatsuka Y, Fujiwara H, Kuzushima K. Improving TCR affinity on 293?T cells.J Immunol Methods. 2018 Nov 22. pii: S0022-1759(18)30305-3. doi: 10.1016/j.jim.2018.11.010. 466:1-8,2019.

Strategical bioengineering for CAR-T cell against solid cancers

Hiroshi Fujiwara

Department of Personalized Cancer Immunotherapy, Mie University Graduate School of Medicine

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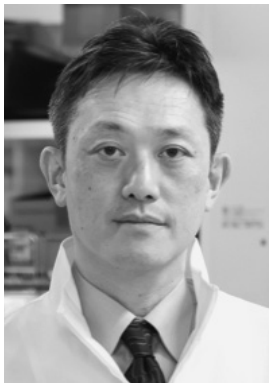
Currently, CD19 specific chimeric antigen receptor (CAR) gene-modified autologous T lymphocyte (CAR-T) therapy against treatment-refractory B-lymphoid blood cancers has successfully become a impressive game changer, and several kinds of CD19 CAR-T cell therapies have become commercially available and been actively introduced into practice all over the world. In addition, BCMA (B cell maturation antigen) specific CAR-T therapy for the treatment of relapsed/refractory multiple myeloma is now chasing after as another success story in the same context. As a corollary, such a powerful therapeutic potential of successful CAR-T cells has been impelling people into development of novel CAR-T therapy for the treatment of refractory solid cancers. However, even at present, CAR-T therapy against solid cancers still remains largely unsuccessful.

Viewing from accumulated knowledge, such difficulty to build an efficacious CAR-T therapy against solid cancers has largely been divided into three categories, i) paucity of clinically-proven target molecules for CAR-T therapy for the treatment of solid cancers, that is, expressed fully immunogenic and only by cancer cells, but not by normal tissues, ii) accumulated disabilities of patient-derived autologous T cells for CAR-T engineering forced by anticancer chemo-radiotherapies, which can cause production failure of CAR-T cells and excessively prolonged time to achieve sufficient number of CAR-T cells, resulting in missing the therapeutic opportunity iii) short life of functional CAR-T cells in vivo due to exhaustion and activation induced cell death (AICD), and iii) suppression of anti-cancer functionality of CAR-T cells forced by the immunosuppressive tumor microenvironment (TME) encompassing inhibitory cellular components such as regulatory T cell, myeloid derived suppressor cell (MDSC), tumor associated macrophage (TAM), tumor associated fibroblast (TAF) ,and environmentally metabolic restrictions. At this very moment, to overcome these impediments, viewing from a multidimensional perspective, huge wisdom of scientists and clinicians all over the world has intensively been put in.

In this talk, I am going to briefly overview the current status of CAR-T therapy against solid cancers and then to introduce some of our own current attempts to overcome those obstacles. In details, I am especially focusing on i) choice of target molecule for CAR-T; disialoganglioside (GD2) for multiple solid cancers and epitope/HLA complexes both aiming at extepanding the spectrum of target molecules for CAR-T therapy against solid cancers, ii) choice of immune cells for CAR engineering; allogeneic gamma/delta T cells inherently being free from risk of graft-vs.-host disease (GVHD) mediated by allo-immunity, and iii) attempts to improve the functional persistence of CAR-T through metabolic fitness and resistance to activation induced cell death (AICD), by modification of intracytoplasmic domain of CAR gene construct.

CURRICULUM VITAE



Name Shin Kaneko

Affiliation Kyoto University
University of Tsukuba

Field of Research Cancer Immunology and Immunotherapy
Regenerative medicine
Stem cell biology

Education

1989-1995 School of Medicine, University of Tsukuba, Tsukuba, Japan (MD)
1998-2002 Graduate School of Medicine (Hematology/Immunology), University of Tsukuba, Tsukuba, Japan (PhD)

Professional Experience

1995-1997 Clinical Resident of internal medicine, Tsukuba University Hospital
2002-2003 Research Fellow, the Japan Society for the Promotion of Science
(Clinical and Experimental Hematology, University of Tsukuba)
2003-2007 Lecturer, Graduate School of Medicine, University of Tsukuba
(Clinical and Experimental Hematology)
2005-2007 Postdoctoral Fellow, Cancer Immunotherapy and Gene therapy program,
San Raffaele Scientific Institute, Milan, Italy
2008-2012 Assistant Professor, Center for Stem Cell Biology and Regenerative Medicine,
Institute of Medical Science, The University of Tokyo
2012- Associate Professor, Center for iPS Cell Research and Application (CiRA), Kyoto University
2013-2017 Facility head, Facility for iPS cell Therapy (FiT), Center for iPS cell Research and Application,
Kyoto University
2020- Professor, CiRA, Kyoto University
Professor, Transborder Medical Research Center (TMRC), University of Tsukuba
2021-2022 Facility head, Facility for animal experimentation, Center for iPS cell Research and Application,
Kyoto University
2022- Deputy Director, CiRA, Kyoto University

Recent Related Publications (5 Papers)

Wang B, Iriguchi S, Waseda M, Ueda N, Ueda T, Xu H, Minagawa A, Ishikawa A, Yano H, Ishi T, Ito R, Goto M, Takahashi R, Uemura Y, Hotta A, Kaneko S. Generation of hypoimmunogenic T cells from genetically engineered allogeneic human induced pluripotent stem cells. Nat Biomed Eng. 2021 May;5(5):429-440. doi: 10.1038/s41551-021-00730-z. Epub 2021 May 17. PMID: 34002062.

Kawai Y, Kawana-Tachikawa A, Kitayama S, Ueda T, Miki S, Watanabe A, Kaneko S. Generation of highly proliferative, rejuvenated cytotoxic T cell clones through pluripotency reprogramming for adoptive immunotherapy. Mol Ther. 2021 Oct 6;29(10):3027-3041. doi: 10.1016/j.ymthe.2021.05.016. Epub 2021 May 21. PMID: 34023508; PMCID: PMC8530944.

Iriguchi S, Yasui Y, Kawai Y, Arima S, Kunitomo M, Sato T, Ueda T, Minagawa A, Mishima Y, Yanagawa N, Baba Y, Miyake Y, Nakayama K, Takiguchi M, Shinohara T, Nakatsura T, Yasukawa M, Kassai Y, Hayashi A, Kaneko S. A clinically applicable and scalable method to regenerate T-cells from iPSCs for off-the-shelf T-cell immunotherapy. Nat Commun. 2021 Jan 18;12(1):430. doi: 10.1038/s41467-020-20658-3. PMID: 33462228; PMCID: PMC7814014.

Ueda T, Kumagai A, Iriguchi S, Yasui Y, Miyasaka T, Nakagoshi K, Nakane K, Saito K, Takahashi M, Sasaki A, Yoshida S, Takasu N, Seno H, Uemura Y, Tamada K, Nakatsura T, Kaneko S. Non-clinical efficacy, safety and stable clinical cell processing of induced pluripotent stem cell-derived anti-glypican-3 chimeric antigen receptor-expressing natural killer/innate lymphoid cells. Cancer Sci. 2020 May;111(5):1478-1490. doi: 10.1111/cas.14374. Epub 2020 Mar 31. PMID: 32133731; PMCID: PMC7226201.

Minagawa A, Yoshikawa T, Yasukawa M, Hotta A, Kunitomo M, Iriguchi S, Takiguchi M, Kassai Y, Imai E, Yasui Y, Kawai Y, Zhang R, Uemura Y, Miyoshi H, Nakanishi M, Watanabe A, Hayashi A, Kawana K, Fujii T, Nakatsura T, Kaneko S. Enhancing T Cell Receptor Stability in Rejuvenated iPSC-Derived T Cells Improves Their Use in Cancer Immunotherapy. Cell Stem Cell. 2018 Dec 6;23(6):850-858.e4. doi: 10.1016/j.stem.2018.10.005. Epub 2018 Nov 15. PMID: 30449714.

CD8 killer T cells and NK cells from iPSC for cancer immunotherapy

Shin Kaneko, M.D., Ph.D[1,2]

[1] CiRA, Kyoto University
[2] TMRC, University of Tsukuba

Important factors that determine the therapeutic efficacy of killer cell-based cell therapy are the maintenance of antigen specificity and memory phenotype of killer cells, and the maintenance of long-term viability and proliferative capacity of killer cells in vivo. Current T-cell production for immunotherapy is individualized autologous setting, which pose certain challenges in terms of manufacturing, quality, and stable supply. Recently, it has become possible to induce CD8 killer T cells and NK cells from iPS cells, and development of CAR-modified killer cells from allogeneic iPS cells has been attempted. In this article, we review the induction of CD8 killer T cells and NK cells from iPS cells, attempts to enhance the safety and reliability of the induction process, and the usefulness of gene editing to reduce allogeneic antigenicity.

CURRICULUM VITAE

Name Katy Rezvani

Affiliation University of Texas, MD Anderson Cancer center

Field of Research NK cell biology and cell therapy

Education

1993 Graduated ,University College London
2005 Ph.D Imperial College London

Professional Experience

University College, London, England	BSC (with honors)	07/1990	Neuroanatomy/Neuroscience
University College, London, England	MBBS (with distinction)	07/1993	Medicine
Hammersmith Hospital, London, United Kingdom	Clinical Fellowship	12/1999	
Hematology Branch NHLBI, NIH, Bethesda, MD	Research Fellowship	12/2007	Stem Cell Allotransplantation (SCAT) Transplant Immunology
Imperial College, London, England	PhD	07/2005	Transplant Immunology

Katy Rezvani M.D, PhD is the Sally Cooper Murray Chair in Cancer Research, Professor of Medicine, Chief of Section for Cellular Therapy, Director of Translational Research and Director of the GMP Facility at MD Anderson Cancer Center. She also serves as the Executive Director of the Adoptive Cell Therapy Platform at MD Anderson. Her research laboratory focuses on the role of natural killer (NK) cells in mediating immunity against hematologic and solid tumors. The goal of this research is to understand mechanisms of tumor-induced NK cell dysfunction and to develop strategies to genetically engineer NK cells in order to enhance their in vivo anti-tumor activity and persistence. Findings from Dr. Rezvani's lab have led to the approval and funding of several investigator-initiated clinical trials of NK cell immunotherapy in patients with hematologic malignancies and solid tumors, as well as the first-in-human clinical trial of off-the-shelf CAR-transduced cord blood NK cells in patients with relapsed/refractory lymphoid malignancies. Dr. Rezvani's work is supported by multiple grants from the National Cancer Institute, the Leukemia and Lymphoma Society, the American Cancer Society, Stand Up to Cancer and the Cancer Prevention & Research Institute of Texas (CPRIT). Dr. Rezvani completed her medical training at University College London, followed by Fellowships of the Royal College of Physicians and the Royal College of Pathologists of the United Kingdom, a Ph.D. in Immunology from Imperial College London and postdoctoral studies at the National Institutes of Health.

NK cells: next generation cell therapies for cancer

Katy Rezvani

University of Texas, MD Anderson Cancer center

Dr. Rezvani will discuss a new frontier in NK cell therapeutics: engineering NK cells with chimeric antigen receptors. She will discuss the opportunities and challenges of NK cell CAR engineering, and present pre-clinical and early phase clinical data on cord blood-derived NK cells expressing CD19 CAR and IL-15 to enhance their in vivo persistence in patients with relapsed or refractory blood cancers. In addition, she will discuss new data on targeting solid tumor with CAR NK cells as well strategies for the gene editing of CAR NK cells to enhance their function by targeting immune checkpoints. Finally, she will discuss the approach of precomplexing NK cells with an anti-CD16 bispecific antibody targeting cancer targets to redirect their specificity, thus providing a rapid approach to translate NK cells with CAR-like characteristics to the clinic

Recent Related Publications (5 Papers)

1. Liu E, Tong Y, Dotti G, Shaim H, Savoldo B, Mukherjee M, Orange J, Wan X, Lu X, Reynolds A, Gagea M, Banerjee P, Cai R, Bdaiwi MH, Basar R, Muftuoglu M, Li L, Marin D, Wierda W6, Keating M6, Champlin R, Shpall E, Rezvani K. Cord blood NK cells engineered to express IL-15 and a CD19-targeted CAR show long-term persistence and potent antitumor activity. Leukemia. 2017 Jul 20. doi: 10.1038/leu.2017.226. PMID: 28725044 PMCID: PMC6063081
2. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, Nassif Kerbauy L, Overman B, Thall P, Kaplan M, Nandivada V, Kaur I, Nunez Cortes A, Cao K, Daher M, Hosing C, Cohen EN, Kebriaei P, Mehta R, Neelapu S, Nieto Y, Wang M, Wierda W, Keating M, Champlin R, Shpall EJ, Rezvani K. Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors. N Engl J Med 382(6):545-553, 2/2020. PMID: 32023374 PMCID: PMC7101242
3. Daher M, Basar R, Gokdemir E, Baran N, Uprety N, Nunez Cortes AK, Mendt M, Kerbauy LN, Banerjee PP, Shanley M, Imahashi N, Li L, Lim FLWI, Fathi M, Rezvan A, Mohanty V, Shen Y, Shaim H, Lu J, Ozcan G, Ensley E, Kaplan M, Nandivada V, Bdiwi M, Acharya S, Xi Y, Wan X, Mak D, Liu E, Jiang XR, Ang S, Muniz-Feliciano L, Li Y, Wang J, Kordasti S, Petrov N, Varadarajan N, Marin D, Brunetti L, Skinner RJ, Lyu S, Silva L, Turk R, Schubert MS, Rettig GR, McNeill MS, Kurgan G, Behlke MA, Li H, Fowlkes NW, Chen K, Konopleva M, Champlin RE, Shpall EJ, Rezvani K.Targeting a cytokine checkpoint enhances the fitness of armored cord blood CAR-NK cells. Blood 137(5):624-636, 2/2021. e-Pub 9/2020. PMCID: PMC7869185.
4. Muftuoglu M, Olson A, Marin D, Ahmed S, Mulanovich V, Tummala S, Chi TL, Ferrajoli A, Kaur I, Li L, Champlin R, Shpall EJ, Rezvani K. Allogeneic BK Virus-Specific T Cells for Progressive Multifocal Leukoencephalopathy. N Engl J Med 379(15):1443-1451, 10/2018. PMCID: PMC6283403.
5. Shaim H, Shanley M, Basar R, Daher M, Gumin J, Zamler DB, Uprety N, Wang F, Huang Y, Gabrusiewicz K, Miao Q, Dou J, Alsuliman A, Kerbauy LN, Acharya S, Mohanty V, Mendt M, Li S, Lu J, Wei J, Fowlkes NW, Gokdemir E, Ensley E, Kaplan M, Kassab C, Li L, Ozcan G, Banerjee PP, Shen Y, Gilbert AL, Jones CM, Bdiwi M, Nunez-Cortes AK, Liu E, Yu J, Imahashi N, Muniz-Feliciano L, Li Y, Hu J, Draetta G, Marin D, Yu D, Mielke S, Eyrich M, Champlin RE, Chen K, Lang FF, Shpall EJ, Heimberger AB, Rezvani K. Targeting the αv integrin-TGF-β axis improves natural killer cell function against glioblastoma stem cells. J Clin Invest. e-Pub 6/2021. PMID: 34138753. 105.

CURRICULUM VITAE

Name Yui Harada

Affiliation Graduate School of Pharmaceutical Sciences,
Kyushu University

Field of Research
- Cancer immunology and immunotherapy
- Regenerative Medicine
- Translational Research

Education

2008. 4 – 2011. 3 Graduate Student at Chiba University
Gradeuate School of Medicine with a Ph.D. (Doctor of Med. Science), working under the Professors Tomohiko Ichikawa (Urology) and Yoshikazu Yonemitsu (Innovative Biotherapeutics) on Cancer immunotherapy.

2006. 4 -2008. 3 Graduate Student at Chiba University
Gradeuate School of Medicine with a M.Sc. (Master of Med. Science), working under the Professors Tomohiko Ichikawa (Urology) and Yoshikazu Yonemitsu (Gene Therapy) on Cancer immunotherapy.

Professional Experience

2019. 8 – present Associate Professor
R&D Laboratory for Innovative Biotherapeutics Graduate School of Pharmaceutical Sciences, Kyushu University

2012. 4 – 2019. 7 Assistant Professor
R&D Laboratory for Innovative Biotherapeutics Graduate School of Pharmaceutical Sciences, Kyushu University

2011. 4 – 2012. 3 Postdoctoral fellow
R&D Laboratory for Innovative Biotherapeutics Graduate School of Pharmaceutical Sciences, Kyushu University

Recent Related Publications (5 Papers)

Ex vivo generation of highly purified and activated NK cells from human peripheral blood.
Saito S, Harada Y, Morodomi Y, Onimaru M, Yoshida K, Kyuragi R, Matsubara H, Yonemitsu Y.
Hum Gene Ther Methods. 4, 241-252, 2013.

Peritoneal dissemination requires an Sp1-dependent CXCR4/CXCL12 signaling axis and extracellular matrix-directed spheroid formation.
*Kasagi Y, *Harada Y, Morodomi Y, Iwai T, Saito S, Yoshida K, Oki E, Saeki H, Ohgaki K, Sugiyama M, Onimaru M, Maehara Y, Yonemitsu Y.
*These two authors contributed equally to this work.
Cancer Research. 76:347-357, 2016.

Natural antibody against neuroblastoma of TH-MYCN transgenic mice does not correlate with spontaneous regression.
Kawakubo N, Harada Y*, Ishii M, Souzaki R, Kinoshita Y, Tajiri T, Taguchi T, Yonemitsu Y.
Biochem Biophys Res Commun 503:1666-1673, 2018.

Highly activated *ex vivo*-expanded natural killer cells in patients with solid tumors in a Phase I/IIa clinical study
Nagai K, Harada Y, Harada H, Yanagihara K, Yonemitsu Y, Miyazaki Y.
Anticancer Res. 40:5687-5700, 2020.

Fc-binding antibody-recruiting molecules targeting prostate-specific membrane antigen: defucosylation of antibody for efficacy improvement
Sasaki K, Harada M, Yoshikawa T, Tagawa H, Harada Y, Yonemitsu Y, Ryujin T, Kishimura A, Mori T, Katayama Y.
ChemBioChem. 22:496-500, 2021.

Off-the-shelf NK-like cell product: GAIA-102 ~ A breakthrough in the immunotherapy against various solid tumors~

Yui Harada

Graduate School of Pharmaceutical Sciences, Kyushu University

Cancer immunotherapy has been established as a new therapeutic category since the recent success of immune checkpoint inhibitors and a type of adoptive immunotherapy, Namely chimeric antigen receptor-modified T cells (CAR-T). Although CAR-T demonstrated impressive clinical results, serious adverse effects (cytokine storm and on-target off-tumor toxicity) and undefined efficacy on solid tumors are important issues to be solved. We've developed a cutting-edge, simple, and feeder-free method to generate highly activated and expanded human NK "like" cells (solid tumor killer/immunogenic cell death inducer), Name d as GAIA-102, from peripheral blood mononuclear cells (US9404083, PCT/JP2019/012744, PCT/JP2020/012386, etc.). GAIA-102 is allogeneic, cryopreserved, off-the-shelf and not genetically modified cell-based medicine being able to defeat solid malignancies.

Although GAIA-102 cells have immature phenotype ($CD3^-/CD56^{bright}/CD57^-$), the cells exhibit highly efficient tumor cell killing irrespective of the HLA expression. GAIA-102 cells express the activating receptors as found in so-called activated NK cells (NKp30/NKp46/NKG2D, etc.) and have specific phenotype ($KIRs^{low/-}/CCR5^+/CCR6^+/CXCR3^+$). GAIA-102 could show efficient accumulation and elimination of various solid tumors, andthe sphere destruction by GAIA-102 was affected neither by myeloid-derived suppressor cells nor regulatory T-lymphocytes. Importantly, GAIA-102 could stimulate innate and acquired immune responses against solid tumors and induce tumor-specific T cells efficiently *in vivo*. Single cell RNA sequencing and trajectory inference data suggested that GAIA-102 has a completely new phenotype while having some subpopulation common to memory like NK cells that are beginning to show good clinical results, and is in a zenithal activated state.

We now just started first-in-human clinical trials against advanced and relapse non-small cell lung cancer (NCT05207371) and will initiate multiple trials against various solid tumors. We expect that the new type of NK like cells will overcome challenges of solid tumor treatment.

Ethics Approval: The animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee of Kyushu University (approval nos. A30-234-0 and A30-359-0).

Special Lecture

Abstract & Curriculum Vitae

CURRICULUM VITAE

Name Anna Maria Ranzoni

Affiliation Nature Medicine

Field of Research Anna is an Associate Editor at Nature Medicine, handling manuscripts in genetics and molecular therapies.

Education

2012 BSc in biological sciences, University of Milan, Italy
2014 MSc in genetics, University of Pavia, Italy
2017 PhD in stem cells and regenerative medicine, University College London, UK

Professional Experience

2017 – 2020 Postdoctoral Researcher, University of Cambridge – Sanger Institute, UK
2020 – present Associate Editor, Nature Medicine, Springer Nature, UK

Publishing Clinical Research at Nature Medicine

Anna Maria Ranzoni

Nature Medicine

In this talk, Dr Anna Maria Ranzoni will give an overview of the process of publishing clinical research at Nature Medicine, with a specific focus on gene therapies. The talk will explain what type of clinical research content Nature Medicine publishes and will go through the guidelines for clinical reporting of gene therapy trials.

Educational Lecture 1

Abstract & Curriculum Vitae

略 歴

名前　　津田　誠

所属　　九州大学

研究分野　　神経薬理学

学歴

1998年　星薬科大学　大学院薬学研究科　博士課程修了

職歴

1999～2002年　JST特別研究員（国立医薬品食品衛生研究所配属）
2002～2004年　トロント小児病院　博士研究員
2004～2005年　厚生労働省　厚生労働技官　（国立医薬品食品衛生研究所配属）
2005～2006年　九州大学　大学院薬学研究院　助手
2006～2014年　九州大学　大学院薬学研究院　助教授（07年より准教授）
2014年～現在　九州大学　大学院薬学研究院　教授（**現職**）
（兼任職）
2016～2020年　九州大学　大学院薬学研究院附属産学官連携創薬育薬センター　センター長
2016年～現在　順天堂大学　大学院環境医学研究所　客員教授
2018年～現在　九州大学　総長補佐
2019年～現在　九州大学　主幹教授
2020年～現在　九州大学　大学院薬学研究院　副研究院長
2021～2022年　京都大学大学院薬学研究科　非常勤講師
2022年～現在　九州大学高等研究院　副研究院長

最近の関連出版物・論文

Kohno et al., A spinal microglia population involved in remitting and relapsing neuropathic pain. Science 376: 86-90 (2022)
Tashima et al., A subset of spinal dorsal horn interneurons crucial for gating touch-evoked pain-like behavior. PNAS 118: e2021220118 (2021)
Kohro et al., Spinal astrocytes in superficial laminae gate brainstem descending control of mechanosensory hypersensitivity. Nat Neurosci 23: 1376-1387 (2020)
Koga et al., Sensitization of spinal itch transmission neurons in a mouse model of chronic itch requires an astrocytic factor. J Allergy Clin Immunol 145: 183-191 (2020)
Inoue K, Tsuda M, Microglia in neuropathic pain: cellular and molecular mechanisms and therapeutic potential. Nat Rev Neurosci 19: 138-152 (2018)

痛みの慢性化に関わる脊髄後角細胞

津田　誠

九州大学

痛覚は有害な刺激から身を守るために必要な生体防御システムのひとつである。皮膚など末梢組織における痛覚受容機構は，昨年ノーベル生理学医学賞の受賞に至ったTRPチャネルの発見を代表に，数多くの研究成果からその理解が進んでいる。一方，中枢神経系における感覚信号の処理・統合の仕組みは依然として不明な点が多い。特に，脊髄後角は末梢からの感覚信号を脳へ送る単なる中継地点と思われがちだが，同部位には複雑な回路が存在し，それに異常が生じると，痛覚信号が強まる（痛覚過敏），あるいは触覚信号が痛覚へ変換される（アロディニア）などの劇的な変容が現れる。これらの現象は，神経の損傷や圧迫で発症する神経障害性疼痛などで認められ，患者の生活の質を極端に低下させる原因となる。すなわち脊髄後角は，慢性疼痛メカニズムや治療薬開発において重要な部位といえる。脊髄後角神経回路の動作異常には，ある特定の細胞の機能変化が引き金になることが最近の研究から明らかにされている。その細胞には，神経だけでなく，その周りに存在するグリアが含まれる。私たちは動物モデルを用いた基礎研究から，その異常につながる神経とグリアのサブセットを複数特定し，AAVベクター等を用いた遺伝子発現制御による介入で神経障害性疼痛を抑制できることを示してきた。本講演では，これまでの研究の流れと上記の新たな成果を含めて，脊髄後角の神経とグリアによる痛みの変調メカニズムを紹介し，細胞および遺伝子治療への応用の可能性について議論したい。

Educational Lecture 2

Abstract & Curriculum Vitae

略 歴

名前　山海　嘉之

所属　筑波大学　システム情報系教授／
サイバニクス研究センター研究統括／
未来社会工学開発研究センターセンター長
CYBERDYNE株式会社 代表取締役社長／CEO

研究分野　サイバニクス、人・AIロボット・情報系の融合複合分野

学歴

1984-1987　筑波大学大学院工学研究科構造工学専攻　博士課程

職歴

2004-　筑波大学システム情報系教授（現任），CYBERDYNE（サイバーダイン社）創設
2010-2015　内閣府 FIRST 最先端 サイバニクス研究拠点 研究統括
2011-2017　筑波大学サイバニクス研究センター センター長
2014-2020　内閣府 ImPACT 革新的研究開発推進プログラム プログラムマネージャー
2016-2021　文科省地域イノベーションエコシステム 事業プロデューサー
2017-　筑波大学サイバニクス研究センター研究統括（現任）
2020-　筑波大学未来社会工学開発研究センター／F-MIRAI センター長（現任）

最近の関連出版物・論文

- 山海嘉之，サイバニクスを駆使した「健康未来社会」― ロボット産業、IT産業に続く新産業「サイバニクス産業」始動，月刊 経団連，2021年12月号，pp.40-43，2021.
- Kazutomo Baba, Andrey Mikhailov, Yoshiyuki Sankai, “Influence of the perfusion bioreactor on Stratified and Distributed approaches for multilayered tissue engineering on biodegradable scaffolds”, Proceedings of 43rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC2021), pp.1181-1186, 2021.
- Kazutomo Baba, Andrey Mikhailov, Yoshiyuki Sankai, “Dynamic flow priming programs allow tuning up the cell layers properties for engineered vascular graft”, Scientific Reports 11, 14666 (2021), DOI:10.1038/s41598-021-94023-9, 19 July, 2021.
- Takashi Nakajima, Yoshiyuki Sankai, Shinjiro Takata, Yoko Kobayashi, Yoshihito Ando, Masanori Nakagawa, Toshio Saito, Kayoko Saito, Chiho Ishida, Akira Tamaoka, Takako Saotome, Tetsuo Ikai, Hisako Endo, Kazuhiro Ishii, Mitsuya Morita, Takashi Maeno, Kiyonobu Komai, Tetsuhiko Ikeda, Yuka Ishikawa, Shinichiro Maeshima, Masashi Aoki, Michiya Ito, Tatsuya Mima, Toshihiko Miura, Jun Matsuda, Yumiko Kawaguchi, Tomohiro Hayashi, Masahiro Shingu & Hiroaki Kawamoto, “Cybernic treatment with wearable cyborg Hybrid Assistive Limb (HAL) improves ambulatory function in patients with slowly progressive rare neuromuscular diseases: a multicentre, randomised, controlled crossover trial for efficacy and safety (NCY-3001)”, Orphanet Journal of Rare Diseases volume 16, Article number: 304 (2021), DOI:10.1186/s13023-021-01928-9, 07 July 2021.

脳神経・筋系疾患の機能再生治療を実現するサイバニクス医療イノベーション ～そして【サイバニクス×再生医療】による更なる挑戦へ～

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『人』と『サイバー・フィジカル空間』を一体的に扱う『サイバニクス』（人・ロボット・情報系の融合複合）を研究開発・駆使することで未来開拓に挑戦している。開拓領域は、細胞培養レベルからロボット技術、サイボーグ技術、AI、IoH/IoT、ヒューマンビッグデータ、クラウド化、スパコンでのデータ解析・AI処理、国際標準化、医療技術の社会実装、治療・診断・予防技術、医療と非医療を繋ぐ健康維持・増進など多岐に渡る。装着型サイボーグ HALは、脳神経系由来の生体電位信号によって人とHALを繋ぎ、人とAI技術・ロボット・情報系を機能的に融合する。HALを装着すると、脳と身体とHALとの間でのインタラクティブ・バイオフィードバック（iBF）が構築され、神経と神経、神経と筋肉の間のシナプス結合が強化・調整される機能再生治療が実現できる。新医療機器として承認された医療用HALによるサイバニクス治療は、我が国ではSMA、ALS、SMBA、CMT、筋ジストロフィーなどの神経・筋難病疾患（８疾患）に対する治験を経て医療保険が適用され、その後の使用成績調査によって長期適用の顕著な治療成果も示された。現在、世界20ヵ国で医療機器プラットフォームとして展開されている。

スピンラザなどの最新の薬剤とサイバニクスとの複合療法に加え、再生医療分野におけるサイバニクス技術の様々な展開も始まっており、ロボット化／サイバニック化されたバイオリアクターに関する取り組み、自立生活できる水準の回復に向けた細胞再生に加えて機能再生という観点を含めた取り組みなど、医療現場で活用できる再生医療／薬剤とHALを含む様々なサイバニクス技術を駆使した取り組みが活発化している。

本講演では、脳神経・筋系疾患の機能再生治療を実現するサイバニクス医療イノベーションについて最新情報を交えて述べると共に、【サイバニクス×再生医療】という新たな革新的医療イノベーションに向けた取り組みについて述べてみたい。

Educational Lecture 3

Abstract & Curriculum Vitae

略 歴

名前　　髙橋　政代

所属　　株式会社ビジョンケア
神戸アイセンター病院 研究センター
立命館大学 RARA

研究分野　　網膜変性疾患、黄斑部疾患、再生医療研究

学歴

1986	京都大学医学部卒業
1992	京都大学大学院医学研究科博士課程修了
1992-2001	京都大学医学部附属病院眼科助手
1995-1996	アメリカソーク研究所研究員
2001-2006	京都大学医学部附属病院探索医療センター開発部助教授
2006-2012	理化学研究所網膜再生医療研究チーム チームリーダー
2012-2019	同研究所網膜再生医療研究開発プロジェクト プロジェクトリーダー
2019-現在	株式会社ビジョンケア代表取締役社長

職歴

1986.3	京都大学医学部卒業
1992.3	京都大学大学院医学研究科博士課程（視覚病態学）修了
1992.4-1994	京都大学医学部附属病院眼科 助手
1995.1-1996.12	アメリカ・サンディエゴ　ソーク研究所研究員
1997.1-2001	京都大学医学部附属病院眼科　助手　復職
2001.10-2006.9	京都大学医学部附属病院探索医療センター開発部 助教授
2006.4-2019.7	理化学研究所 膜再生医療研究開発プロジェクト プロジェクトリーダー
2019.8-現在	株式会社ビジョンケア　代表取締役社長

最近の関連出版物・論文

1. Maeda T, Mandai M, Sugita S, Kime C, Takahashi M.
Strategies of pluripotent stem cell-based therapy for retinal degeneration: update and challenges. Trends Mol Med. 2022 Mar 31:S1471-4914(22)00053-3.
doi: 10.1016/j.molmed.2022.03.001. Online ahead of print.
2. Ochiai K, Motozawa N, Terada M, et al.
A variable-scheduling maintenance culture platform for mammalian cells
SLAS Technology 2021 Apr;26(2):209-217. doi: 10.1177/2472630320972109.
3. Mandai M, Watanabe A, Kurimoto Y, et al.
Autologous induced stem-cell-derived retinal cells for macular degeneration.
The New England Journal of Medicine 376. 1038–1046 (2017) doi:10.1056/NEJMoa1608368

網膜再生医療とサステナブルメディスン

高橋　政代[1,2,3]

[1]株式会社ビジョンケア
[2]神戸アイセンター病院 研究センター
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我々は網膜外層疾患の細胞治療や遺伝子治療の開発を目指している。2013年からiPS細胞の安全な利用を実証するために、自家iPS由来網膜色素上皮（RPE）細胞シート移植を行ったが、細胞シートは7年後の今もまだ機能し視細胞を維持している。2017年からの同種移植臨床試験では、HLAミスマッチを回避すれば局所のステロイド剤のみで免疫反応を制御できることを確認し、HLA部分KO iPS細胞を用いたパイプライン開発に繋がっている。現在、対象疾患をRPE不全症に拡大し、懸濁液から紐状のRPEストリップに進化した形で、小さな穴から安全に移植する臨床研究を行っている。網膜外層の再建には、視細胞の再生も重要な課題だが、昨年、網膜色素変性症の2例に対してiPS細胞網膜オルガノイド移植を行い、結果を解析中である。

これらの経験をもとに、網膜外層疾患の病名ではなく症例の状態によるカテゴリーごとに治療法を作っていくべきだと考えている。また、replacement therapyは外科的治療であるため、医薬品開発にはない最終製品とのギャップが存在する。したがって、効果のある理想的な治療とするためには、それぞれの治療法に適した症例を厳密に選び、検査や手術手技などの臨床側の準備する必要がある。そうでなければ、細胞治療は高価なギャンブルになってしまいサステナブルな医療とはならない。

また、細胞製造に関しては、通常、CPC内の装置はすべてバリデーションが行われているが、細胞培養の技術は検証されていないため、細胞の状態が培養する人によって異なることがある。この問題に対して、技術者と同じ操作をヒューマノイドロボットにさせることで、常に安定した品質の細胞を得ることができるように技術もバリデーションを行える方向に進んでいる。網膜再生医療の現状とこれからをお話しする。

NATSJ-JSGCT Joint Symposium

Abstract & Curriculum Vitae

Non-viral Therapeutics

CURRICULUM VITAE

Name Takanori Yokota, MD, PhD

Affiliation Professor
Department of Neurology and Neurological Science,
Tokyo Medical and Dental University

Professional Experience

Takanori Yokota received Japanese National License of Medical Doctors in 1984 and the Ph.D. degree from Tokyo Medical and Dental University,Tokyo, Japan, in 1990.
From 1984, he served as a Neurologist at Tokyo Medical and Dental University ; from 1998, as a Research Fellow in Bredesen's lab in Burham Institute (CA, USA); from 1999, as a Research Fellow in Bredesen's lab in Buck Center for Aging Research (CA, USA); from 2000, as a Assistant Professor in Neurology at Tokyo Medical and Dental University; from 2004, as a Associate Professor in Neurology at Tokyo Medical and Dental University.
From 2014 to the present, he serves as a Professor in Neurology at Tokyo Medical and Dental University.
His major interest includes physiology of neurological diseases.
Dr. Yokota is a member of Japanese Neurological Society.

Award:

2001 Award of 42nd Japananese Neurological Association
2003 Award of 31th Japananese the Naito memorial Foundation
2003 Award of 56th Japananese Vitamin Association
2006 Award of 20th Meeting of Japanese Society of Neuroimmunology

Recent Related Publications (5 Papers)

2021

1. Ohyagi M, Nagata T, Ihara K, Yoshida-Tanaka K, Nishi R, Miyata H, Abe A, Mabuchi Y, Akazawa C, Yokota T.DNA/RNA heteroduplex oligonucleotide technology for regulating lymphocytes in vivo. Nat Communs 2021 Dec;12(1):7344.
2. Nagata T, Dwyer CA, Yoshida-Tanaka K, Ihara K, Ohyagi M, Kaburagi H, Miyata H, Ebihara S, Yoshioka K, Ishii T, Miyata K, Miyata K, Powers B, Igari T, Yamamoto S, Arimura N, Hirabayashi H, Uchihara T, Hara RI, Wada T, Bennett CF, Seth PP, Rigo F, Yokota T. Cholesterol-functionalized DNA/RNA heteroduplexes cross the blood-brain barrier and knock down genes in the rodent CNS. Nat Biotechnol 2021 Dec;39(12):1529-36.
3. Asada K, Sakaue F, Nagata T, Zhang JC, Yoshida-Tanaka K, Abe A, Nawa M, Nishina K, Yokota T. Short DNA/RNA heteroduplex oligonucleotide interacting proteins are key regulators of target gene silencing. Nucleic Acids Res. 2021 May;49(4):4864-76.

2019

1. Yoshioka K, Kunieda T, Asami Y, Guo H, Miyata H, Yoshida-Tanaka K, Sujino Y, Piao W, Kuwahara H, Nishina K, Iwata Hara R, Nagata T, Wada T, Obika S, Yokota T. Highly efficient silencing of microRNA by heteroduplex oligonucleotides. Nucleic Acids Res 2019 Aug;47(14):7321-32.

2017

1. Nishina T, Numata J, Nishina K, Yoshida-Tanaka K, Nitta K, Piao W, Iwata R, Ito S, Kuwahara H, Wada T, Mizusawa H, Yokota T. Chimeric Antisense Oligonucleotide Conjugated to α- Tocopherol. Mol Ther Nucleic Acids 2015 Jan 13;4:e220.

Blood-brain-barrier (BBB) crossing heteroduplex oligonucleotide

Takanori Yokota

Department of Neurology and Neurological Science, Tokyo Medical and Dental University

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Achieving regulation of endogenous gene expression in the central nervous system (CNS) with antisense oligonucleotides (ASOs) administered systemically would facilitate the development of ASO-based therapies for neurological diseases. We demonstrate that DNA/RNA heteroduplex oligonucleotides (HDOs) conjugated to cholesterol or α-tocopherol at the 5′ end of the RNA strand reach the CNS after subcutaneous or intravenous administration in mice and rats. The HDOs distribute throughout the brain, spinal cord and peripheral tissues and suppress the expression of four target genes by up to 90% in the CNS, whereas single-stranded ASOs conjugated to cholesterol have limited activity. Gene knockdown was observed in major CNS cell types and was greatest in neurons and microglial cells. Side effects, such as thrombocytopenia and focal brain necrosis, were limited by using subcutaneous delivery or by dividing intravenous injections. By crossing the blood–brain barrier more effectively, cholesterol-cconjugated HDOs may overcome the limited efficacy of ASOs targeting the CNS without requiring intrathecal administration. (Nat Biotech, 2021)

CURRICULUM VITAE

Name Takeshi Wada

Affiliation Tokyo University of Science

Field of Research Organic synthesis of artificial biomolecules:
1) Stereocontrolled synthesis of backbone modified DNA and RNA analogs,
2) Design and synthesis of artificial oligosaccharides,
3) Design and synthesis of artificial oligopeptides

Education
1986, BS, Tokyo University of Science
1988, MS, Tokyo Institute of Technology
1991, PhD, Tokyo Institute of Technology

Professional Experience
1991, Assistant Professor, Tokyo Institute of Technology
1999, Associate Professor, The University of Tokyo
2008, Founder and Scientific Adviser, Chiralgen, Ltd.
2013, Founder and Director, Wave Life Sciences
2013-Present, Professor, Tokyo University of Science
2017-Present, Founder and Scientific Adviser, Wave Life Sciences

Recent Related Publications (5 Papers)
1) Yuhei Takahashi, Kazuki Sato, Takeshi Wada, Solid-phase synthesis of boranophosphate/phosphorothioate/phosphate chimeric oligonucleotides and yheir potential as antisense oligonucleotides, *J. Org. Chem.* **2022**, *87*, 3895-3909.
2) Hiromasa Matsuda, Erina Yoshida, Takaaki Shinoda, Kazuki Sato, Rintaro Iwata Hara, Takeshi Wada, Solution-phase synthesis of oligodeoxyribonucleotides using the *H*-phosphonate method with *N*-unprotected 5'-phosphite monomers, *RSC Adv.* **2021**, *11*, 38094-38107.
3) Hiromasa Matsuda, Hiroaki Ito, Yohei Nukaga, Sho Uehara, Kazuki Satoa, Rintaro Iwata Hara, Takeshi Wada, Solid-phase synthesis of oligouridine boranophosphates using the H-boranophosphonate method with 2′-O-(2-cyanoethoxymethyl) protection, *Tetrahedron Lett.* **2021**, *87*, 153526.
4) Rintaro Iwata Hara, Takeshi Wada, Inhibition of off-target cleavage by RNase H using an artificial cationic oligosaccharide, *Org. Biomol. Chem.* **2021**, *19*, 6865-6870.
5) Tetsuya Nagata, Chrissa A. Dwyer, Kie Yoshida-Tanaka, Kensuke Ihara, Masaki Ohyagi, Hidetoshi Kaburagi, Haruka Miyata, Satoe Ebihara, Kotaro Yoshioka, Takashi Ishii, Kanjiro Miyata, Kenichi Miyata, Berit Powers, Tomoko Igari, Syunsuke Yamamoto, Naoto Arimura, Hideki Hirabayashi, Toshiki Uchihara, Rintaro Iwata Hara, Takeshi Wada, C. Frank Bennett, Punit P. Seth, Frank Rigo, Takanori Yokota, Cholesterol-functionalized DNA/RNA heteroduplexes cross the blood–brain barrier and knock down genes in the rodent CNS, *Nature Biotech.* **2021**, *39*, 1529-1536.

New molecular technologies to improve efficacy and safety of oligonucleotide therapeutics

Takeshi Wada

Tokyo University of Science

Current issues in the development of nucleic acid drugs are establishment of effective drug delivery systems and reduction of off-target effects. In order to overcome these issues, we have developed stereopure backbone-modified DNA and RNA analogs. A proper backbone modification of oligonucleotides increaces their drug efficacy as well as biological stability. However, it also results in the generation of numerous diastereomers. Since the *P*-chirality affects nearly all the critical properties of nucleic acid drugs, stereocontrolled synthesis of *P*-chiral oligonucleotides is of great importance. In this presentation, I wish to describe a recent progress of stereocontrolled synthesis of *P*-chiral DNA and RNA analogs and their unique properties. Further, we have also developed artificial cationic oligosaccharides and cationic oligopeptides that bind specifically to A-type nucleic acid duplexes such as RNA/RNA and DNA/RNA. These molecules are expected to be useful as new carriers for siRNA and DNA/RNA heteroduplex oligonucleotide (HDO) drugs. Properties and medicinal applications of these molecules will also be described.

CURRICULUM VITAE

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Graduate School of Engineering, The University of Tokyo

Field of Research Biomaterial, Nanomedicine

Education

2006 PhD (Engineering), Graduate School of Engineering,
The University of Tokyo

Professional Experience

2022– present Professor, Graduate School of Engineering, The University of Tokyo
2016–2021 Associate Professor, Graduate School of Engineering, The University of Tokyo
2013–2017 Associate Professor, Graduate School of Medicine, The University of Tokyo
2009–2012 Assistant Professor, Graduate School of Medicine, The University of Tokyo
2006–2009 Assistant Professor, Graduate School of Engineering, The University of Tokyo

Recent Related Publications (5 Papers)

1. S. Min, H. J. Kim, M. Naito, S. Ogura, K. Toh, K. Hayashi, B. S. Kim, S. Fukushima, Y. Anraku, K. Miyata, K. Kataoka, Systemic brain delivery of antisense oligonucleotides across the blood — brain barrier with a glucose — installed polymeric nanocarrier. *Angew. Chem. Int. Ed.*59, 8173-8180 (2020)
2. S. Kim, S. Osawa, M. Naito, S. Ogura, R. Kamegawa, H. Ishida, H. J. Kim, S. Uchida, K. Miyata, A 50 nm-sized micellar assembly of thermoresponsive polymer-antisense oligonucleotide conjugates for enhanced gene knockdown in lung cancer by intratracheal administration. *Adv. Therap.*3,1900123 (2020)
3. J. Kim, S. Ogura, T. Otabe, R. Kamegawa, M. Sato, K. Kataoka, K. Miyata, Fine-tuning of hydrophobicity in amphiphilic polyaspartamide derivatives for rapid and transient expression of messenger RNA directed toward genome engineering in brain. *ACS Cent. Sci.*5, 1866-1875 (2019)
4. Watanabe, K. Hayashi, K. Toh, H. J. Kim, X. Liu, H. Chaya, S. Fukushima, K. Katsushima, Y. Kondo, S. Uchida, S. Ogura, T. Nomoto, H. Takemoto, H. Cabral, H. Kinoh, H. Tanaka, M. R. Kano, Y. Matsumoto, H. Fukuhara, S. Uchida, M. Nangaku, K. Osada, N. Nishiyama, K. Miyata, K. Kataoka, In vivo rendezvous of small nucleic acid drugs with charge-matched block catiomers to target cancer. *Nat. Commun.*10,1894 (2019)
5. S. Kim, S. Chuanoi, T. Suma, Y. Anraku, K. Hayashi, M. Naito, H. J. Kim, I. C. Kwon, K. Miyata, A. Kishimura, K. Kataoka, Self-assembly of siRNA/PEG-b-catiomer at integer molar ratio into 100 nm-sized vesicular polyion complexes (siRNAsomes) for RNAi and codelivery of cargo macromolecules. *J. Am. Chem. Soc.*141, 3699-3709 (2019)

Polymer-based nanomedicines for nucleic acid delivery and retention

Kanjiro Miyata

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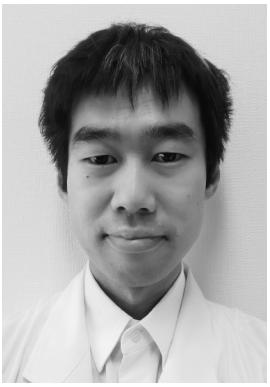
Recently, nucleic acid drugs have been extensively highlighted as a next generation biopharmaceutical. Indeed, new oligonucleotide drugs are approved each year. Also, the first mRNA drugs are widely used as a vaccine. However, their therapeutic use remains limited due to undesired biodistribution or low tissue-targetability, except for the liver.

To enhance the targetability of nucleic acid drugs, we have developed polymer (or polypeptide)-based nanomedicines, which will be introduced in the presentation. One of them is an ultra-small nanomedicine that consists of a single oligonucleotide molecule with one or two molecules of Y-shaped block copolymers (YBCs) via electrostatic interactions, termed unit polyion complex (uPIC). uPICs showed a hydrodynamic diameter of approximately 15 nm and were equilibrated (or stabilized) with free YBCs in the bloodstream, thereby eliciting the prolonged blood circulation with high tissue permeability. As a result, oligonucleotide-loaded uPICs allow for the efficient accumulation in various cancer models, e.g., fibrotic pancreatic tumor tissues and brain tumor tissues [1]. This nanomedicine formulation is clinically tested for triple negative breast cancer patients. Additionally, we further investigated the impacts of chemical structures of oligonucleotides [2] and chain lengths of YBCs [3] on the blood circulation property of uPICs. These studies demonstrated that the blood circulation property of uPICs was dramatically enhanced by utilizing the chemically modified oligonucleotides with the phosphorothioate backbone and 2'-F/OMe and by optimizing the number of cationic sites in YBCs. On the other hand, we developed an oligonucleotide-conjugated nanoparticle with a size ranging 50–800 nm (termed nanoball) as a nanomedicine staying at the administration site. When intratracheally administrated, nanoballs were more effectively retained in lung tissue compared to naked oligonucleotides, which were rapidly distributed throughout the body, presumably due to their small size (< 5 nm). The nanoball resulted in the enhanced gene silencing effect in the lung because of the prolonged retention in the lung, compared to naked oligonucleotides [4]. In summary, polymer-based nanomedicines have strong potentials for controlling the biodistribution of nucleic acid drugs.

References

[1] S. Watanabe, et al., *Nat. Commun.* 10, 1894 (2019).
[2] M. Naito, et al., *J. Control. Release* 330, 812–820 (2021)
[3] H. Chaya, et al., *Biomacromolecules* 23, 388–397 (2021)
[4] B. S. Kim, et al., *Adv. Therap.* 3, 1900123 (2020)

CURRICULUM VITAE



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Field of Research Neurology, Neurodegenerative disorders,
Parkinson's disease, alpha-synuclein,
therapeutic strategies for neurodegenerative diseases,
translational research

Education

2002-2008 M.D. -Faculty of Medicine, Osaka University, Japan
2012-2016 Ph.D. (Dr. of medical science) - Graduate school of Medicine, Osaka University, Japan

Professional Experience

2008-2010 Resident in Medicine, Higashiosaka City General Hospital, Osaka, Japan
2010-2012 Resident in Neurology, Higashiosaka City General Hospital, Osaka, Japan
2012-2013 Resident in Neurology, Osaka University Hospital, Osaka, Japan
2013-2016 Attending Neurologist, Osaka University Hospital, Osaka, Japan
2016-2017 Specially appointed Assistant Professor, Department of Pathology (Toru Nakano Lab.), Osaka University Graduate School of Medicine, Osaka, Japan
2017-2019 Postdoctral Research fellow, Institute for Cell Engineering, Johns Hopkins University School of Medicine (Ted M Dawson Lab.), Baltimore, MD, USA
2019-2020 Senior head physician of Department of Neurology, Higashiosaka city medical center, Osaka, Japan
2020 Specially appointed Assistant Professor, Department of Neurology, Osaka University Graduate School of Medicine, Osaka, Japan
2021-Current Assistant Professor, Department of Neurology, Osaka University Graduate School of Medicine, Osaka, Japan

Recent Related Publications (5 Papers)

1. Hideshima M, Kimura Y, Aguirre C, et al. Two-step screening method to identify α-synuclein aggregation inhibitors for Parkinson's disease. Sci rep 2022; 12: 351
2. Maeda T, Kimura Y, Nakano T, Yamaguchi S. Ribosomal stress induces 2-cell embryo-like state transition of the mouse ESCs through p53 activation. Biochem Biophys Res Commun 2021; 579: 175-180
3. Choong CJ, Kimura Y, Mochizuki H. Disturbed mitochondria-lysosome crosstalk in GBA1-associated Parkinson's disease. Mov Disord 2021; 36: 1792
4. Zhang S, Liu YQ, Jia C, et al. Mechanistic basis for receptor-mediated pathological α-synuclein fibril cell-to-cell transmission in Parkinson's disease. Proc Nat Acad Sci USA 2021; 118: e2011196118
5. Kimura Y, Shofuda T, Higuchi Y, et al. Human genomic safe harbors and the suicide gene-based safeguard system for iPSC-based cell therapy. Stem Cells Transl Med 2019; 8: 627-638

Oligonucleotide therapeutics for intractable neurological disorders: Recent Advances

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Oligonucleotide therapeutics are an emerging new class of therapeutics that demonstrate potential to treat various intractable diseases. While the capacity of antisense oligonucleotides (ASOs) to suppress gene and protein expression was confirmed in the 1970s, recent years have witnessed remarkable advances in basic science and translation into the clinic. In the last six years, a number of nucleic acid drugs have been approved for hard-to-treat diseases including neurological disorders.

Based on the structures, targets, and mechanisms of action, oligonucleotide drugs can be divided into categories including ASO, siRNA, miRNA mimic, aptamers, decoy and CpG-oligodeoxynucleotides. Oligonucleotide therapeutics have a wide range of potential applications in the clinic owing to their highly different modes of action such as antisense, ligands and protein inhibitors. In the field of neurology, ASOs that modulate splicing of *SMN2* and *Dystrophin* pre-mRNA were approved for spinal muscular atrophy and Duchenne muscular dystrophy, respectively. siRNA encapsulated in lipid nanoparticle that degrades *TTR* mRNA was also available for hereditary ATTR amyloidosis. Many others are being developed worldwide, targeting neurological disorders caused by gene mutations and neurodegenerative diseases such as Huntington's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), multiple system atrophy (MSA) and Alzheimer's disease. We have been developing ASO suppressing *SNCA* expression as a potential therapy for MSA and PD. Beyond pre-clinical trials, several clinical trials of oligonucleotide therapeutics are ongoing for neurological diseases, whereas phase III trials of tominersen (for Huntington's disease) and tofersen (for ALS) closed due to failing to attain their primary efficacy endpoints in 2021.

In addition to remarkable advances in basic science and development of oligonucleotides themselves, recent years have witnessed marked development in drug delivery systems and technologies. Notably, recent innovation starts to open up a new avenue of applications in which the central nervous system protected by the intact blood-brain barrier can be targeted by systemic administration of ligand-conjugated oligonucleotides.

In this symposium, we will review the recent state of oligonucleotide therapeutics for neurological diseases including basic science, approved drugs and clinical trials.

CURRICULUM VITAE

Name Tojo Nakayama

Affiliation Harvard Medical School, Boston Children's Hospital, MA, USA
Tokyo Medical and Dental University, Tokyo, Japan

Field of Research Antisense oligonucleotide therapy for *KCNT1* epileptic encephalopathy
Identification of novel genes associated with amino acid metabolism for microcephaly
Characterizing *SCN1A* genetics in Dravet syndrome
Characterizing *RFX* gene family members in autism and ADHD

Education

1996-2002	Tohoku University School of Medicine, Sendai, Japan
2007-2011	Tohoku University Graduate School of Medicine, Sendai, Japan

Professional Experience

2002-2004	Resident, Pediatrics, Sendai City Hospital; Sendai, Japan
2004-2005	Resident, Neonatology, Miyagi Children's Hospital, Sendai, Japan
2005-2007	Resident, Pediatric Neurology, National Center for Neurology and Psychiatry, Tokyo, Japan
2007-2010	Trainee, RIKEN Brain Science Institute, Saitama, Japan
2011-2012	Clinical Fellow, Pediatrics, Tohoku University Hospital, Sendai, Japan
2012-2014	Assistant Professor, Pediatrics, Tohoku University Hospital, Sendai, Japan
2011-2012	Clinical Fellow, Pediatrics, Tohoku University Hospital, Sendai, Japan
2014-2014	Research Fellow, Genetics and Genomics, Harvard Medical School Boston Children's Hospital, MA, USA
2022-present	Associate Professor, Neurology and Neurological Science, Tokyo Medical and Dental University, Tokyo, Japan
2022-present	Visiting Researcher, Genetics and Genomics, Harvard Medical School Boston Children's Hospital, MA, USA

Recent Related Publications (5 Papers)

1. Harris, HK*, Nakayama T*, Lai, J*, et al. Disruption of RFX family transcription factors causes autism, attention-deficit/hyperactivity disorder, intellectual disability, and dysregulated behavior. ***Genetics in Medicine.*** 2021;23(6):1028-1040. (*these authors contributed equally to this work)
2. Nakayama T*, Wu J*, Galvin-Parton P, Weiss J, Andriola MR, Hill RS, Vaughan D, El-Quessny M, Barry BJ, Partlow JN, Barkovich AJ, Ling J, Mochida GH. Deficient activity of alanyl-tRNA synthetase underlies an autosomal recessive syndrome of progressive microcephaly, hypomyelination, and epileptic encephalopathy. ***Hum Mutat.*** 2017;38(10):1348-1354. (*these authors contributed equally to this work)
3. Ouyang Q*, Nakayama T*, Baytas O, Davidson SM, Yang C, Schmidt M, Lizarraga SB, Mishra S, Ei-Quessny M, Niaz S, Gul Butt M, Imran Murtaza S, Javed A, Chaudhry HR, Vaughan DJ, Hill RS, Partlow JN, Yoo SY, Lam AT, Nasir R, Al-Saffar M, Barkovich AJ, Schwede M, Nagpal S, Rajab A, DeBerardinis RJ, Housman DE, Mochida GH, Morrow EM. Mutations in mitochondrial enzyme *GPT2* cause metabolic dysfunction and neurological disease with developmental and progressive features. ***Proc Natl Acad Sci USA.*** 2016;113(38):E5598-607. (*these authors contributed equally to this work) 4.
4. Nakayama T*, Al-Maawali A*, El-Quessny M, Rajab A, Khalil S, Stoler JM, Tan WH, Nasir R, Schmitz-Abe K, Hill RS, Partlow JN, Al-Saffar M, Servattalab S, LaCoursiere CM, Tambunan DE, Coulter ME, Elhosary PC, Gorski G, Barkovich AJ, Markianos K, Poduri A, Mochida GH. Mutations in *PYCR2*, encoding pyrroline-5-carboxylate reductase 2, cause microcephaly and hypomyelination. ***Am J Hum Genet.*** 2015 May 7;96(5):709-19. (*these authors contributed equally to this work)
5. Nakayama T*, Ogiwara I*, Ito K, Kaneda M, Mazaki E, Osaka H, Ohtani H, Inoue Y, Fujiwara T, Uematsu M, Haginoya K, Tsuchiya S, Yamakawa K. Deletions of *SCN1A* 5' genomic region with promoter activity in Dravet syndrome. ***Hum Mutat.*** 2010 Jul;31(7):820-9. (*these authors contributed equally to this work)

Individualized medicine with antisense oligonucleotides for rare neurological disorders

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Rare diseases are defined as those affecting less than <50,000 individuals in Japan. Despite their Name, they are not rare: in aggregate, rare diseases affect ~7 million individuals in Japan, half of whom are children. Only ~5% of these conditions have any treatment; thirty percent of children affected with a rare disease will not survive beyond the age of four. Finding new and faster ways of developing drugs for patients with rare disease has been long recognized as a critical national priority.

Individualized medicine leverages the patient's genome to design therapies that result in improved outcomes. The relatively mature technology behind antisense oligonucleotides (ASOs) allows for truly personalized therapy but requires coordination between families, health care professionals, industry, and regulatory bodies.

Our work began with a pioneering effort in 2017, in which we developed and manufactured a genetically targeted, patient-customized medicine for a young girl with Batten Disease, a fatal neurodegenerative disorder, and began treating her with it, all in just one year's time. This work was published in 2019 and has been the subject of extensive media coverage worldwide.

Since this initial effort, our team has turned to developing additional genetic medicines for a host of rare diseases. Three of these efforts are in active clinical trials, and several more are progressing towards launch in the next one or two years. In these projects, we have developed or are developing first-of-breed medicines for several genetic disorders: Batten Disease, Ataxia Telangiectasia, *KCNT1* infantile epilepsy, and several others.

In this session, we will discuss early forays in the application of ASOs as individualized medicine. Through these projects, we are pioneering novel approaches to the rapid development of treatments for rare diseases, an area of research that substantially contributes to the welfare throughout the world. Our ongoing projects will advance our practical knowledge of gene therapeutics for ultimately broader fields of rare neurological diseases.

Symposium 1

Abstract & Curriculum Vitae

Ocular gene and cell therapy

CURRICULUM VITAE

Name Yoshinori Oie

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Field of Research Ophthalmology, cornea, tissue engineering, Fuchs endothelial corneal dystrophy

Education

-2001 graduated from Osaka University Medical School
-2010 finished Osaka University Graduate School of Medicine, Major in phthalmology

Professional Experience

2001-2003	Resident, Department of Ophthalmology, Osaka University Hospital
2003-2006	Clinical fellow, Department of Ophthalmology, Osaka Rosai Hospital
2006-2010	Graduate school student, Osaka University Graduate School of Medicine.
2010-2011	Clinical fellow, Department of Ophthalmology, Tohoku Univeristy Hospital
2011-2013	Clinical fellow, Department of Ophthalmology, Osaka Univeristy Hospital
2013-present	Assistant professor, Department of Ophthalmology, Osaka University Graduate School of Medicine

Regenerative medicine for corneal epithelium

Yoshinori Oie, Kohji Nishida

Osaka University

Recently, a novel therapeutics utilizing tissue engineering and stem cell technology, called regenerative medicine, has been emphasized. Complete loss of corneal epithelial stem cells because of severe eye disease leads to limbal stem cell deficiency (LSCD) that causes corneal vascularization and opacification with severe visual loss. We have developed a unique method using tissue-engineered epithelial cell sheets. Ocular surface reconstruction using autologous epithelial cell sheets has drastically changed the treatment of LSCD because it can prevent potential problems associated with limbal transplantation, including immune rejection and donor tissue shortages. We conducted clinical trials using cultivated limbal and oral mucosal epithelial cell sheets, and confirmed the efficacy and safety. They are now approved as Cellular and Tissue-Based Products called “Nepic” and “Ocural” under Pharmaceutical and Medical Device Act (PMD Act). Moreover, we are conducting clinical study using induced pluripotent stem cell derived corneal epithelium.

CURRICULUM VITAE

Name Michiko Mandai

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Field of Research ophthalmology, regenerative medicine

Education

1988	MD, Faculty of Medicine, Kyoto University, Japan
1990-1994	PhD, Graduate School of Medicine, Kyoto University, Japan

Professional Experience

1988-1989	Resident, Ophthalmology, Kyoto University Hospital
1989-1990	Resident, Ophthalmology, Kansai Electric Power Hospital
1994-2000	Assistant professor, Ophthalmology, Kyoto University
2000-2002	Visiting researcher, NIH, Bethesda, MD, USA
2002-2006	Assistant professor, Translational Research Center, Kyoto University Hospital
2006-2012	Researcher, Laboratory for Retinal Regeneration, RIKEN Center for Developmental Biology
2006-2017	Medical Doctor (macula specialist), Kobe City Medical Center General Hospital
2013-2022	Deputy Project Leader, Laboratory for Retinal Regeneration, RIKEN Center for Biosystems Dynamics Research
2017-	Medical Doctor (macula specialist), Kobe City Eye Hospital
2022-	Director, Research Center, Kobe City Eye Hospital

Recent Related Publications (5 Papers)

Yamasaki S, Tu H-Y, Matsuyama T, Horiuchi M, Hashiguchi T, Sho J, Kuwahara A, Kishino A, Kimura T, Takahashi M, Mandai M. A Genetic modification that reduces ON-bipolar cells in hESC-derived retinas enhances functional integration after transplantation. iScience. 25 (1):103657, 2022

Matsuyama T, Tu HY, Sun J, Hashiguchi T, Akiba R, Sho J, Fujii M, Onishi A, Takahashi M, Mandai M. Genetically engineered stem cell-derived retinal grafts for improved retinal reconstruction after transplantation. iScience, 2021, 24(8):102866

Yamasaki S, Sugita S, Horiuchi M, Masuda T, Fujii S, Makabe K, Kawasaki A, Hayashi T, Kuwahara A, Kishino A, Kimura T, Takahashi M, Mandai M. Low Immunogenicity and Immunosuppressive Properties of Human ESC- and iPSC-Derived Retinas. Stem Cell Reports. 16(4):851-867, 2021

Iraha S, Tu HY, Yamasaki S, Kagawa T, Goto M, Takahashi R, Watanabe T, Sugita S, Yonemura S, Sunagawa GA, Matsuyama T, Fujii M, Kuwahara A, Kishino A, Koide N, Eiraku M, Tanihara H, Takahashi M, Mandai M. Establishment of Immunodeficient Retinal Degeneration Model Mice and Functional Maturation of Human ESC-Derived Retinal Sheets after Transplantation. Stem Cell Reports, 2018, 10(3):1059-1074

Mandai M, Fujii M, Hashiguchi T, Sunagawa GA, Ito S, Sun J, Kaneko J, Sho J, Yamada C, Takahashi M. iPSC-derived retina transplants improve vision in rd1 end-stage retinal degeneration mice Stem Cell Reports.8: 69–83, 2017

Regenerative therapy using ESC/iPSC-derived retinas for retinal degeneration

Michiko Mandai

Kobe City Eye Hospital

Retinitis pigmentosa is a group of hereditary diseases in which rod photoreceptors are progressively degenerate, followed by a subsequent loss of cone photoreceptors. No substantial therapy for the disease is established and the number of disease-causing genes identified so far exceeds 100. Our challenge is to develop cell therapy utilizing ESC/iPSC-derived retinal tissues. We used animal models with end-stage retinal degeneration to obtain proof of concept data that included 1) maturation of photoreceptors in transplanted ESC/iPSC-retinas, 2) synaptic formation between host bipolar cells and graft photoreceptors, 3) restoration of light responses in the host retinal ganglion cells in the transplanted area as recorded by ex-vivo multiple electrode array system, and 4) a light perception by behavior test after transplantation. Based on these, we started a clinical study at Kobe City Eye Hospital in 2020 to test the safety and feasibility of the treatment, and two patients underwent transplantation surgery. The graft survived well in the long-standing disease environment of human retinitis pigmentosa, and there was no sign of rejection or any adverse events so far. In order to further enhance the functional integration of retina grafts, we are also developing the genetically modified grafts where we delete graft bipolar cells or some inner cells from the graft while retaining the photoreceptor cells, horizontal cells, and Muller cells to support graft maturation and function. The photoreceptor cells in these grafts matured and made a better contact and more synapses with host bipolar cells than those in the wildtype grafts. These grafts also improved functional outcomes compared to the wildtype grafts. In clinical application, how to assess therapeutic outcomes is also important. We combine various visual functional tests for low vision, to see if it may help evaluate progress of the disease or therapeutic efficacy.

CURRICULUM VITAE

Name Koji Nishiguchi

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Field of Research Ophthalmology
Gene therapy

Education
2005 PhD in Medical Science, Nagoya University Graduate School of Medicine

Professional Experience
1999 Resident of Ophthalmology, Nagoya University Hospital
2000 Medical Fellow, Schepens Eye Research Institute, Harvard Medical School
2002 Research Fellow, Massachusetts Eye and Ear Infirmary, Harvard Medical School
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2011 Research Fellow, Medical Genetics, University of Lausanne
2012 Clinical Research Associate, Institute of Ophthalmology, University College London
2014 Associate Professor, Advanced Ophthalmic Medicine, Tohoku University
2020 Professor, Ophthalmology, Nagoya University

Recent Related Publications (5 Papers)
Nishiguchi KM, Miya F, Mori Y, Fujita K, Akiyama M, Kamatani T, Koyanagi Y, Sato K, Takigawa T, Ueno S, Tsugita M, Kunikata H, Cisarova K, Nishino J, Murakami A, Abe T, Momozawa Y, Terasaki H, Wada Y, Sonoda KH, Rivolta C, Tsunoda T, Tsujikawa M, Ikeda Y, Nakazawa T. A hypomorphic variant in EYS detected by genome-wide association study contributes toward retinitis pigmentosa. ***Commun Biol***. 2021;4(1):140. doi: 10.1038/s42003-021-01662-9.
Nishiguchi KM, Fujita K, Miya F, Katayama S, Nakazawa T. Single AAV-mediated mutation replacement genome editing in limited number of photoreceptors restores vision in mice. ***Nat Commun.*** 2020;11(1):482. doi: 10.1038/s41467-019-14181-3.
Koyanagi Y, Akiyama M, Nishiguchi KM, Momozawa Y, Kamatani Y, Takata S, Inai C, Iwasaki Y, Kumano M, Murakami Y, Omodaka K, Abe T, Komori S, Gao D, Hirakata T, Kurata K, Hosono K, Ueno S, Hotta Y, Murakami A, Terasaki H, Wada Y, Nakazawa T, Ishibashi T, Ikeda Y, Kubo M, Sonoda KH.Genetic characteristics of retinitis pigmentosa in 1204 Japanese patients. ***J Med Genet.*** 2019;56(10):662-670.
Nishiguchi KM, Fujita K, Tokashiki N, Komamura H, Takemoto-Kimura S, Okuno H, Bito H, Nakazawa T. Retained Plasticity and Substantial Recovery of Rod-Mediated Visual Acuity at the Visual Cortex in Blind Adult Mice with Retinal Dystrophy. ***Mol Ther***. 2018;26(10):2397-2406. doi: 10.1016/j.ymthe.2018.07.012.
Nishiguchi KM, Carvalho LS, Rizzi M, Powell K, Holthaus SM, Azam SA, Duran Y, Ribeiro J, Luhmann UF, Bainbridge JW, Smith AJ, Ali RR. Gene therapy restores vision in *rd1* mice after removal of a confounding mutation in ***Nat Commun***. 2015;6:6006.

Genome editing gene therapy for retinitis pigmentosa

Koji Nishiguchi

Nagoya University

Retinitis pigmentosa (RP) is an inherited retinal degeneration with no effective treatment. Recently, the efficacy of adeno-associated virus (AAV)-mediated gene supplementation therapy in which wildtype copies of a defective gene were delivered to the diseased cells has been demonstrated. However, relatively small cargo limit of AAV restricts the application of the therapy to only up to a fraction of Japanese patients with RP. Therefore, devising gene therapy approach other than gene supplementation is important. Genome editing can treat mutations locally regardless of the size of the gene involved, thus harbors the potential to complement the current approach.
Herein, the development of a versatile single AAV-mediated mutation replacement therapy that have been shown to be equally effective as gene supplementation therapy in restoring the visual function in an animal model of RP is described. Furthermore, with the final goal of clinical application, the development of a genome editing therapeutic vector targeting the founder mutation S1653Kfs in EYS gene that is exceedingly frequent in Japanese RP patients, carried by more than 10% of them, is outlined.

CURRICULUM VITAE

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Field of Research Retina, Gene Therapy

Education

1992 M.Ag., Plant Pathology, Kyoto Prefectural University, Japan
1998 Ph.D., Tohoku University Graduate school of Medicine, Japan

Professional Experience

1997-2002 Assistant professor, Department of Ophthalmology, Tohoku University School of Medicine, Japan
2002-2004 post-doctoral fellowship in Department of Ophthalmology, Dean A. McGee Eye Institute, Prof. Robert Eugene Anderson Lab. University of Oklahoma, USA
2004-2008 Associate Professor, Tohoku University Biomedical Engineering Research Organization, Japan
2008-2012 Associate Professor, Tohoku University Institute for International Advanced Interdisciplinary Research, Japan
2012- present Professor, Iwate University Department of Chemistry and Biological Sciences, Japan

Recent Related Publications (5 Papers)

Watanabe Y, Sugano E, Tabata K, Hatakeyama A, Sakajiri T, Fukuda T, Ozaki T, Suzuki T, Sayama T, Tomita H*, Development of an optogenetic gene sensitive to daylight and its implications in vision restoration, npj Regenerative Medicine, 6(1): 64. 2021

Tabata K, Sugano E, Hatakeyama A, Watanabe Y, Suzuki T, Ozaki T, Fukuda T, Tomita H*, Phototoxicities Caused by Continuous Light Exposure Were Not Induced in Retinal Ganglion Cells Transduced by an Optogenetic Gene, Int J Mol Sci, 22(13): 6732, 2021

Tomita H*, Sugano E, Optogenetics-mediated gene therapy for retinal diseases, Optogenetics 'Light-Sensing Proteins and Their Applications in Neuroscience and Beyond', Yawo H, Kandori H, Koizumi A, Kageyama R, eds. Advances in Experimental Medicine and Biology;1293:535-543. 2021

Sugano E, Endo Y, Sugai A, Kikuchi Y, Tabata K, Ozaki T, Kurose T, Takai Y, Mitsuguchi Y, Honma Y, Tomita H*, Geranylgeranyl acetone prevents glutamate-induced cell death in HT-22 cells by increasing mitochondrial membrane potential, Eur J Pharmacol. 883:173193, 2020

Sakajiri Y, Sugano E, Watanabe Y Sakajiri T, Tabata K, Kikuchi T, Tomita H*, Natronomonas pharaonis halorhodopsin Ser81 plays a role in maintaining chloride ions near the Schiff base, Biochem Biophys Res Commun, 503(4), 2326-2332, 2018

Watanabe Y, Sugano E, Tabata K, Ozaki T, Saito T, Tamai M, Tomita H*, Kinetic profiles of photocurrents in cells expressing two types of channelrhodopsin genes, Biochem Biophys Res Commun, 496(3), 814-819, 2018

Gene therapy using an optogenetic gene for restoring vision

Hiroshi Tomita, Akito Hatakeyama, Kitako Tabata, Tatsuki Sayama, Kazuya Kitabayashi, Reina Onoguchi, Eriko Sugano

Iwate University

Channelrhodopsin-2 (ChR2) derived from the green algae, Chlamydomonas, functions as a light-gated cation channel, which is utilized for optogenetic mediated research in neuroscience. In the field of ophthalmology, ChR2 has been developed as gene therapy to restore vision for patients with blindness, and a clinical trial has been performed in the U.S since 2016. Recently, the clinical trial of another channelrhodopsin gene, ChrimsonR, that has the sensitivity to the red spectrum has been performed and the recovery of vision has been partially demonstrated. We have developed the new channelrhodopsin gene that has the sensitivity to the broad spectrum of visible light, mVChR1. However, it still has the problem that the light sensitivity is weaker than the native vision. Therefore, we have been making the effort to develop a higher sensitivity than these channelrhodopsins. Recently, we succeeded to develop ComV1 that has the sensitivity to the daylight by the modification of mVChR1.
In this symposium, Id like to introduce the visual function of the gene therapy using the ComV1 gene in the animal model of retinitis pigmentosa and the limitation of recovered visual functions.

CURRICULUM VITAE

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Graduate Shool of Medical Sciences, Kyushu University

Field of Research Retinal gene therapy, inherited retinal diseases, neuroinflammation, nano-particle medicine

Education

1997-2003 M.D., Faculty of Medicine, Kyushu University
2005-2009 Ph.D., Department of Pathology, Graduate School of Medical Sciences, Kyushu University.

Professional Experience

2003-2005 Residency in Ophthalmology, Kyushu University Hospital
2009-2012 Research fellow, Angiogenesis Laboratory, Massachusetts Eye and Ear Infirmary, Harvard Medical School.
2012-2014 Retina fellow, Department of Ophthalmology, Kyushu University Hospital.
2015-2019 Assistant Professor in Ophthalmology, Graduate School of Medical Sciences, Kyushu University
2019-Present Lecturer in Ophthalmology, Graduate School of Medical Sciences, Kyushu University

Recent Related Publications (5 Papers)

1. Funatsu J, Murakami Y (Corresponding Author), Shimokawa S, Nakatake S, Fujiwara K, Okita A, Fukushima M, Shibata K, Yoshida N, Koyanagi Y, Akiyama M, Notomi S, Hisatomi T, Takeda A, Paschalis EI, Vavvas DG, Ikeda Y, Sonoda KH. Circulating inflammatory monocytes oppose microglia and contribute to cone cell death in retinitis pigmentosa. ***PNAS Nexus***. 2022 (in press)
2. Murakami Y (Corresponding Author), Koyanagi Y, Fukushima M, Yoshimura M, Fujiwara K, Akiyama M, Momozawa Y, Ueno S, Terasaki H, Oishi A, Miyata M, Ikeda H, Tsujikawa A, Mizobuchi K, Hayashi T, Fujinami K, Tsunoda K, Park JY, Han J, Kim M, Lee CS, Kim SJ, Park TK, Joo K, Woo SJ, Ikeda Y, Sonoda KH. Genotype and Long-term Clinical Course of Bietti Crystalline Dystrophy in Korean and Japanese Patients. ***Ophthalmology Retina***. 2021; S2468-6530.
3. Murakami Y, Ishikawa K, Nakao S, Sonoda KH. Innate immune response in retinal homeostasis and inflammatory disorders. ***Prog Retin Eye Res***. 2020; 74: 100778.
4. Okita A, Murakami Y (Corresponding Author), Shimokawa S, Funatsu J, Fujiwara K, Nakatake S, Koyanagi Y, Akiyama M, Takeda A, Hisatomi T, Ikeda Y, Sonoda KH. Changes of Serum Inflammatory Molecules and Their Relationships with Visual Function in Retinitis Pigmentosa. ***Invest Ophthalmol Vis Sci***. 2020; 61: 30.
5. Nakatake S, Murakami Y (Corresponding Author), Ikeda Y, Morioka N, Tachibana T, Fujiwara K, Yoshida N, Notomi S, Hisatomi T, Yoshida S, Ishibashi T, Nakabeppu Y, Sonoda KH. MUTYH promotes oxidative microglial activation and inherited retinal degeneration. ***JCI Insight***. 2016; 1: e87781

Retinal gene therapy using anti-angiogenic and neuroprotective factors

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Retinal gene therapy using adeno-associated viral (AAV) or lentiviral vectors can mediate sustained transgene expression in the diseased loci, and serves as a promising strategy for chronically progressive retinal diseases. Following the success of AAV gene therapy to supplement normal *RPE65* gene for patients with Leber's congenital amaurosis, a number of clinical trials have been conducted worldwide to achieve the clinical POC for inherited retinal diseases. In addition to these strategies for mendelian diseases, retinal gene therapy can be applicable to more general conditions by delivering therapeutic genes to combat key disease pathologies. For example, recent clinical trials have shown that AAV-mediated sustained expression of anti-VEGF molecules efficiently suppressed the choroidal neovascularization in patients with age-related macular degeneration and reduce the treatment burden of multiple intravitreal injection. Our group have conducted a phase 1/2a clinical trial of neuroprotective lentiviral gene therapy that oxerexpress pigment epithelium-derived factor for patients with retinitis pigmentosa, a progressive retinal degenerative disease that leads to blindness. In this symposium, I will summarize the recent advances of retinal gene therapy, especially focusing on the strategies using therapeutic factors, that will substantially change the clinical practice in the next decade.

Symposium 2

Abstract & Curriculum Vitae

Gene Editing & Mitochondrial Manipulation

CURRICULUM VITAE

Name Beverly Y. Mok

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Field of Research Genome editing, Protein Engineering, Directed Evolution

Education

2015 B.A., University of Cambridge
2021 Ph.D (Chemistry and Chemical Biology), Harvard University

Professional Experience

2016-2021 Graduate student, Harvard University
2022- Present Postdoctoral Research, Institute of Molecule and Cell Biology (A*STAR), Singapore

Recent Related Publications (5 Papers)

1. **Mok, B. Y**., Raguram, A., Huang, T.P., Liu, D.R. CRISPR-free base editors with enhanced activity and expanded targeting scope in mitochondrial and nuclear DNA. *Nature Biotechnology,* doi: 10.1038/s41587-022-01256-8 (2022)
2. Koblan, L. W.*, Arbab, M.*, Shen, M.W.*, Hussmann, J.A., Anzalone, A.V., Doman, J.L., Newby, G.A., Yang, D., **Mok, B**., Replogle, J.M., Xu, A., Sisley, T.A., Weissman, J.S., Adamson, B., Liu, D.R. Development of C • G-to-G • C transversion base editors from CRISPRi screens, target- library analysis and machine learning. *Nature Biotechnology* **39***,* 1414-1425 (2021).
3. **Mok, B. Y**.*, de Moraes M.H.*, Zeng, J., Bosch, D.E., Kotrys, A.V., Raguram, A., Hsu, F., Radey, M.C., Peterson S.B., Mootha, V.K., Mougous, J.D., Liu, D.R. A bacterial cytidine deaminase toxin enables CRISPR-free mitochondrial base editing. *Nature* **583**, 631-637, (2020).
4. Arbab, M., Shen M.W., **Mok, B**., Wilson, C., Matuszek, Ż., Cassa, C.A., Liu, D.R. Determinants of Base Editing Outcomes from Target Library Analysis and Machine Learning. *Cell* **182**, 463-489 e430, (2020)
5. Maji, B., Gangopadhyay, S.A., Lee, M., Shi, M., Wu, P., Heler, R., **Mok, B**., Lim, D., Siriwardena, S.U., Paul, B., Dancik, V., Vetere, A., Mesleh, M.F., Marraffini, L.A., Liu, D.R., Clemons, P.A., Wagner, B.K., Choudhary, A. A High-Throughput Platform to Identify Small-Molecule Inhibitors of CRISPR-Cas9. *Cell* **177**, 1067-1079 e1019, (2019)

CRISPR-free Base Editor for Precise Editing of Mitochondrial and Nuclear DNA

Beverly Y. Mok[1,2,3]

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Programmable DNA nucleases result in targeted destruction of mitochondrial DNA (mtDNA) copies but cannot introduce specified sequence changes. Owing to the challenge of importing guide RNAs into the mitochondria, CRISPR-based systems have not been used reliably to install precise nucleotide changes in target mtDNA. To begin to address this challenge, we recently developed an all-protein base editor (DdCBE) to enable targeted C•G-to-T•A conversions within mtDNA and nuclear DNA. DdCBE contains an interbacterial toxin (DddA) that catalyzes the deamination of cytidines within dsDNA. We engineered split-DddA halves that are non-toxic and inactive until brought together on target DNA. Each TALE is fused to a non-toxic half of DddA and one copy of uracil glycosylase inhibitor (UGI) protein. Binding of two TALE fused split-DddA–UGI fusions to adjacent sites promotes reassembly of functional DddA for deamination of target cytosines within the dsDNA spacing region.

In our initial studies, we observed a range of mtDNA editing efficiencies (4.6–49%) depending on the position of the target C within the spacing region between the DNA-bound DdCBE halves. To improve editing efficiency and overcome the strict T<u>C</u> sequence-context constraint of DddA, we used phage-assisted non-continuous and continuous evolution to evolve DddA variants with improved activity and expanded targeting scope. Compared to canonical DdCBEs, base editors with evolved DddA6 improved mitochondrial DNA (mtDNA) editing efficiencies at T<u>C</u> by 3.3-fold on average. DdCBEs containing evolved DddA11 offered a broadened H<u>C</u> (H = A, C or T) sequence compatibility for both mitochondrial and nuclear base editing, increasing average editing efficiencies at A<u>C</u> and C<u>C</u> targets from less than 10% for canonical DdCBE to 15–30% and up to 50% in cell populations sorted to express both halves of DdCBE. We used these evolved DdCBEs to efficiently install disease-associated mtDNA mutations in human cells at non-T<u>C</u> target sites. DddA6 and DddA11 substantially increase the effectiveness and applicability of all-protein base editing.

CURRICULUM VITAE

Name Yuma Yamada

Affiliation Faculty of Pharmaceutical Sciences, Hokkaido University
FOREST Program, Japan Science and Technology Agencya

Field of Research Drug delivery system, Nanotechnology,
Mitochondria biology, Gene therapy, Cell therapy

Education

2003.3 Completion of Bachelor degree, School of Pharmaceutical Sciences and Pharmacy, Hokkaido University, Japan.
2005.3 Completion of Master degree, Graduate School of Pharmaceutical Sciences, Hokkaido University, Japan.
2005.4- Entrance into the Ph. D course, Graduate School of Pharmaceutical Sciences, Hokkaido University, Japan.
2008. 9. Ph.D. in Pharmaceutical Sciences, Hokkaido University, Japan.

Professional Experience

2007.1- Instructor, Faculty of Pharmaceutical Sciences, Hokkaido University, Japan.
2008.10- Assistant professor, Faculty of Pharmaceutical Sciences, Hokkaido University, Japan.
2009.4- present Pharmacist in the Department of Pharmacy, Hokkaido University Hospital, Japan.
2016.4- present Associate professor, Faculty of Pharmaceutical Sciences, Hokkaido University, Japan.
2019.4- present Scientific Adviser, LUCA Science Inc., Japan.
2020.4- present Principal Investigator, Hokkaido University's Industry Creation Laboratories (Laboratory for Biological Drug Development based on DSS Technology), Japan.
2021.4- present FOREST program researcher, Japan Science and Technology Agency, Japan.

Recent Related Publications (5 Papers)

¶equal contribution, *Corresponding author

1. Sasaki D, Abe J, Takeda A, Harashima H, Yamada Y¶, *, Transplantation of MITO cells, mitochondria activated cardiac progenitor cells, to the ischemic myocardium of mouse enhances the therapeutic effect. *Rep.* 12: 4344 (2022). *International co-authored paper, Research Press Release*
2. Satrialdi, Takano Y*, Hirata E, Ushijima N, Harashima H, Yamada Y*, The effective in vivo mitochondrial-targeting nanocarrier combined with a π-extended porphyrin-type photosensitizer. *Nanoscale Advances* 3: 5919-5927 (2021). *International co-authored paper, Research Press Release*
3. Kawamura E¶, Maruyama M, Abe J, Sudo A, Takeda A, Takada S, Yokota T, Kinugawa S, Harashima H, Yamada Y¶;*, Validation of gene therapy for mutant mitochondria by delivering mitochondrial RNA using a MITO-Porter, a liposome-based nano device. *Ther. – Nuceic Acids* 20: 687-698 (2020). *Independent clinical research*
4. Yamada Y*, Somiya K, Miyauchi A, Osaka H, Harashima H*, Validation of a mitochondrial RNA therapeutic strategy using fibroblasts from a Leigh syndrome patient with a mutation in the mitochondrial ND3 gene. *Rep*. 10: 7511 (2020). *Independent clinical research*
5. Yamada Y*, Satrialdi, Hibino M, Sasaki D, Jiro A, Harashima H. Power of mitochondrial drug delivery systems to produce innovative nanomedicines. *Drug. Deliv. Rev.* 154-155: 187-209 (2020). *International co-authored paper*

Challenge to gene-cell therapy based on mitochondrial DDS

Yuma Yamada[1,2], Hideyoshi Harashima[1]

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[2] FOREST Program, Japan Science and Technology Agency

Drug delivery systems (DDS), which deliver target molecules to mitochondria with various functions, are expected to make a significant contribution to medicine and life sciences. Although several mitochondria-targeted DDSs have been reported so far, mitochondrial delivery of therapeutic exogenous proteins and nucleic acids has been difficult due to limitations in the physical properties and size of the cargoes. Focusing on the fact that mitochondria actively repeat fusion and fission and share biomolecules with each other inside cells, we designed MITO-Porter, a nano capsule that can fuse mitochondrial membranes. This strategy *via* membrane fusion does not limit the physical properties or size of the cargoes. To realize this strategy, the lipid membrane composition was explored and mitochondrial highly fusible lipid based nano capsule (MITO-Porter) were identified and successfully delivered macromolecules to mitochondria [*Y. Yamada, et al, Biochim. Biophy. Acta 1778: 423-432, 2008; Y. Yamada, et al, Adv. Drug Deliv. Rev. 154-155: 187-209, 2020*]. Currently, based on the nanotechnology for mitochondrial DDS established in the MITO-Porter development study, we are expanding collaborative research targeting mitochondria with researchers and clinicians from various fields. This presentation will focus on our latest research results on "Mitochondria targeted gene therapy" [*E. Kawamura, et al, Mol. Ther. – Nuceic Acids. 20: 687-698, 2020*] and "Cell transplantation therapy" [*D. Sasaki, et al, Sci. Rep. 12: 4344, 2022*]. Moreover, I would like to discuss "Gene Editing & Mitochondrial Manipulation", the theme of this symposium.

CURRICULUM VITAE

Name Ken-Ichi Wada

Affiliation Kyushu University
RIKEN

Field of Research Cell Biology, Molecular Biology, Microfluidics

Education

1991-1996 College of Biological Science, University of Tsukuba
1996-2002 Doctoral Program in Health and Sport Sciences, University of Tsukuba

Professional Experience

2002-2007 Biomaterials Center, National Institute for Materials Science (NIMS)
2008-2011 Center for Developmental Biology (CDB), RIKEN
2011-2019 Bioengineering Laboratory/Nano Medical Engineering Laboratory, RIKEN
2019-present R&D Laboratory for Innovative Biotherapeutics, Kyushu University

Recent Related Publications (5 Papers)

Ken-Ichi Wada, Kazuo Hosokawa, Yoshihiro Ito, Mizuo Maeda. "A microfluidic device for modulation of organellar heterogeneity in live single cells", ***Anal Sci 37: 499-505, 2021***
Ken-Ichi Wada, Kazuo Hosokawa, Yoshihiro Ito, Mizuo Maeda. "Quantitative control of mitochondria transfer between live single cells using a microfluidic device", ***Biol Open 6: 1960-1965, 2017***
Ken-Ichi Wada, Kazuo Hosokawa, Yoshihiro Ito, Mizuo Maeda. "Effects of ROCK inhibitor Y-27632 on cell fusion through a microslit", ***Biotechnol Bioeng 112: 2334-2342, 2015***
Ken-Ichi Wada, Kazuo Hosokawa, Eitaro Kondo, Yoshihiro Ito, Mizuo Maeda. "Cell fusion through a microslit between adhered cells and observation of their nuclear behavior". ***Biotechnol Bioeng 111: 1464-1468, 2014***
Ken-Ichi Wada, Kazuyoshi Itoga, Teruo Okano, Shigenobu Yonemura, Hiroshi Sasaki. "Hippo pathway regulation by cell morphology and stress fibers". ***Development 138: 3907-3914, 2011***

Mitochondrial genome manipulation using a microfluidic device

Ken-Ichi Wada[1,2], Kazuo Hosokawa[2], Yoshihiro Ito[2], Mizuo Maeda[2], Yui Harada[1], Yoshikazu Yonemitsu[1]

[1] Kyushu University
[2] RIKEN

Mitochondria have their own genome (mitochondrial DNA: mtDNA) which is essential for mitodhondrial functions. mtDNA is a small circular DNA of 16.5 kbp in size, encodes 13 oxidative phosphorylation-related proteins, 22 tRNAs and 2 rRNAs for their translation, and several thousands copies exist in a cell. To date, many studies have shown that various mutations on mtDNA cause mitochondrial dysfunctions and are related with wide ranged pathological abnormalities such as tumor metastasis, diabetes and neurological disorders as well as mitochondrial diseases. Therefore, genetic manipulation-based functional analyses on mtDNA contribute to development of therapeutic treatments for those disorders. However, the multicopy feature of mtDNA limits the analyses because dysfunctions of the mutated mtDNA are compensated by other mtDNA existing within the cell. Therefore, for genetic manipulation on mtDNA, introducing the same mutation(s) in all copies (i.e., achieving homoplasmy of mutated mtDNA) is required. In this symposium, I will talk about our recent activities toward developing a novel method for introducing homoplasmic mtDNA mutations using a microfluidic device.
Replacement of mtDNA by cytoplast (enucleated cell) fusion with a mtDNA-less (ρ^0) cell, or generation of a transmitochondrial cybrid, is one of few practical mtDNA manipulation methods. Since it has been reported that synaptosome (a very small cytoplast derived from neurons) fusion to ρ^0 cell permitted clonal propagation of a minor population of mtDNA variants due to the transfer of a small number of mitochondria, we speculated that generating transmitochondrial cybrids through single mitochondrion transfer from a mtDNA mutation-accumulated cell to a ρ^0 cell, or mitochondrial cloning, achieves homoplasmy of mutated mtDNA. Accordingly, we first developed a microfluidic device that can perform single mitochondrion transfer between live single cells. In this device, paired single cells were fused through a micro tunnel (2 μm in width, 10 μm in length) by Sendai virus envelope-based method to promote a strictured cytoplasmic connection between them. As a result, a few mitochondria, in some cases only one mitochondrion, moved to the fusion partner passing through the cytoplasmic connection, and subsequent recovery culture disconnected the fused cells to recover into single cells. These data suggest that our microfluidic device can perform single mitochondrion transfer between live single cells. Furthermore, we succeeded in generating transmitochondrial cybrids using our microfluidic device even under a pyruvate and uridine-supplemented condition. Based on these data, we conclude that our microfluidic device system has the potential to generate transmitochondrial cybrids harboring homoplasmic mtDNA mutations including mitochondrial respiratory dysfunctional ones. Therefore, our method provides a promising approach for mtDNA manipulation, and will be a useful experimental platform for mitochondrial genetics.

CURRICULUM VITAE

Name Tsukasa Ohmori

Affiliation Department of Biochemistry,
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Field of Research Gene Therapy, Thrombosis and Hemostasis, Genome editing

Education

1994 M.D., Jichi Medical University
2003 Ph.D., Yamanashi Medical University

Professional Experience

1994-1995	Resident in the Department of Internal Medicine, Yamanashi Prefectural Hospital
1996-1998	Department of Internal Medicine, Nanbu Clinic, Yamanashi, Japan
1999	Research Fellow, Department of Laboratory Medicine, Yamanashi Medical University
2000-2003	Department of Internal Medicine, Minobusan Hospital, Yamanashi, Japan
2004-2007	Research Associate, Research Division of Cell and Molecular Medicine, Center for Molecular Medicine, Jichi Medical University
2007-2015	Assistant Professor, Research Division of Cell and Molecular Medicine, Center for Molecular Medicine, Jichi Medical University
2015-2016	Associate Professor, Department of Biochemistry, Jichi Medical University School of Medicine
2017-present	Professor, Department of Biochemistry, Jichi Medical University School of Medicine

Recent Related Publications (5 Papers)

1) Reiss UM, Mahlangu J, Ohmori T, Ozalo MC, Srivastava A, Zhang L. Hemophilia gene therapy – Update on New country initiatives. Haemophilia (in press).
2) Hayakawa M, Sakata A, Hayakawa H, Matsumoto H, Hiramoto T, Kashiwakura Y, Baatartsogt N, Fukushima N, Sakata Y, Suzuki-Inoue K, and Ohmori T. Characterization and visualization of murine coagulation factor VIII-producing cells in vivo. Sci Rep 2021;11:14824. doi.org/10.1038/s41598-021-94307-0.
3) Baatartsogt N, Kashiwakura Y, Hayakawa M, Kamoshita N, Hiramoto T, Mizukami H, and Ohmori T. Development of a sensitive and reproducible cell-based assay via secNanoLuc to detect neutralizing antibody against adeno-associated virus vector capsid. Mol Ther Method and Clin Dev 2021;22:162-172.
4) Byambaa S, Uosaki H,Ohmori T, Hara H, Endo H, Nureki O, Hanazono Y. Non-viral *ex vivo* genome-editing in mouse bona fide hematopoietic stem cells with CRISPR/Cas9. Mol Ther Methods Clin Dev. 2021;20:451-462. doi:10.1016/j.omtm.2021.01.001.
5) Reiss UM, Zhang L, Ohmori T. Hemophilia gene therapy – New country initiatives. Haemophilia 2021 Feb;27 Suppl 3:132-141. doi: 10.1111/hae.14080.

The Potential of Genome Editing Treatment with an Engineered Cas

Tsukasa Ohmori[1], Takafumi Hiramoto[1], Tomoki Togashi[1,2], Yuji Kashiwakura[1], Nemekhbayar Baatartsogt[1], Morisada Hayakawa[1], Nobuhiko Kamoshita[1], Eriko Morishita[2], Osamu Nureki[3]

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[2] Department of Clinical Laboratory Science, Division of Health Sciences, Graduate School of Medical Science, Kanazawa University
[3] Department of Biological Sciences, Graduate School of Science, The University of Tokyo

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CRISPR-Cas9 system is a powerful technology for the treatment of genetic diseases. Recently, excellent therapeutic efficacies of genome editing targeting *TTR* locus for transthyretin amyloidosis and *BCL11A* locus for β-thalassemia and sickle cell disease were reported in human Phase I/II clinical trials. These genome editing treatments are based on non-homologous end joining (NHEJ) accompanied by double strand break (DSB), leading to the disruption of gene expression. We have previously developed a gene-editing approach for hemophilia B (coagulation factor IX mutation) using the CRISPR-Cas9 system in mice. We inserted cDNA for exons 2–8 into intron 1 of *F9* at the DSB site by Cas9 expressed by AAV vector.

The NHEJ-mediated disruption of a target gene or the insertion of an ectopic gene is a main strategy for the current genome editing treatment. However, DSBs by a DNA-cutting enzyme could generate large deletions and shuffle genes, which may result in possible genome toxicity. Therefore, an alternative approach to correct disease-specific mutations without DSBs would be advantageous.

Base editing is a genome-editing technology that can convert a specific DNA base into another at a targeted genome. Base editors are chimeric proteins that comprise a DNA-targeting module and a catalytic domain capable of deaminating a cytidine or adenine base (C to T or A to G). Cas9 nickase (that does not cause a DSB) could be used as a targeting module to bind specific DNA and deliver base editors. Although several studies have reported the treatment of disease-specific mutations using base editing, PAM sequence requirement by Cas9 (NGG for SpCas9 or NNGRRT for SaCas9) hinders its application in various mutations. Thus, a more flexible module is required to bind the target DNA to apply the base-editing approach.

To enable the base-editing treatment for various point mutations, we employed a base-editing based on SpCas9-NG, an engineered Cas9 with broad PAM flexibility (only "G" is required as the PAM sequence). We recently succeeded in the repair of the point mutations in induced pluripotent stem cells (iPSCs) derived from a severe hemophilia B patient. The base editor fused with SpCas9-NG, but not wild-type SpCas9, induced the C to T conversion at the mutation. The repair of the patient mutation in HEK293cells restored the coagulation factor IX production in the supernatant. In this symposium, we introduced the base editing approach to treat a point mutation with engineered Cas, and the recent development of genome editing treatment targeting various diseases in our laboratory.

CURRICULUM VITAE

Name Matthew Porteus

Affiliation Department of Pediatrics,
Stanford University School of Medicine

Field of Research

Education

Professional Experience

Recent Related Publications (5 Papers)

Advancing Homologous Recombination based Genome Editing of Hematopoietic Stem Cells

Matthew Porteus

Department of Pediatrics, Stanford University School of Medicine

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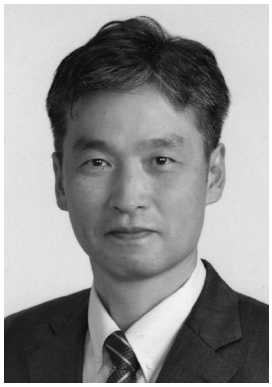
Genome editing by homologous recombination remains the most precise and versatile approach to genetically engineering cells. Hematopoietic stem cells (HSCs) have the biologic property of giving rise to multiple blood types for the lifetime of a patient (lifetime durability). We have developed a highly active ex vivo genome editing system that robustly stimulates targeted integration of genes at frequencies of >40% in HSCs. Moreover, we have recently developed improvements in the system such that the ratio of HDR to INDELs is markedly improved with ratios changing from less than 1 to greater than 10. We have applied these systems to both directly genetically correct pathologic mutations as well as to use the system to rewire the genome to create new biologic properties. In this talk, I will review some of the progress we have made on using homologous recombination to create HSC based cellular therapies.

Symposium 3

Abstract & Curriculum Vitae

Vector Development

CURRICULUM VITAE



Name Keiji Itaka

Affiliation Institute of Biomaterials and Bioengineering,
Tokyo Medical and Dental University (TMDU)

Field of Research mRNA medicine, mRNA vaccine, DDS, Biomaterials, gene therapy, regenerative medicine, orthopaedic surgery

Education

1991 Graduate from Department of Medicine, The University of Tokyo
2003 Graduate from Graduate School of Medicine, The University of Tokyo

Professional Experience

1991 M.D. (orthopaedic surgery)
1997 Assistant Professor, University or Tokyo Hospital
2004 Lecturer, Graduate School of Engineering, The University or Tokyo
2008 Associate Professor, Graduate School of Medicine, The University or Tokyo
2017 Professor, Institute of Biomaterials and Bioengineering, Tokyo Medical and Dental University (TMDU)

Recent Related Publications (5 Papers)

Hashimoto Y, Itaka K, Aoki Y, et al. Brain Dp140 alters glutamatergic transmission and social behaviour in the mdx52 mouse model of Duchenne muscular dystrophy. Progress in Neurobiology, 2022 (in press)
Kida H, Itaka K, Tachibana K, et al. Influence of Nanobubble Size Distribution on Ultrasound-Mediated Plasmid DNA and Messenger RNA Gene Delivery. Front. Pharmacol. 13:855495, 2022
Nakanishi H, Saito H, Itaka K. Versatile design of intracellular protein-responsive translational regulation system for synthetic mRNA. ACS Synth Biol. 11(3): 1077-1085, 2022
Pezzotti G, Terai Y, Itaka K, et al. Raman spectroscopic insight into osteoarthritic cartilage regeneration by mRNA therapeutics encoding cartilage-anabolic transcription factor Runx1. Materials Today Bio 13: 100210, 2022
Fukushima Y, Itaka K, et al. Treatment of ischemic neuronal death by introducing brain-derived neurotrophic factor mRNA using polyplex nanomicelle. Biomaterials 270: 120681, 2021

mRNA as a new modality for gene therapy

Keiji Itaka

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mRNA is a promising modality as an alternative to gene therapy. Not only the flexibility for designing the nucleic acid sequences, mRNA is applied theoretically for any cell type. The risk of insertional mutagenesis is negligible, allowing mRNA to be potentially applied for treatment of many common diseases.
Now there are several pipelines of mRNA medicine, mostly for enzyme replacement therapy. However, several important issues are remained. The first would be the transient protein translation from the mRNA. To extend the duration of protein translation, various modified mRNA design such as circular mRNA is under investigation. On the one hand, mRNA can be injected repeatedly as appropriate, which would compensate for the limited duration of translation. In this regard, the immune responses induced by the mRNA injection should be carefully addressed. Besides immunogenicity of mRNA itself, which has been dealt well with the use of modified nucleotides such as pseudouridine, the immune responses are largely due to lipid nanoparticle (LNP), which are widely used for mRNA delivery. For vaccines, it is mandatory to enhance the immune response, and LNP plays an important role as adjuvants. However, for therapeutic purposes, it should be avoided to induce inflammation at the administration site.
In this presentation, I would like to present a brief overview of the technologies used for development of mRNA vaccines and medicines, and introduce studies on mRNA and DDS including ourselves. For example, we recently developed a system to achieve cell-specific protein translation from the administered mRNA, based on Caliciviral VPg-based translational activator (CaVT) combined with inteins and target protein-binding nanobodies (ACS Synth. Biol. 2022, 11, 1077). This system allows translational activation and repression in response to the protein in the cytoplasm of the target cells. This system is expected to enhance the efficacy and improve the safety of the mRNA medicines.
Regarding DDS, we developed a non- or less- immunogenic polymer-based carriers called polyplex nanomicelle. This carrier allows mRNA administration to e.g. the joints without inducing inflammation. Now we are doing a preclinical study to develop an mRNA medicine encoding a chondrogenic transcription factor, RUNX1, for treatment of osteoarthritis.
Finally, the most critical point to develop mRNA medicines would be the choice of therapeutic factor(s). Any protein including secretory proteins or intracellular proteins (or both) is available for encoding to the mRNA. These are medical issues, and would be the specialties for most members in JSGCT here. I believe that, for developing mRNA medicines, the three issues of mRNA design, DDS, and the therapeutic factors are equally important. I hope we can discuss in detail about the potentials of mRNA medicine.

CURRICULUM VITAE

Name Michinori Kohara

Affiliation Tokyo Metropolitan Institute of Medical Science

Field of Research molecular-biological analysis (replication and pathogenesis), prevention and therapy of infectious disease

Education

1969-1973: The faculty of Science, Kochi University.
1982-1984: The faculty of pharmacology, Kitasato University. "Molecular-biological analysis of polio virus virulence". Work supervised by Professor Akio Nomoto.
1984-1986: The faculty of Medicine, Tokyo University.
Awarded the degree of Ph. D. in Medicine for a this entitled "Molecular-biological analysis of polio virus virulence". Work supervised by Professor Akio Nomoto.
1986: Doctor of Medicine (Ph. D.): University of Tokyo.

Professional Experience

1973-1989: Researcher at the Japan Poliomyelitis Research Institute, working on molecular-biological analysis of polio virus pathogenesis.
1989-1992: Research head at the Fundamental Research Laboratory, Tonen Co., working on molecular-biological analysis of hepatitis C virus.
1992-present: Project reader (now; Emeritus Investigator) at the Department of Microbiology and Cell Biology, Tokyo Metropolitan Institute of Medical Science. I am working on molecular-biological analysis (replication and pathogenesis), prevention and therapy (low molecule substance, vaccine) of infectious disease, such as Poliomyelitis virus, hepatitis C virus, hepatitis B virus, Influenza virus, Dengue virus, SARS-CoV.

Recent Related Publications (5 Papers)

Kyoko Tsukiyama-Kohara, and Michinori Kohara*. Basic Study for Vaccine Development Targeting Virus Infections. ***Viruses*** 2022, 14, 57. https://doi.org/10.3390/v14010057 Editorial.Takahiro Sanada, Tomoko Honda, Fumihiko Yasui, Kenzaburo Yamaji, Tsubasa Munakata, Naoki Yamamoto, Makoto Kurano, Yusuke Matsumoto, Risa Kohno, Sakiko Toyama, Yoshiro Kishi, Takuro Horibe, Yudai Kaneko, Mayumi Kakegawa, Kazushige Fukui, Takeshi Kawamura, Wang Daming, Chungen Qian, Fuzhen Xia, Fan He, Syudo Yamasaki, Atsushi Nishida, Takayuki Harada, Masahiko Higa, Yuko Tokunaga, Asako Takagi, Masanari Itokawa, Tatsuhiko Kodama, and Michinori Kohara*. Serologic Survey of IgG Against SARS-CoV-2 Among Hospital Visitors Without a History of SARS-CoV-2 Infection in Tokyo, 2020–2021. ***J. Epidemiol***. 2021 Nov 13. doi: 10.2188/jea.JE20210324.Asako Takagi, Yutaka Amako, Daisuke Yamane, Bouchra Kitab, Yuko Tokunaga, Ahmed El-Gohary, Michinori Kohara*, Kyoko Tsukiyama-Kohara*. Longer Poly(U) Stretches in the 3'UTR Are Essential for Replication of the Hepatitis C Virus Genotype 4a Clone in in vitro and in vivo. ***Frontiers in Microbiology*** 2021 Nov 25;12:764816.doi: 10.3389/fmicb.2021.764816Tomoko Honda, Sumiko Gomi, Daisuke Yamane, Fumihiko Yasui, Takuya Yamamoto, Tsubasa Munakata, Yasushi Itoh, Kazumasa Ogasawara, Takahiro Sanada, Kenzaburo Yamaji, Yasuhiro Yasutomi, Kyoko Tsukiyama-Kohara, and Michinori Kohara*. Development and characterization of a highly sensitive NanoLuciferase-based immunoprecipitation system for the detection of anti-influenza virus HA antibodies. ***mSphere*** 2021 Volume 6 Issue 3 e01342-20.Makoto Saito, Yasushi Itoh, Fumihiko Yasui, Tsubasa Munakata, Daisuke Yamane, Makoto Ozawa, Risa Ito, Takayuki Katoh, Hirohito Ishigaki, Misako Nakayama, Shintaro Shichinohe, Kenzaburo Yamaji, Naoki Yamamoto, Ai Ikejiri, Tomoko Honda, Takahiro Sanada, Yoshihiro Sakoda, Hiroshi Kida, Le Thi Quynh Mai, Yoshihiro Kawaoka, Kazumasa Ogasawara, Kyoko Tsukiyama-Kohara*, Hiroaki Suga*, and Michinori Kohara*. Macrocyclic peptides exhibit antiviral effects against influenza virus HA and prevent pneumonia in animal models. ***Nature Communications*** 2021 May 11;12(1):2654. doi: 10.1038/s41467-021-22964-w.

COVID-19 pathological analysis and control using mice transduced with an adenoviral vector expressing human ACE2 and attenuated vaccinia vaccine encoding the SARS-CoV-2 spike protein

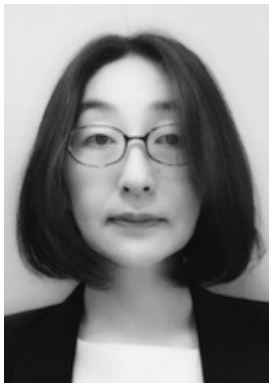
Michinori Kohara, Fumihiko Yasui, Yusuke Matsumoto

Tokyo Metropolitan Institute of Medical Science

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is currently causing a worldwide pandemic. Development of animal models that recapitulate coronavirus disease 2019 (COVID-19) are essential for evaluating vaccines and antivirals, and for understanding the pathogenesis of the disease. To analyze the molecular pathogenesis of SARS-CoV-2, a small animal model such as mice is needed: human ACE2, the receptor of SARS-CoV-2, needs to be expressed in the respiratory tract of mice. We conferred SARS-CoV-2 susceptibility in mice by using an adenoviral vector expressing hACE2 driven by an EF1α promoter with a leftward orientation. In this model, severe pneumonia like human COVID-19 was observed in SARS-CoV-2-infected mice, which was confirmed by dramatic infiltration of inflammatory cells in the lung with efficient viral replication. We demonstrated that an early circulating SARS-CoV-2 specifically induces the manifestation of severe symptoms and is associated with dramatically altered host responses.

As long as COVID-19 pandemic continues, new variants of SARS-CoV-2 with altered antigenicity will emerge. It is urgently required to develop vaccines that elicit robust, broad, and durable protection against the SARS-CoV-2 variants. We developed a vaccine that contains the SARS-CoV-2 S gene on the attenuated vaccinia virus DIs strain platform (rDIs-S). rDIs-S induced neutralizing antibody and T-lymphocyte responses in human angiotensin converting enzyme 2 (hACE2) transgenic mice and cynomolgus macaques, and showed broad protection against the early SARS-CoV-2 Wuhan strain to the recent Omicron BA.1 variant. Recent reports have shown that the antibody levels induced by the current mRNA vaccine dramatically declined at 6 months after 2nd vaccination. Thus, additional periodic vaccination would be required for the control of COVID-19 in the current vaccination. However, since the current mRNA vaccines often cause undesirable adverse events, the long-term immune memory response conferred by rDIs-S may be one of the useful advantages for the development of new vaccines. In the present study, we demonstrated the efficacy of rDIs-S, an attenuated vaccinia virus carrying the SARS-CoV-2 *S* gene. Furthermore, given that vaccination with rDIs-S effectively induced antibody and T-lymphocyte responses that also reacted with variant strains, rDIs-S may be useful for conferring protection against new variants by use as a booster after vaccination with 1st-generation vaccines.

CURRICULUM VITAE



Name Hiromi Hayashita-Kinoh

Affiliation Division of Molecular and Medical Genetics,
Center for Gene and Cell Therapy,
The Institute of Medical Science,
The University of Tokyo

Field of Research Molecular Biology, AAV vector, Gene therapy

Education

2002 Ph.D., The University of Tokyo, Graduate School of Medicine
1998 M.S., Hiroshima University, Graduate School of Science
1996 B.S., Hiroshima University

Professional Experience

2020-present Project Assistant Professor, Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo, Japan (Prof. Takashi Okada)
2017-2020 Assistant Professor, Division of Cell and Gene Therapy, Nippon Medical School, Japan (Prof. Takashi Okada)
2014-2017 Researcher, Department of Biochemistry and Molecular Biology, Nippon Medical School, Japan (Prof. Takashi Okada)
2008-2014 Postdoc, Department of Molecular Therapy, National Institute of Neuroscience, NCNP, Japan (Chairman Shin'ichi Takeda, Section Chief, Takashi Okada)
2007-2008 Postdoc, Cell-Matrix Frontier Laboratory, Hiroshima University, Hiroshima, Japan (Associate Prof. Yasuyuki Yokosaki)
2002-2007 Postdoc, University Research Institute for Diseases of Old Age, Juntendo University School of Medicine, Tokyo, Japan (Prof. Yoshikuni Mizuno, Lecturer Hideki Mochizuki)

Recent Related Publications (5 Papers)

1. Improved transduction of canine X-linked muscular dystrophy with rAAV9-microdystrophin via multipotent MSC pretreatment. Hayashita-Kinoh H, Posadas-Herrera Guillermo, Nitahara-Kasahara Y, Kuraoka M, Okada H, Chiyo T, Takeda S, Okada T. Molecular therapy. Methods & clinical development 20: 133-141, 2021
2. rAAV8 and rAAV9-Mediated Long-Term Muscle Transduction with Tacrolimus (FK506) in Non-Human Primates. Ishii A, Okada H, Hayashita-Kinoh H, Shin JH, Tamaoka A, Okada T, Takeda S. Molecular therapy. Methods & clinical development 18: 44-49, 2020
3. Intra-amniotic rAAV-mediated microdystrophin gene transfer improves canine X-linked muscular dystrophy and may induce immune tolerance.Hayashita-Kinoh H, Yugeta N, Okada H, Nitahara-Kasahara Y, Chiyo T, Okada T, Takeda S. Molecular therapy 23(4): 627-637, 2015
4. Robust Long-term Transduction of Common Marmoset Neuromuscular Tissue With rAAV1 and rAAV9. Okada H, Ishibashi H, Hayashita-Kinoh H, Chiyo T, Nitahara-Kasahara Y, Baba Y, Watanabe S, Takeda S, Okada T. Molecular therapy Nucleic acids 2: e95, 2013
5. Improvement of cardiac fibrosis in dystrophic mice by rAAV9-mediated microdystrophin transduction. Shin JH, Nitahara-Kasahara Y, Hayashita-Kinoh H, Ohshima-Hosoyama S, Kinoshita K, Chiyo T, Okada H, Okada T, Takeda S. Gene therapy 18: 910-919, 2011

Development of dosing protocol to reduce the required dose of rAAV using adult stem cells

Hiromi Hayashita-Kinoh[1], Guillermo Posadas-Herrera[1], Yuko Nitahara-Kasahara[1], Akiko Ishii[2], Shin'ichi Takeda[2], Takashi Okada[1]

[1] Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, The Institute of Medical Science, The University of Tokyo
[2] Department of Molecular Therapy, National Institute of Neurosciences, NCNP

Backgrounds: AAV vectors are safe and can express therapeutic genes for long periods of time. rAAV-based gene therapy has been tried for hereditary diseases such as SMA, XLMTM, and DMD. When rAAV is administered intravenously in clinical trials, doses of $3x10^{13}$ to $1x10^{14}$ v.g./kg BW are common. Systemic administration of more than $1x10^{14}$ v.g./kg BW can cause direct liver injury, thrombocytopenia, TMA, immune response to the virus vector and germline transmission. Therefore, vector doses should be reduced, or safer dosing protocols should be considered.
Duchenne muscular dystrophy (DMD) is a congenital disease in which mutations in the dystrophin gene cause gradual loss of skeletal and cardiac muscle. However, in dogs and monkeys, the expression of AAV vector-derived genes decreases with time due to immune response. Here, we investigated the strategy of using somatic stem cells in combination with rAAV to induce immune tolerance to the rAAV9 vector and to express sufficient transgene at a lower dose. Somatic stem cells have been used in various inflammatory diseases, including graft-versus-host disease (GvHD), due to their immunosuppressive effects to regulate dendritic cells. In addition, we investigated the immunomodulatory effect of MSCs in rAAV transduction.
Methods: Eight-week-old normal or $CXMD_J$ (DMD) dogs were intravenously injected with bone marrow-derived MSCs and rAAV9-luciferase or rAAV9-microdystrophin (2×10^{12} v.g./kg BW). Seven days after the injection, MSCs were injected systemically again. Eight days after the first injection, rAAV9-luciferase or rAAV9-microdystrophin was injected intravenously into the same dogs. qRT-PCR was used to analyze IFN-γ expression in purified canine peripheral leukocytes to examine the immune response to rAAV9. Expression of luciferase or microdystrophin in the skeletal muscle of transgenic animals was confirmed by immunohistochemistry. Furthermore, MSCs-treated $CXMD_J$ transduced with rAAV9-microdystrophin were compared with non-treated $CXMD_J$ to evaluate gait function and limb lameness.
Results: MSCs treatment followed by low dose of rAAV9 resulted in higher expression of transgenes (luciferase or microdystrophin) in skeletal and cardiac muscle compared to rAAV alone. rAAV9 treatment followed by IFN-γ expression in purified peripheral blood leukocytes was not enhanced by the combination of rAAV9 and MSCs. $CXMD_J$ treated with MSCs and rAAV9-microdystrophin showed superior functional improvement compared to other DMD dogs of the same age.
Conclusions: Our results demonstrate that MSCs pretreated rAAV injection improves the expression of rAAV-derived transgene in dogs. This strategy would be a practical approach to analyze the expression and function of transgenes *in vivo*. These findings also support the future feasibility of rAAV-mediated protein supplementation strategies in the treatment of DMD and various genetic diseases.

CURRICULUM VITAE

Name Jessica M. Tate

Affiliation Thermo Fisher Scientific, Patheon

Field of Research Viral vector gene therapy manufacturing and analytical technologies.

Education

2011 Ph.D University at Buffalo
Biochemical and molecular characterization of pox virus early gene transcription termination.

Professional Experience

2011-2014 Post-doctoral Researcher
2014-2016 Florida Biologix, Assistant Director, Downstream Process Development
2016-2019 Brammer Bio, Director Tech Transfer & Technical Project
2019-present Thermo Fisher Scientific, Director of Viral Vector R&D

CV

Jessica M. Tate, Ph.D., Director of. Viral Vector R&D, Science and Technology, Pharma Services
Dr. Tate leads the viral vector R&D group within the Science and Technology Team, which is driving the company-wide initiative to develop manufacturing and analytical platforms for GMP production of viral vector-based gene therapies utilized in in vivo and ex vivo human therapeutics. She joined the company through the acquisition of Brammer Bio in 2019.
Dr. Tate previously served in roles as Assoc. Director of Downstream Process Development and Director of Technology Transfer for Brammer Bio. Dr. Tate has been studying virus production, purification and analytics for 15+ years, and developing cGMP vector platforms for 8 years. Dr. Tate was a postdoctoral researcher at University or Florida and University of Rochester, received her doctoral degree in Biological Sciences from The State University of New York at Buffalo, and obtained bachelor's degrees in both Chemistry and Biology from The State University of New York at Buffalo.

Robust AAV Purification Platform Tolerant of Changes in Harvest Material

Jessica M. Tate, Hetal Brahmbhatt, Zahra Karjoo

Thermo Fisher Scientific, Patheon

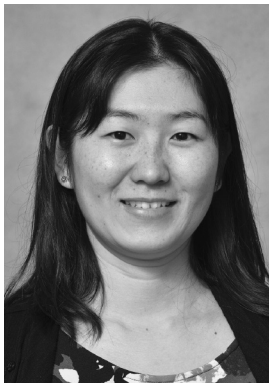
Often times small changes in viral vector manufacturing upstream process can have significant impacts to the performance of the downstream purification process. Due to this, a downstream process usually needs to be co-developed with the upstream process, and deviations in upstream unit operations can lead to failure of the entire manufacturing run, both of which result in increases in costs and timelines for getting these products to market. Thermo Fisher Scientific has created a robust downstream purification platform for AAV that can tolerate a variety of upstream conditions. Leveraging this purification platforms will enable our clients to bring their products to market on a de-risked, accelerated pathway.

Symposium 4

Abstract & Curriculum Vitae

Cancer Gene Therapy 1

CURRICULUM VITAE



Name Mizuho Sato-Dahlman

Affiliation University of Minnesota

Field of Research Cancer gene therapy

Education

2002-2006 BS, Tokyo University of Pharmacy and Life Sciences, Japan. Bioscience

2006-2011 PhD, Nara Institute of Science and Technology, Japan. Bioscience

Professional Experience

2011-2013 Postdoctoral Fellow, Osaka University Research Institute for Microbial Diseases, Department of Cell Biology

2013-2019 Postdoctoral Fellow, University of Minnesota, Department of Surgery, Division of basic and translational research

2019-Present Research Assistant Professor, University of Minnesota, Department of Surgery, Division of basic and translational research

Recent Related Publications (5 Papers)

LaRocca, C., Salzwedel, A., Sato-Dahlman, M., Romanenko, M., Andrade, R., Davydova, J., & Yamamoto, M. (2021). Interferon Alpha-Expressing Oncolytic Adenovirus for Treatment of Esophageal Adenocarcinoma. *Ann Surg Oncol*.

Sato-Dahlman, M., LaRocca, C. J., Yanagiba, C., & Yamamoto, M. (2020). Adenovirus and Immunotherapy: Advancing Cancer Treatment by Combination. *Cancers, 12*(5). PMC7281656 doi: 10.3390/cancers12051295

Sato-Dahlman, M., Wirth, K., & Yamamoto, M. (2018). Role of Gene Therapy in Pancreatic Cancer-A Review. *Cancers, 10*(4). PMC5923358 doi: 10.3390/cancers10040103

Sato-Dahlman, M., & Yamamoto, M. (2018). The Development of Oncolytic Adenovirus Therapy in the Past and Future - For the Case of Pancreatic Cancer. *Current cancer drug targets, 18*(2), 153-161. PMC6186423 doi: 10.2174/1568009617666170222123925

Sato-Dahlman, M., Miura, Y., Huang, J. L., Hajeri, P., Jacobsen, K., Davydova, J., & Yamamoto, M. (2017). CD133-targeted oncolytic adenovirus demonstrates anti-tumor effect in colorectal cancer. *Oncotarget, 8*(44), 76044-76056. PMC5652684 doi: 10.18632/oncotarget.18340

Engineering of the cancer-targeted oncolytic adenovirus for systemic therapy of advanced cancers

Mizuho Sato-Dahlman, Shuhei Shinoda, Naohiko Nakamura, Praveensingh Hajeri, Brett Roach, Kari Jacobsen, Masato Yamamoto

University of Minnesota

An ultimate goal of the developers of cancer genetherapy/virotherapy is to develop a device enabling systemic treatment of the patients with advanced or spread diseases. Oncolytic adenovirus (OAd) is a one of the promising anti-cancer agents under development. Oncolytic adenovirus (OAd) has high transduction efficiency *in vitro* and *in vivo* mediated by specific protein-to-protein binding and the exponential replication causing oncolysis. However, many cancer cells are resistant to wild-type Ad infection due to the low expression of adenovirus primary receptor (coxsackie-adenovirus receptor, CAR). In order to overcome this issue, several infectivity-enhanced oncolytic adenoviruses (OAds) with modified fibers has been developed (e.g. RGD, Ad5/3). These vectors confer augmented anti-tumor effect in CAR-negative cancer cells. However, these OAds still lack binding selectivity for cancer cells and are at risk for causing various adverse effects by increasing distribution to normal organs (e.g. innate immune response, hepatotoxicity) with systemic administration. Therefore, precise targeting of oncolytic virus at the level of infection (transductional targeting) is needed to achieve *in vivo* therapeutic benefits and safety for systemic treatment of cancer. To generate the cancer-targeting OAd, we have previously developed a novel system to identify the cancer-specific binding OAd by Ad-based library screening approach. To generate Ad library, we placed a 21 base pair random sequence (7 amino acids) in the AB-loop of the fiber-knob region that is responsible for the binding affinity to the Ad receptor, CAR. To date, we have identified several cancer-targeted OAds by using this screening system. Among them, the Mesothelin-targeted OAd (MSLN-OAd), and the CD133-targeted OAd (CD133-OAd) showed strong anti-tumor effect with systemic injection in human tumor xenograft models. Here, we characterized these two fiber-modified cancer-targeted OAds (MSLN-OAd and CD133-OAd) toward systemic application. Additionally, we will also discuss about our recent progress about the combination therapy with oncolytic adenovirus and chemo/radiotherapy in pancreatic cancer. We have developed IFN-alpha expressing OAd, and the combination therapy with IFN-OAd and chemo/radiation showed remarkable inhibition in pancreatic cancer animal models. These data suggest that Cancer-targeted OAds has great potential for developing new systemic treatment options for advanced cancers.

CURRICULUM VITAE

Name Hiroaki Wakimoto

Affiliation Massachusetts General Hospital, Harvard Medical School

Professional Experience

Dr. Wakimoto graduated Tokyo Medical and Dental University in 1989 and was a board certified neurosurgeon in Japan, when he decided to focus on laboratory sciences on brain tumors. Since 2009, Dr. Wakimoto has been a faculty member of the department of Neurosurgery at Massachusetts General Hospital, Harvard Medical School, and is currently an associate professor and Tawingo endowed research scholar of neurosurgery. Dr. Wakimoto's research interest is in preclinical modeling of malignant central nervous system tumors, and application of clinically relevant models to develop oncolytic viruses and molecular targeted agents for clinical translation. Patient-derived malignant glioma cells established by his group have been widely distributed to laboratories across the globe, leading to discoveries of disease biology and novel therapies. Dr. Wakimoto serves on the editorial board of journals such as Cancers, Journal of Neuro-oncology and Molecular Therapy Oncolytics, and his research is partly supported by NIH R01.

Preclinical development of oncolytic virus therapies for malignant brain tumors using cancer stem-like cell-based models

Hiroaki Wakimoto

Massachusetts General Hospital, Harvard Medical School

It has become increasingly evident that clinical translation of novel anti-cancer therapeutics requires application of preclinical cancer models that appropriately represent clinical cancers. Cancer stem or stem-like cells play key roles in the maintenance and recurrence of the tumor, and this feature renders cancer stem cell-based preclinical cancer models potentially useful to better predict clinical efficacy of exploratory therapeutic approaches. Cancer stem cells appear particularly relevant for the malignant brain tumor glioblastoma (GBM), in which functional cellular hierarchy has been reported. Using fresh surgical biomaterials, we have established a large panel of patient-derived glioblastoma (GBM) stem-like cell models that have been phenotypically and molecularly characterized, enabling distribution to the wide research community for research use. Using these models both in vitro and in vivo, we have shown the impact of particular genetic mutations present in oncolytic herpes simplex virus type 1 (oHSV) on the ability of oHSV to replicate in GBM cancer stem cells. We have further demonstrated the therapeutic benefits of mechanistic combination of oHSV therapy with arming with proapoptotic molecule or with targeted agents (PARP inhibitor or TGFbeta inhibitor) in orthotopic GBM models in mice. On the other hand, murine GBM stem-like cells provide genetically defined models that mimic the biological hallmarks of human GBM in immune competent settings. These have served as a powerful model to study oncolytic virus immunotherapy employing oHSV and oncolytic adenovirus, and its enhancement by immune checkpoint blockade. At the symposium, I will discuss the advancements our research has made with cancer stem cell models, and challenges that will need to be addressed.

CURRICULUM VITAE

Name Naoki Hosen

Affiliation Osaka University Graduate School of Medicine

Field of Research Hematology, Cancer Immunology

Education

1998 - 2002 Osaka University Graduate School of Medicine, Osaka, Japan
1988 - 1994 Osaka University Medical School, Osaka, Japan

Professional Experience

2020 – Present Professor, Laboratory of Cellular Immunotherapy,
World Premier Interenational Immunology Frontier Research Center, Osaka University, Japan
2020 – Present Professor, Department of Hematology and Oncology,
Osaka University Graduate School of Medicine, Japan
2014 – 2019 Associate Professor, Department of Cancer Stem Cell Biology,
Osaka University Graduate School of Medicine, Japan
2009 – 2014 Associate Professor, Department of Functional Diagnostic Science,
Osaka University Graduate School of Medicine, Japan
2007 – 2009 Associate Professor, Department of Cancer Stem Cell Biology,
Osaka University Graduate School of Medicine, Japan
2003 – 2007 Postdoctoral fellow, Department of Pathology, Stanford University School of Medicine

Recent Related Publications (5 Papers)

1 Hasegawa, K. et al. Selective targeting of multiple myeloma cells with a monoclonal antibody recognizing the ubiquitous protein CD98 heavy chain. Sci Transl Med 14, eaax7706, doi:10.1126/scitranslmed.aax7706 (2022).
2 Shingai, Y. et al. Autonomous TGFbeta signaling induces phenotypic variation in human acute myeloid leukemia. Stem Cells 39, 723-736, doi:10.1002/stem.3348 (2021).
3 Toda, J. et al. Signal-transducing adapter protein-1 is required for maintenance of leukemic stem cells in CML. Oncogene 39, 5601-5615, doi:10.1038/s41388-020-01387-9 (2020).
4 Hosen, N. et al. The activated conformation of integrin beta7 is a novel multiple myeloma-specific target for CAR T cell therapy. Nat Med 23, 1436-1443, doi:10.1038/nm.4431 (2017).
5 Wagner, K. D. et al. The Wilms' tumour suppressor Wt1 is a major regulator of tumour angiogenesis and progression. Nat Commun 5, 5852, doi:10.1038/ncomms6852 (2014).

CAR-T cell therapy for multiple myeloma

Naoki Hosen

Osaka University Graduate School of Medicine

The effects of CAR T cells targeting CD19 on B-cell hematological cancers are tremendous and can cure many previously incurable patients. As cytokine release syndrome can be controlled with anti-IL6 receptor antibody treatment, the number of facilities that carry out CD19 CAR-T cell therapy is increasing. Multiple myeloma is one of the most refractory blood cancers. Recently, high efficacy of CAR T cells targeting BCMA has been shown, and approved in Japan. Under such circumstances, the development of new CAR-T cells has become a major competition worldwide. We have shown that CAR T cells specific for activated integrin beta 7 is effective for multiple myeloma and are proceeding with their clinical development. In this talk, we will describe the current status and future prospects of CAR-T cell therapy for multiple myeloma, including the introduction of the novel CAR-T cells that we are developing.

Symposium 5

Abstract & Curriculum Vitae

Oncolytic virus

CURRICULUM VITAE

Name Khalid Shah

Affiliation BWH, Harvard Medical School

Field of Research Dr. Shah is the Vice Chair of Research at BWH and a Professor at Harvard Medical School. He is also the Director of the Center for Stem Cell Therapeutics and Imaging at BWH and the joint Center of Excellence in Biomedicine. He and a Principal Faculty at Harvard Stem Cell Institute in Boston.

Dr. Shah and his team have pioneered major developments in translational cell therapy field, successfully developing experimental models to understand basic cancer biology and therapeutic cells for cancer. These studies have been published in a number of very high impact journals. Previously, Dr. Shah's translational stem cell work has caught the attention in the public domain and as such it has been highlighted in the media world-wide including features on BBC and CNN. Recently, Dr. Shah's laboratory has reverse engineered cancer cells using CRISPR/Cas9 technology and utilized them as therapeutics to treat cancer. This work was published in journal *Science Translation Medicine* and highlighted world-wide including features on Scientific American, New York Times and Scientific American. Dr. Shah holds current positions on numerous councils, advisory and editorial boards in the fields of stem cell therapy and oncology. In an effort to translate the exciting therapies develped in his laboratory into clinics, he has founded two biotech companies whose main objective is the clinical translation of therapeutic stem cells in cancer patients.

Education
MS: Wageningen University and Research Center, The Netherlands 1997
PhD: Wageningen University and Research Center, The Netherlands 2001

Professional Experience
Postdoctoral Fellow- 2002-2005- MGH, Harvard Medical School

Recent Related Publications (5 Papers)

1. Bhere, D., Choi, S.H., van de Donk, P, Hope, D., Gortzak, K., Kunnummal, A, Khalsa, J, K., Reinshagen, C., Nissar, N., Ling, H, Vasdev, N, Essayed, W.I,, Golby, A., Bi, L., Feng, C., Zhang, Y.S., Lowe A, Revai Lechtich E Essayed, W, I., Quevedo, P.V., Banouni, M., Palagina, A., Abdi, R., Fury, B., Smirnakis, S., Lowe, A., Reeve, B., Hiller, A., Chiocca, E.A., Prestwich, G.A., Wakimoto, H,, Bauer, G., and Shah K. Stratification and subsequent local treatment of resected brain tumors with encapsulated and engineered allogeneic stem cells. ***Nature Communications*** 2022 In Press
2. Kitamura Y, Moleirinho S, Kanaya N, Du, W., Reinshagen C, Brastianos P., Falcone, J. L., Hofer, A. M., Franco, A., and Shah K. Anti-EGFR VHH-armed death receptor ligand-engineered allogeneic stem cells have therapeutic efficacy in diverse brain metastatic breast cancers ***Science Advances***. 2021; 7, eabe8671, doi: 10.1126/sciadv.abe8671
3. Khalsa J, Cheng N, Keegan J, Chaudry A, Driver J, Bi L, Lederer J and Shah, K. Immune profiling genetically diverse syngeneic murine GBM tumors identifies immunologically active and inert types. ***Nature Communication***s 2020. 11, 3912. doi: 10.1038/s41467-020-17704-5.
4. Kiyokawa J, Kawamura Y, Ghouse SM, Acar S, Barçın E, Martínez-Quintanilla J, Martuza RL, Alemany R, Rabkin SD, Shah K*, Wakimoto H*. Modification of extracellular matrix enhances oncolytic adenovirus immunotherapy in glioblastoma. ***Clin Cancer Res***. 2020 doi: 10.1158/1078-0432.CCR-20-2400. * Co-corresponding authors
5. Reinshagen, C., Bhere, D., Choi, SH, Hutten, S., Nesterenko, I., Wakimoto, H., Le Roux, E., Rizvi, A., Du, W. Minucci, C and Shah, K CRISPR-enhanced engineering of therapy-sensitive cancer cells for self-targeting of primary and metastatic tumors ***Science Translational Medicine*** 2018; doi: 10.1126/scitranslmed.aao3240.

Gene Edited and Engineered Cell based therapies for Cancer: From Bench to Bedside

Khalid Shah

BWH, Harvard Medical School

Cell based therapies are emerging as a promising strategy for cancer. We have developed cell surface receptor targeted adult stem cells, cancer cells and T cells expressing novel bi-functional immunomodulatory proteins and releasing oncolytic viruses. Using our recently established tumor models that mimic clinical settings, we have explored the fate and efficacy of different engineered cell based therapies. Our findings demonstrate the strength of using innovative approaches and clinically relevant preclinical models that pave a path for clinical translation. This presentation provides data and rationale for assessing combined cell based studies in preclinical studies and translating the most promising studies into the clinic.

CURRICULUM VITAE

Name Chae-Ok Yun

Affiliation Department of Bioengineering, College of Engineering,
Hanyang University, Seoul, Korea
GeneMedicine Co., Ltd., Seoul, Korea

Field of Research Chae-Ok Yun is serving as a Distinguished Professor at Hanyang University and a CEO of GeneMedicine Co., Ltd. in Korea. Her field of research is in gene therapy, immunotherapy, cell therapy, nanomedicine, and tumor biology.

Education

1992-1996	Ph.D. in Cell and Molecular Biology Illinois Institute of Technology, Chicago, IL, USA
1986-1988	M.S. in Molecular Biology Sogang University, Seoul, Korea
1982-1986	B.S. in Biology with a minor in Chemistry Sogang University, Seoul, Korea

Professional Experience

PRIMARY APPOINTMENTS

2014 – present	Founder & CEO GeneMedicine Co., Ltd., Seoul, Korea
2011 – present	Professor Dept. of Bioengineering, School of Engineering Hanyang University, Seoul, Korea
2000 – 2011	Professor Institute for Cancer Research Yonsei University of College of Medicine, Seoul, Korea

PROPFESSIONAL EXPERIENCE

2017– 2018	Visiting Scientist (Sabbatical) Robert Langer's Lab, Koch Institute of Cancer Massachusetts Institute of Technology, Cambridge, USA
2013 – present	Guest Professor Sichuan University, China
2012 – present	Adjunct Professor Medical School, University of Washington, Seattle, USA
2010 – present	Adjunct Professor Dept. of Pharmaceutics and Pharmaceutical Chemistry The University of Utah, Salt Lake City, Utah, USA
1998 – 2000	Research Associate Dept. of Radiation Oncology (Rakesh K. Jain, Ph.D.) MGH, Harvard Medical School, Boston, MA, USA
1996 – 1998	Post-doctoral Fellow Biotherapeutics Development Lab (Richard P. Junghans, M.D., Ph.D.) Dept. of Hematology/Oncology Harvard Medical School, Boston, MA, USA

Recent Related Publications (5 Papers)

- Thavasyappan Thambi, Jeongmin Lee, A-Rum Yoon, Dayananda Kasala, Chae-Ok Yun, A pH- and Bioreducible Cationic Copolymer with Amino Acids and Piperazines for Adenovirus Delivery, ***Pharmaceutics***, 14(3), 597, 2022
- Thai Minh Duy Le. A-Rum Yoon, Thavasyappan Thambi, Chae-Ok Yun, Polymeric Systems for Cancer Immunotherapy: A Review, ***Frontiers in Immunology***, 13, 826876, 2022 (corresponding author)
- DaeYong Lee, JongHoon Ha, Hyomin Ahn, Seong Dong Jeong, MoonKyoung Jeong, Ji-Ho Park, Chae-Ok Yun, Yeu-Chun, Kim Polypeptide-Based K+ Ionophore as a Strong Immunogenic Cell Death Inducer for Cancer Immunotherapy, ***Applied Biomaterials***, 4(12), 8333-8342, 2021
- Han-Gyu Chang, Yong-Hyeon Choi, JinWoo Hong, Joung-Woo Choi, A-Rum Yoon, Chae-Ok Yun, GM101 in Combination with Histone Deacetylase Inhibitor Enhances Anti-Tumor Effects in Desmoplastic Microenvironment, ***Cells***, 10(11), 2811, 2021
- SeongDong Jeong, Bo-Kyeong Jung, DaeYong Lee, Hyomin Ahn, JongHoon Ha, Ilkoo Noh, Chae-Ok Yun, Yeu-Chun Kim, Immunogenic Cell Death Inducing Fluorinated Mitochondria-Disrupting Helical Polypeptide Synergizes with PD-L1 Immune Checkpoint Blockade, ***Advanced Science***, 8(7):2001308, 2021

Addressing the challenges of conventional oncolytic virotherapy

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Recently, increasing number of oncolytic viruses (OV)s have been entering clinical trials each year due to their unique abilities, like inflaming of the immunologically 'cold' tumor microenvironment, that are difficult to achieve by standard cancer therapy. Still, there are several inherent limitations and barriers, like (1) poor intratumoral distribution of the virus away from the administration site, (2) suboptimal antitumor efficacy against distant metastases, and (3) rapid clearance from the tumor due to viral shedding and induction of antiviral immunity, that abate the therapeutic potential of conventional oncolytic viruses in clinical environment. GeneMedicine's key oncolytic adenovirus pipelines, GM101, GM102, GM103, and GM104, have been precisely engineered to address these challenges in an innovative manner to maximize the induction of antitumor immune response, therapeutic efficacy against distant metastases, longevity of the virus' biological activity in tumor tissues, and safety profile of the virus.

CURRICULUM VITAE

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Education

1983-1989 Tohoku University School of Medicine
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Professional Experience

1997-2000 Massachusetts General Hospital/Harvard Medical School, Research fellow
2000-2002 Department of Dermatology, Tohoku University Graduate School of Medicine, Assistant professor
2003-2005 Department of Dermatology, Tohoku University Graduate School of Medicine, Associate professor / Lecturer
2005-2009 Department of Dermatology, Tohoku University School of Medicine, Associate professor
2010- Department of Dermatology, Shinshu University School of Medicine, Professor and Chairman
2014- Center for Clinical Research, Shinshu University Hospital, Director

Recent Related Publications (5 Papers)

Omodaka T, Minagawa A, Okuyama R. Ultraviolet-related skin cancers distribute differently on the face surface. ***Br J Dermatol*** 185: 205-207, 2021.

Mikoshiba A, Ashida A, Sakaizawa K, Kiniwa Y, Okuyama R. Detecting copy number alteration of oncogenes in cell-free DNA to monitor treatment response in acral and mucosal melanoma. ***J Dermatol Sci*** 97: 172-178, 2020.

Shirai T, Kiniwa Y, Sano T, Nakamura K, Mikoshiba Y, Ohashi N, Sekijima Y, Okuyama R. Presence of antibodies to striated muscle and acetylcholine receptor in association with occurrence of myasthenia gravis with myositis and myocarditis in a patient with melanoma treated with an anti-PD-1 antibody: a case report. ***Eur J Cancer*** 106: 193-195, 2019.

Hidaka T, Ogawa E, Kobayashi EH, Suzuki T, Funayama R, Nagashima T, Fujimura T, Aiba S, Nakayama K, Okuyama R, Yamamoto M. AhR links atopic dermatitis and air pollution via Artemin induction. ***Nat Immunol*** 18: 64-73, 2017.

Minagawa A, Omodaka T, Okuyama R. Melanomas favor mechanical stress points on the plantar surface. ***N Engl J Med*** 374: 2404-2406, 2016.

An investigator-initiated clinical trial of third generation oncolytic virus armed with IL-12 against malignant melanoma

Ryuhei Okuyama[1], Kazuhiko Matsumoto[2], Hiroshi Koga[1], Kenta Nakamura[1], Minoru Tanaka[3], Tomoki Todo[3]

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Oncolytic viral therapy is a therapeutic method using viruses with replication in cancer, but not normal tissues, by unique modification to viral genome. The oncolytic virus destroys cancer by direct cell lysis through virus replication. It also induces anti-tumor immunity in the process of immunological elimination, and acts as an efficient cancer vaccine. A major advantage of oncolytic viruses is the limited toxicity and ability to induce antitumor immune response using each individual tumor as a source of antigen. A variety of viruses are now at various stages of clinical and pre-clinical development for the treatment of cancers. Todo *et al*. have established third generation oncolytic virus, G47Δ (teserpaturev), from herpes simplex virus type 1. By artificial triple mutations, G47Δ has acquired not only high safety, but also replication ability specific for cancer which leads to powerful anti-tumor effect. G47Δ demonstrated an improvement in 1-year survival rate in patients with recurrent or residual glioblastoma, and was approved for insurance in Japan last year, ahead of the rest of the world.
Furthermore, Todo *et al*. have developed armed oncolytic virus T-hIL12 from G47Δ for enhancing the induction of anti-tumor immunity by incorporation of IL-12 DNA. We aim at clinical development of T-hIL12 for melanoma, a highly metastatic skin cancer. The non-clinical study showed high anti-tumor effect of T-hIL12, and interestingly, anti-tumor activity of the virus was enhanced by the combination with anti-PD-1 antibody, which is the standard remedy for advanced melanoma. Since T-hIL12 is expected to show extremely high utility in clinical practice, we have designed an investigator-initiated clinical trial (phase 1/2 test) using a clinical lot of T-hIL12 manufactured according to GMP standard. Inclusion patients are stage 3B · C/4 melanoma patients with metastasis to the skin, subcutis, and/or lymph node. T-hIL12 is administered intratumorally. In phase 1, we mainly examine the safety of T-hIL12. In the phase 2, T-hIL12 is administered in addition to anti-PD-1 antibody, and the effectiveness of T-hIL12 is chiefly investigated.
Currently, we are conducting clinical trial smoothly. We will develop the armed oncolytic virus originating in Japan and aim for the development of an innovative cancer therapy responding to various cancers including melanoma.

CURRICULUM VITAE

Name Ken-ichiro Kosai

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Education

M.D., Kurume University School of Medicine (March, 1988)
Ph.D., Kurume University Graduate School of Medicine (March, 1992)

Professional Experience

1988 Resident (Pediatrics) and Graduate Student (Pathology), Kurume University School of Medicine
1992 Assistant Professor, Department of Pathology, Kurume University School of Medicine
1993 Post Doc.> Visiting Assistant Professor (1994-), Baylor Collage of Medicine, USA
1996 Visiting Assistant Professor, Division of Biochemistry, Osaka University Medical School
1997 Assistant Professor, Kurume University Research Center for Innovative Cancer Therapy, (and Departments of Pediatrics, Human Genetics and Surgery), Kurume University
2000 Associate Professor, Department of Gene Therapy and Regenerative Medicine, Gifu University School of Medicine
2003 Professor, Cognitive and Molecular Research Institute for Brain Diseases, (and Department of Pediatrics), Kurume University
2006 Professor and Chairman, Department of Gene Therapy and Regenerative Medicine, Kagoshima University Graduate School of Medicine and Dental Sciences

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Director, Center for Innovative Therapy Research and Application,
Kagoshima University Graduate School of Medicine and Dental Sciences
Director, Center for Clinical and Translational Research, Kagoshima University Hospital
Visiting Faculty, Kurume University School of Medicine
Special Affiliated Research Associate, Gifu University School of Medicine
Advisory Board Member, Biologics Center for Research and Training

Recent Related Publications (5 Papers)

Mitsui K, Takahashi T, Ide K, Matsuda E, Kosai K.: Optimization of Adenoviral Gene Transfer in Human Pluripotent Stem Cells. ***Biochem Biophys Res Commun***. 541:78-83, 2021

Matsuda E, Obama Y, Kosai K.: Safe and low-dose but therapeutically effective adenovirus-mediated hepatocyte growth factor gene therapy for type 1 diabetes in mice. ***Life Sci.*** 268:119014. 2021

Suzuki S, Kofune H, Uozumi K, Yoshimitsu M, Arima N, Ishitsuka K, Ueno S and Kosai K.: A survivin-responsive, conditionally replicating adenovirus induces potent cytocidal effects in adult T-cell leukemia/lymphoma. ***BMC Cancer.***19(1):516. doi:10.1186/s12885-019-5730-1, 2019

Ide K, Mitsui K, Irie R, Matsushita Y, Ijichi N, Toyodome S, Kosai K.: A Novel Construction of Lentiviral Vectors for Eliminating Tumorigenic Cells from Pluripotent Stem Cells. ***Stem Cells***. 36:230-239, 2018 ***(Featured Article in this issue; Best of Japan, Virtual Issues, Stem Cells)***

Sakamoto K, Khai NC, Wang Y, Irie R, Takamatsu H, Matsufuji H, Kosai K.: Heparin-binding epidermal growth factor–like growth factor and hepatocyte growth factor inhibit cholestatic liver injury in mice via different actions. ***Int J Mol Med***. 38(6):1673-1682, 2016

Survivin-Responsive Conditionally Replicating Adenovirus Regulated with Multiple Factors (Surv.m-CRA): From Basic Research to Phase II Clinical Trials

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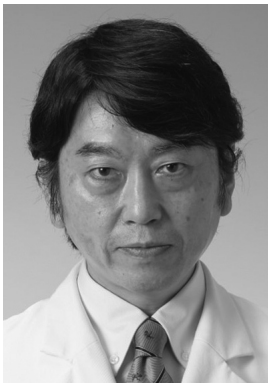
Kagoshima University

There is a worldwide race to develop the best performing oncolytic viruses (OVs), which selectively replicate in and kill cancer cells, and OV immunotherapies, including OV armed with immune genes. We first developed combination immune gene therapy strategies, which efficiently induced systemic antitumor immunity after a local injection of replication-defective adenovirus vector expressing a suicide gene and cytokine genes. This idea is, at least in part, the basis for the development of OV immunotherapy. We next developed an original platform technology of "m-CRA", that is, "Conditionally replicating adenovirus (CRA) that is regulated and/or treats cancer cells with multiple factors", for efficiently developing candidates in the best OV immunotherapies. Using this technology, we generated numbers of m-CRAs and found that one of the best was survivin-responsive m-CRAs (Surv.m-CRAs) in terms of the following advantages. Surv.m-CRAs induced not only more potent and cancer-specific cytotoxic effects than competitors but also induced increased effectiveness against cancer stem cells, which are resistant to conventional therapies. In addition, Surv.m-CRA propagated vigorously and exerted a strong cytotoxic effect specifically on undifferentiated and tumorigenic human pluripotent stem cells (hPSCs), but not on the well differentiated normal cells that derived from hPSCs. This feature and the data in nonclinical GLP studies strongly suggest the strictly cancer-specific viral replication of Surv.m-CRA, *i.e.*, high safety.

We completed ICH-GCP First-In-Human clinical trials of Surv.m-CRA-1 (Surv.m-CRA without transgene) in Kagoshima University Hospital. Patients underwent a single intratumoral injection of either 1×10e10 viral particle (vp), 1×10e11 vp or 1×10e12 vp. As a result, Surv.m-CRA-1 was well tolerated and showed more remarkable antitumor effects for prolonged periods than previously reported CRAs. Based on this result, we are doing Phase II multicenter clinical trial of Surv.m-CRA-1 for malignant bone tumors toward approval. We also performed Phase I/II clinical trial of Surv.m-CRA-1 for pancreatic cancer patients.

Furthermore, we are developing a series of next-generation OV immunotherapies, including Surv.m-CRA-2s (armed with diverse immune genes and promoters), which more strongly induced systemic antitumor immunity for metastatic cancers with high safety. Nonclinical study of one of Surv.m-CRA-2s is underway.

CURRICULUM VITAE



Name Hiroshi Tazawa

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Professional Experience

2002-2005 Visiting Scientist, International Agency for Research on Cancer, France
2005-2007 Staff Scientist, National Cancer Center Research Institute, Japan
2007-2010 Assistant Professor, Okayama University Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Japan
2010-2011 Assistant Professor, Center for Gene and Cell Therapy, Okayama University Hospital, Japan
2011-2016 Assistant Professor, Center for Innovative Clinical Medicine, Okayama University Hospital, Japan
2016-Present Associate Professor, Center for Innovative Clinical Medicine, Okayama University Hospital, Japan

Recent Related Publications (5 Papers)

1. Koujima T, Tazawa H, Ieda T, Araki H, Fushimi T, Shoji R, Kuroda S, Kikuchi S, Yoshida R, Umeda Y, Teraishi F, Urata Y, Mizuguchi H, Fujiwara T. Oncolytic virus-mediated targeting of the ERK signaling pathway inhibits invasive propensity in human pancreatic cancer. ***Mol. Ther. Oncolytics***, 17:107-117, 2020.
2. Tanimoto T, Tazawa H, Ieda T, Nouso H, Tani M, Oyama T, Urata Y, Kagawa S, Noda T, Fujiwara T. Elimination of MYCN-amplified neuroblastoma cells by telomerase-targeted oncolytic virus via MYCN suppression. ***Mol. Ther. Oncolytics***, 18:14-23, 2020.
3. Kakiuchi Y, Kuroda S, Kanaya N, Kumon K, Tsumura T, Hashimoto M, Yagi C, Sugimoto R, Hamada Y, Kikuchi S, Nishizaki M, Kagawa S, Tazawa H, Urata Y, Fujiwara T. Local oncolytic adenovirotherapy produces an abscopal effect via tumor-derived extracellular vesicles. ***Mol. Ther.***, 29:2920-2930, 2021.
4. Shirakawa Y, Tazawa H, Tanabe S, Kanaya N, Noma K, Koujima T, Kashima H, Kato T, Kuroda S, Kikuchi S, Kagawa S, Katsui K, Kanazawa S, Urata Y, Fujiwara T. Phase I study of endoscopic intratumoral injection of OBP-301 (Telomelysin) with radiotherapy in esophageal cancer patients unfit for standard treatments. ***Eur. J. Cancer***, 153:98-108, 2021.
5. Masuda T, Tazawa H, Ieda T, Hashimoto Y, Kikuchi S, Kuroda S, Tanabe S, Noma K, Urata Y, Kagawa S, Shirakawa Y, Fujiwara T. Overexpression of adenovirus E1A reverses transforming growth factor-β-induced epithelial-mesenchymal transition in human esophageal cancer cells. ***Acta Med. Okayama***, 76:203-215, 2022.

Recent progress in clinical application of telomerase-specific oncolytic virotherapy

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Oncolytic virotherapy has emerged as a novel antitumor treatment for eliminating tumor cells without affecting normal cells. Adenovirus serotype 5 (Ad5) is widely used as a virus vector in oncolytic virotherapy. For inducing tumor-specific cell death using Ad5, replacement of the wild-type *E1* promoter with the promoters of cancer-related genes is a useful method for enhancing the tumor tropism of virus replication.

Telomerase is an enzyme that adds the telomere, a region of repeated nucleotides (TTAGGG), to the ends of chromosomes. Tumor cells frequently exert high telomerase activity, which contributes to unlimited proliferation and tumor development via telomere elongation, whereas normal somatic cells are telomerase-negative. Telomerase activity is closely correlated with human telomerase reverse transcriptase (hTERT) expression Therefore, to target tumor cells with telomerase activity, we generated two types of telomerase-specific replication-competent oncolytic adenoviruses, OBP-301 (suratadenoturev) and OBP-702 (tumor suppressor p53-armed OBP-301).

OBP-301 is a telomerase-specific replication-competent oncolytic adenovirus, which drives the expression of adenoviral *E1A* and *E1B* genes under the control of the *hTERT* promoter, providing tumor-specific virus replication. A phase I study has confirmed the safety and biological activity of intratumoral administration of OBP-301 in patients with advanced solid tumors in the United States (Nemunaitis et al., Mol Ther 2010). Preclinical experiments demonstrated that OBP-301 sensitizes human esophageal cancer cells to ionizing radiation via adenoviral E1B-mediated inhibition of DNA repair machinery (Kuroda et al., Cancer Res 2010). To determine the feasibility, efficacy, and pharmacokinetics of OBP-301 in combination with radiotherapy, a phase I study was conducted in elderly patients with esophageal cancer in Japan (Shirakawa et al., Eur J Cancer 2021). A phase II study of intratumoral injection of OBP-301 and radiotherapy as a first-line treatment for esophageal cancer is underway as a multicenter trial in Japan.

OBP-702 is a modified OBP-301, which induces the Egr1 promoter-driven wild-type p53 expression. Preclinical experiments demonstrated that OBP-702 exhibits more profound antitumor effect than OBP-301 in a variety of human cancer cells, including lung cancer (Yamasaki et., Eur J Cancer 2012), osteosarcoma (Hasei et al., Mol Cancer Ther 2013), and pancreatic cancer (Koujima et al., Mol Ther Oncolytics 2020). OBP-702 induces apoptosis and autophagy-related cell death via induction of p53 signaling pathway. For clinical application of OBP-702, the production of Good Manufacturing Practice-grade OBP-702 is ongoing by grant support of Japan Agency for Medical Research and Development.

In this symposium, we present recent progress of clinical study of OBP-301, preclinical data of OBP-702, and future perspective in clinical application of telomerase-specific oncolytic virotherapy.

Symposium 6

Abstract & Curriculum Vitae

Young investigators session:
explore a new era of gene therapy research

略 歴

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研究分野 ゲノム生物学、遺伝子治療学、腫瘍治療学

学歴

2001年　名古屋大学理学部生命理学科卒業
2003年　奈良先端科学技術大学院大学バイオサイエンス研究科・博士前期課程修了
2007年　大阪大学大学院医学系研究科医学専攻・博士後期課程修了

職歴

2010年　大阪大学大学院医学系研究科遺伝子治療学・助教
2014年　大阪大学大学院医学系研究科遺伝子治療学・准教授
2014-2015年　米国NIH NIAMS, Visiting fellow

最近の関連出版物・論文

1. Peroxisomal membrane protein PMP34 is involved in the human papillomavirus infection pathway
   Ito R, Kitamura K, Inohara H, Yusa K, Kaneda Y, Nimura K(corresponding author)
   Frontiers in Virology, in press
2. A simple method using CRISPR-Cas9 to knock-out genes in murine cancerous cell lines
   Ishibashi A, Saga K, Hisatomi Y, Li Y, Kaneda Y & Nimura K(corresponding author)
   Sci Rep. 2020 Dec 18;10(1):22345. doi: 10.1038/s41598-020-79303-0.
3. Evaluation of HPV16 E7 expression in head and neck carcinoma cell lines and clinical specimens
   Kitamura K, Nimura K(corresponding author), Ito R, Saga K, Inohara H & Kaneda Y
   Sci Rep. 2020 Dec 17;10(1):22138. doi: 10.1038/s41598-020-78345-8.
4. SF3B2-mediated RNA splicing drives human prostate cancer progression. Kawamura N*, Nimura K* (corresponding author), Saga K, Ishibashi A, Kitamura K, Nagano H, Yoshikawa Y, Ishida K, Nonomura N, Arisawa M, Luo J, Kaneda Y. (*co-first)
   Cancer Res. 2019 Oct 15;79(20):5204-5217. doi: 10.1158/0008-5472.CAN-18-3965.
5. Myc Regulates Chromatin Decompaction and Nuclear Architecture during B Cell Activation.
   Kieffer-Kwon KR*, Nimura K*, Rao SSP*, Xu J*, Jung S, Pekowska A, Dose M, Stevens E, Mathe E, Dong P, Huang SC, Ricci MA, Baranello L, Zheng Y, Tomassoni Ardori F, Resch W, Stavreva D, Nelson S, McAndrew M, Casellas A, Finn E, Gregory C, St Hilaire BG, Johnson SM, Dubois W, Cosma MP, Batchelor E, Levens D, Phair RD, Misteli T, Tessarollo L, Hager G, Lakadamyali M, Liu Z, Floer M, Shroff H, Aiden EL, Casellas R. (*co-first)
   Mol Cell. 2017 Aug 17;67(4):566-578.e10. doi:10.1016/j.molcel.2017.07.013.

生体内における非増殖性ウイルスHVJ-Eによる抗腫瘍効果誘導メカニズムの解明

二村　圭祐

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がん患者体内で抗腫瘍免疫を誘導し、標的腫瘍のみならず非標的腫瘍にも抗腫瘍効果を誘導することができれば有効ながん治療法となる。腫瘍溶解性ウイルス療法は新たながん治療法として着目されているが、非増殖性ウイルス療法が抗腫瘍効果を誘導する機序の全貌は不明である。今回、私達はUVを照射することで非増殖性となったセンダイウイルス（HVJ-E）が腫瘍細胞において転写因子IRF7を中心としたシグナル経路を活性化することで抗腫瘍効果を発揮することを見出した。さらにHVJ-EとT細胞補助刺激因子OX40に対するアゴニスト抗体を組み合わせた腫瘍内投与によって、全身性の抗腫瘍効果を誘導できた。トランスクリプトームとT細胞受容体レパトア解析によって、HVJ-E+OX40療法がCD4、CD8 T細胞を活性化し、腫瘍間での直接的な移動を促進することがわかった。HVJ-E+OX40療法は腫瘍細胞でのNKG2Dリガンドと T細胞でのNKG2Dの発現を誘導し、NKG2DリガンドとNKG2Dとの相互作用によって全身性の抗腫瘍効果を発揮していることが示唆された。さらに、HVJ-Eの抗腫瘍効果は腫瘍細胞でのIRF7の標的因子の発現に完全に依存していた。IRF7標的因子はそれ自体が抗腫瘍効果を持ち、OX40抗体との組み合わせによって、T細胞を活性化・増幅し、全身性の抗腫瘍効果を誘導することができた。私達の結果から、非増殖性ウイルス療法が抗腫瘍効果を誘導する機序が明らかになり、さらに全身性の抗腫瘍効果を誘導する機序の一端が明らかになった。

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職歴

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平成15年4月　京都市立病院 小児科 医員
平成17年4月　都府立医科大学附属病院 小児科 医員
平成21年4月　京都府立与謝の海病院小児科 副医長
平成23年4月　京都府立医科大学大学院医学研究科 小児発達医学 助教
平成25年2月　Center for Cell and Gene Therapy, Baylor College of Medicine Postdoctoral Associate
平成27年8月　京都府立医科大学大学院医学研究科 小児科学 助教
平成31年4月　京都府立医科大学大学院医学研究科 小児科学 講師（学内）
令和4年4月　信州大学　学術研究・産学官連携推進機構　教授

最近の関連出版物・論文

1. Suematsu M, Yagyu S*, Nagao N, Kubota S, Shimizu Y, Tanaka M, Nakazawa Y, Imamura T. PiggyBac Transposon-Mediated CD19 Chimeric Antigen Receptor-T Cells Derived From CD45RA-Positive Peripheral Blood Mononuclear Cells Possess Potent and Sustained Antileukemic Function. Front. Immunol. 13:770132. doi: 10.3389/fimmu.2022.770132, 2022
2. Tomida A, Yagyu S*, Nakamura K, Kubo H, Yamashima K, Nakazawa Y, Hosoi H, Iehara T. Inhibition of MEK pathway enhances the antitumor efficacy of chimeric antigen receptor T cells against neuroblastoma. Cancer Sci. Oct;112(10):4026-4036. 2021
3. Yagyu S*, Mochizuki H, Yamashima K, Kubo H, Saito S, Tanaka M, Sakamoto K, Shimoi A, Nakazawa Y. Lymphodepleted non-human primate model for the assessment of acute on-target and off-target toxicity of human CAR-T cells. Clin Transl Immunol 10(6):e1291, 2021
4. Nakamura K, Yagyu S*, Hirota S, Tomida A, Kondo M, Shigeura T, Hasegawa A, Tanaka M, Nakazawa Y. Autologous antigen-presenting cells efficiently expand piggyBac transposon chimeric antigen receptor T cells with predominant memory phenotype. Mol Ther Methods Clin Dev 21:315-324, 2021 (IF=6.698)
5. Kubo H, Yagyu S*, Nakamura K, Yamashima K, Tomida A, Kikuchi K, Iehara T, Nakazawa Y, Hosoi H. Development of non-viral, ligand-dependent, EPHB4-specific chimeric antigen receptor T cells for treatment of rhabdomyosarcoma. Mol Ther Oncolytics 20:646-658, 2021

固形腫瘍に対する非ウイルス遺伝子改変CAR-T細胞療法の開発

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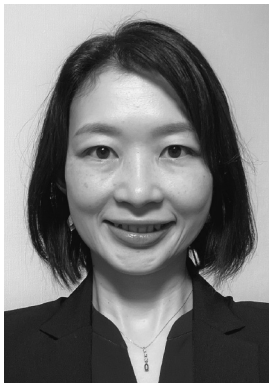
遺伝子改変キメラ抗原受容体T細胞（CAR-T細胞）は、B細胞性腫瘍に対して劇的な治療効果を示し、現在までに我が国でも複数の製剤が承認されるに至った。しかしながら、CD19-CAR-T細胞療法の劇的な治療効果を比較すると、固形腫瘍に対するCAR-T細胞療法の効果は未だ限定的である。この原因としては、1）標的となる腫瘍特異的抗原が少ない、2）固形腫瘍組織へのCAR-T細胞の集積と浸潤が弱い、3）腫瘍抗原の発現低下による免疫逃避、4）免疫抑制的な腫瘍微小環境におけるT細胞の機能喪失、5）CAR分子の過剰活性化による免疫疲弊など、様々な原因が複合的に考えられており、これらの問題点の克服のために、現在も工夫が重ねられている。最近の研究の進歩により、免疫疲弊因子の発現が少なく、メモリー機能の高いCAR-T細胞分画を含む製剤を投与された患者で、持続的な抗腫瘍効果を示すことが明らかとなってきた。つまり、固形腫瘍に対するCAR-T細胞の抗腫瘍効果の改善のためには「質の良いCAR-T細胞」を製造することが重要である。我々は、非ウイルス遺伝子改変法、とくにピギーバックトランスポゾンを用いたCAR-T細胞製造法の開発に取り組んでいる。その中でも、遺伝子導入法、細胞培養法を最適化することによって、メモリー機能が高く、免疫疲弊を受けにくいCAR-T細胞の製造法開発を行ってきた。実際に、血液腫瘍、固形腫瘍に対する複数のピギーバックトランスポゾンCAR-T細胞製剤を開発し、強く持続的なin vitro、in vivo抗腫瘍効果を証明することができた。また、一部のCAR-T細胞については、我が国での臨床応用を目指して、非臨床安全性試験を推進し、臨床応用間近となっている。本講演では、固形腫瘍に対するCAR-T細胞療法開発の潮流と、克服すべき問題点について概説し、われわれが取り組む固形腫瘍CAR-T細胞製剤開発について紹介する。

略 歴



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最近の関連出版物・論文

1. Gene and oligonucleotide delivery via micro- and nanobubbles by ultrasound exposure. Endo-Takahashi Y, Negishi Y, Drug Metab Pharmacokinet. 2022, 44:100445
2. Ternary Complexes of pDNA, Neuron-Binding Peptide, and PEGylated Polyethyleneimine for Brain Delivery with Nano-Bubbles and Ultrasound. Endo-Takahashi Y, Kurokawa R, Sato K, Takizawa N, Katagiri F, Hamano N, Suzuki R, Maruyama K, Nomizu M, Takagi N, Negishi Y. Pharmaceutics. 2021, 13(7):1003
3. Microbubbles and Nanobubbles with Ultrasound for Systemic Gene Delivery. Endo-Takahashi Y, Negishi Y, Pharmaceutics. 2020, 12(10):964
4. Preparation of Angiopep-2 Peptide-Modified Bubble Liposomes for Delivery to the Brain. Endo-Takahashi Y, Ooaku K, Ishida K, Suzuki R, Maruyama K, Negishi Y, Biol Pharm Bull. 2016;39(6):977-83
5. Systemic delivery of miR-126 by miRNA-loaded Bubble liposomes for the treatment of hindlimb ischemia. Endo-Takahashi Y, Negishi Y, Nakamura A, Ukai S, Ooaku K, Oda Y, Sugimoto K, Moriyasu F, Takagi N, Suzuki R, Maruyama K, Aramaki Y, Sci Rep. 2014, 4:3883

超音波応答性ナノバブルの開発と核酸・遺伝子治療への応用

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遺伝子・核酸医薬による疾患治療法の開発において、有用なDDS開発は重要課題とされている。体外からの外部刺激に応答したDDSは、そのエネルギーの適用エリアにより、比較的容易に標的組織特異的デリバリーが可能となる。なかでも超音波エネルギーは、その安全性の高さから注目され、マイクロバブル・ナノバブルなどの微小気泡と併用することで、超音波造影効果、遺伝子・核酸デリバリー効果の増強が可能とされている。これまでに我々は、脂質をベースとした超音波応答性ナノバブルを開発し、超音波造影、および遺伝子・核酸の細胞内導入を可能とすることを明らかとしてきた。さらに、造影診断と治療の両者に有用なバブル製剤の開発には、全身投与への適応が重要と考え、遺伝子・核酸搭載型ナノバブルの開発を進めてきた。全身投与を介した導入では、特に核酸は分解・排泄されやすいことが克服すべき課題であるうえ、超音波を利用した導入の駆動力となるナノバブルと核酸の同一挙動での循環が重要となる。これまでに、全身投与で有用な遺伝子・核酸搭載型ナノバブルとして、コレステロール修飾核酸を脂質膜に組み込んだナノバブル、核酸をカチオン性脂質により静電的に搭載したナノバブル、標的指向性を有するペプチドを利用した核酸複合体搭載ナノバブル、カチオン性多糖類コーティングを利用した核酸搭載ナノバブルなどを開発し、下肢筋組織・脳・腫瘍などにおける超音波造影や遺伝子・核酸デリバリーに成功した。本発表では、下肢虚血モデルや腫瘍モデルマウスを用いた検討における治療効果を交えながら、これらナノバブルと超音波併用による微小血管を介した遺伝子・核酸デリバリーについて紹介したい。

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最近の関連出版物・論文

1. Delivery of CRISPR-Cas tools for in vivo genome editing therapy: Trends and challenges Taha EA, Lee J, Hotta A. J Control Release. 2022 Feb;342:345-361. doi: 10.1016/j.jconrel.2022.01.013.
2. Low immunogenicity of LNP allows repeated administrations of CRISPR-Cas9 mRNA into skeletal muscle in mice Kenjo E, Hozumi H, Makita Y, Iwabuchi KA, Fujimoto N, Matsumoto S, Kimura M, Amano Y, Ifuku M, Naoe Y, Inukai N, Hotta A. Nat Commun. 2021 Dec 8;12(1):7101. doi: 10.1038/s41467-021-26714-w.
3. Efficient ssODN-Mediated Targeting by Avoiding Cellular Inhibitory RNAs through Precomplexed CRISPR-Cas9/sgRNA Ribonucleoprotein Kagita A, Lung MSY, Xu H, Kita Y, Sasakawa N, Iguchi T, Ono M, Wang XH, Gee P, Hotta A. Stem Cell Reports. 2021 Apr 13;16(4):985-996. doi: 10.1016/j.stemcr.2021.02.013.
4. Extracellular nanovesicles for packaging of CRISPR-Cas9 protein and sgRNA to induce therapeutic exon skipping Gee P, Lung MSY, Okuzaki Y, Sasakawa N, Iguchi T, Makita Y, Hozumi H, Miura Y, Yang LF, Iwasaki M, Wang XH, Waller MA, Shirai N, Abe YO, Fujita Y, Watanabe K, Kagita A, Iwabuchi KA, Yasuda M, Xu H, Noda T, Komano J, Sakurai H, Inukai N, Hotta A. Nat Commun. 2020 Mar 13;11(1):1334. doi: 10.1038/s41467-020-14957-y.
5. Targeted Disruption of HLA Genes via CRISPR-Cas9 Generates iPSCs with Enhanced Immune Compatibility Xu H, Wang B, Ono M, Kagita A, Fujii K, Sasakawa N, Ueda T, Gee P, Nishikawa M, Nomura M, Kitaoka F, Takahashi T, Okita K, Yoshida Y, Kaneko S, Hotta A. Cell Stem Cell. 2019 Apr 4;24(4):566-578.e7. doi: 10.1016/j.stem.2019.02.005.

ゲノム編集治療実現を目指した独自送達技術開発

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CRISPR-Cas9を始めとするゲノム編集技術の目覚ましい進歩により、多くの遺伝子変異疾患に対するゲノム編集療法が開発されつつある。しかし、ウイルスベクターが主流である従来の遺伝子“補充”療法とは異なり、ゲノム編集酵素の特性に合わせた一過性の送達技術が必要である。我々はデュシェンヌ型筋ジストロフィーの原因であるジストロフィンタンパク質を回復させるため、CRISPR-Cas9を用いたエクソンスキッピング法を開発し、患者由来iPS細胞でその有効性を確認した。また、ウイルス様粒子を用いたNanoMEDICタンパク質送達システムや、ワクチンで有名となった脂質ナノ粒子(LNP)によるmRNA送達技術など、様々なCRISPR送達技術を開発し、幅広く検討している。送達効率だけでなく、投与方法、生体内の動体や、免疫応答を含めた安全性など、様々な角度から検討ことで、より良い形での治療法を開発したい。我々が取り組んでいるCRISPRゲノム編集および送達技術の最新の知見を紹介する。

Symposium 7

Abstract & Curriculum Vitae

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他

最近の関連出版物・論文

山本武範，内田恵理子，山下拓真，井上貴雄：遺伝子治療用製品・遺伝子導入／改変細胞製品の品質・安全性に関する海外規制動向. *医薬品医療機器レギュラトリーサイエンス* 2022;53(2):72-84.
内田恵理子: 日本と海外規制の違い，カルタヘナ法について―遺伝子治療にかかるカルタヘナ法規制－. *薬理と治療* 2021;49 (11):1803-1806.
Uchida E：Regulations and safety assessment of genome editing technologies for human gene therapy. ***Translat Regulat Sci.*** 2020;2(3): 107-114.
Yamaguchi T, Uchida E, Okada T, Ozawa K, Onodera M, Kume A, Shimada T, Takahashi S, Tani K, Nasu Y, Mashimo T, Mizuguchi H, Mitani K, Maki K: Aspects of gene therapy products using current genome-editing technology in Japan. ***Human Gene Ther.*** 2020;31:1043-1053.
内田恵理子：日本における遺伝子治療の規制. *実験医学* 2020;38(2): 169-175.

遺伝子細胞治療の開発にかかる規制の最新動向

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遺伝子細胞治療は、対象疾患によっては極めて高い有効性を示す新たなモダリティとして期待されている。遺伝子治療製品は既に日米欧で17品目、日本でも7品目が承認されるなど実用化が進み、多様な製品開発が活発化している。一方、臨床開発を迅速に進めるには安全性や有効性を担保するための規制要件の理解が必要である。

現在、アカデミアが臨床研究を行う場合、in vivo遺伝子治療は「臨床研究法」と「遺伝子治療等臨床研究に関する指針」、ex vivo遺伝子治療は「再生医療等安全性確保法」に沿った開発を進める必要がある。このうち「遺伝子治療等臨床研究に関する指針」は「人を対象とする生命科学・医学系研究に関する倫理指針」と共に、個人情報保護法の令和2、3年度の改正施行を受けて今年3月に一部改正された。現在、「再生医療等安全性確保法」はin vivo遺伝子治療も対象とし、自由診療による遺伝子治療も法規制する方向で見直しが行われているが、法制化に伴い遺伝子治療指針もまた見直しが必要と考えられる。

一方、遺伝子治療は希少疾患を対象とする場合も多く国際共同治験が増加しているが、グローバル開発の促進には規制の国際調和が重要である。医薬品規制調和国際会議（ICH）では、遺伝子治療に関してこれまでに３つのICH見解を発出しているが、規制当局への拘束力があるICHガイドラインは作成されていなかった。しかし、遺伝子治療の実用化の進展を受け、初めて遺伝子治療製品を対象とするガイドライン案（ICH-S12遺伝子治療製品の非臨床生体内分布試験)が作成された。また、ICH-Q5Aガイドライン（ヒト又は動物細胞株を用いて製造されるバイオテクノロジー応用医薬品のウイルス安全性評価）は、遺伝子治療製品も対象とする方向で改正案の作成が進められている。本講演では、以上のような遺伝子治療の国内外の規制に関する最新の動向について紹介したい。

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再生医療等安全性確保法の見直しについて

岡本　圭祐、笹渕　美香

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平成26年11月に再生医療等の安全性の確保等に関する法律（平成25年法律第85号。以下「再生医療法」という。）及び薬事法等の一部を改正する法律が施行された。再生医療法は、再生医療等の迅速かつ安全な提供や普及の促進を図ることを目的としており、再生医療等（再生医療及び細胞治療）を臨床研究や自由診療として行う場合は、再生医療法の対象となる。同法では細胞培養加工施設の構造設備基準や、細胞を培養加工する上での基準が設けられたが、原則として医療機関内に制限されていた細胞培養加工を外部委託することが可能となった。再生医療法は現在、施行後5年を目処とした法の見直しのとりまとめが行われたところである。その中で、遺伝子治療技術については、遺伝子治療等臨床研究指針で定義する *in vivo* 遺伝子治療を含めた技術や、その関連技術を法の範囲に含めることとした。

令和２年度から５年間となる健康・医療戦略及び医療分野研究開発計画においては、モダリティ（技術・手法）等を軸とした６つの統合プロジェクトに再編され、３つ目の統合プロジェクトとして「再生・細胞医療・遺伝子治療プロジェクト」が位置付けられた。統合プロジェクトを踏まえて設置された協議会における議論も踏まえ、文部科学省・厚生労働省・経済産業省が研究等の支援を行っており、厚生労働省は、臨床研究に移行する研究に対する研究費の支援等を行っている。

本講演では、再生医療法の見直しや協議会での議論について、遺伝子治療技術に関する話題を中心に解説する。

略 歴

名前　　櫻井　陽

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研究分野　　ウイルス学
病原体診断学
レギュラトリーサイエンス（生物学製剤、先進医療医薬品）

学歴

1994-1998　京都大学薬学部
1999-2001　京都大学大学院理学研究科　修士課程
2001-2004　北海道大学大学院理学研究科　博士課程

職歴

2004-2007　ウイスコンシン大学マディソン校　博士研究員
2007-2009　警察庁 科学警察研究所　非常勤研究員
2009-2013　公益財団法人 東京都医学総合研究所　常勤研究員
2013-2014　同　主任研究員
2014-2017　独立行政法人 医薬品医療機器総合機構　ワクチン等審査部　審査専門員
2017-2019　同　ワクチン等審査部　主任専門員
2019-2020　同　ワクチン等審査部　審査役補佐
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2021-　同　スペシャリスト（バイオ品質担当）

最近の関連出版物・論文

1. Yoshiaki Maruyama, Akira Sakurai, Masaki Kasai *et al.*
Current status and future perspective of gene therapy products in JapanCell & Gene Therapy Insights, 7(3), 131-140 (2021)
2. 小川孝、櫻井陽、吉田智志　他　感染症の予防を目的とした組換えウイルスワクチンの開発に関する考え方医薬品医療機器レギュラトリーサイエンス, Vol. 51 No.12 658-668 (2020)
3. Akira Sakurai, Takashi Ogawa, Jun Matsumoto *et al.*
Regulatory aspects of quality and safety for live recombinant viral vaccines against infectious diseases in Japan
Vaccine 37 (43), 6573-6579 (2019)
4. Akira Sakurai, Katsuyoshi Takayama, Namiko Nomura et al.
Fluorescent immunochromatography for rapid and sensitive typing of seasonal influenza viruses
PLoS One 10 (2), e0116715 (2015)
5. Akira Sakurai, Katsuyoshi Takayama, Namiko Nomura et al.
Multi-colored immunochromatography using nanobeads for rapid and sensitive typing of seasonal influenza viruses
Journal of virological methods 209, 62-68 (2014)

カルタヘナ法の運用改善について

櫻井　陽

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本邦において、遺伝子組換え生物を医薬品等として治験を行う又は治療に用いる場合は、薬機法のみならずカルタヘナ法の規制も受ける。カルタヘナ法は、生物の多様性に関する条約におけるカルタヘナ議定書に対して国内担保法として制定された法律であるが、遺伝子組み換え生物を用いる医薬品等の開発においては障壁となっているのではないかという議論がかねてより存在する。しかしながら、カルタヘナ法は本邦独自の法ではなく、EUのDIRECTIVE 2001/18/ECに基づいて作成された欧州各国の国内法を参考に制定されている。したがって、規制の枠組みは欧州と大きな差はない。また、生物の多様性に関する条約を批准していない米国においても、国内法によってカルタヘナ法で要求されるものと同等の環境影響評価は必須とされており、承認申請までに必要な情報に日米欧に差はない。したがって、カルタヘナ法が開発障壁と感じられる原因は、法的な規制の問題ではなく、①行政・申請者ともに経験が乏しかったこと、②カルタヘナ法の申請に関する情報が少なかったこと、③カルタヘナ法の運用が医薬品等の開発の実情と一部かみ合っていなかったこと、が考えられた。

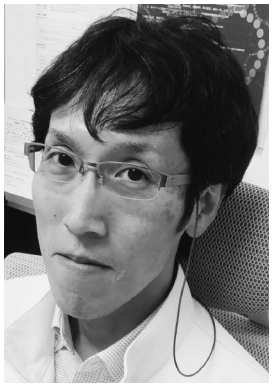
以上から、厚生労働省とPMDAは2019年以降、医薬品等の開発にかかるカルタヘナ法の運用改善に着手し、特に2021年以降は大きな運用改善を複数実施している。これらの改善については、改善着手後に申請を行った多くの申請者から高い評価を受ける一方で、まだ認知していない開発者が多く存在することも事実である。本発表では、厚生労働省とPMDAが2019年以降に実施した運用改善について説明する。

Symposium 8

Abstract & Curriculum Vitae

Neuromuscular Disorders

略 歴



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研究分野　　小児科学、神経科学、小児神経学

学歴
1998年　　：群馬大学医学部卒業
2004-2008年：群馬大学大学院医学系研究科博士課程修了

職歴
1998-2003年：日本赤十字社医療センター小児科・新生児未熟児科
2003-2004年：群馬大学附属病院
2005-2008年：日本学術振興会特別研究員DC1
2008-2009年：群馬大学附属病院小児科
2009-2015年：群馬大学大学院医学系研究科助教
2011-2013年：Dept. Neurology, Philipps University Marburg, Germany
2015-2016年：群馬大学大学院医学系研究科部内講師
2016-2017年：群馬大学附属病院講師
2017年-　　：自治医科大学小児科・遺伝子治療研究センター准教授

最近の関連出版物・論文

1. Kuwajima M, Kojima K, Osaka H, Hamada Y, Jimbo E, Watanabe M, Aoki S, Sato-Shirai I, Ichimoto K, Fushimi T, Murayama K, Ohtake A, Kohda M, Kishita Y, Yatsuka Y, Uchino S, Mimaki M, Miyake N, Matsumoto N, Okazaki Y, Ogata T, Yamagata T, Muramatsu K. Valine metabolites analysis in ECHS1 deficiency. ***Mol Genet Metab Rep***. 2021; 29:100809.
2. Kurokawa Y, Osaka H, Kouga T, Jimbo E, Muramatsu K, Nakamura S, Takayanagi Y, Onaka T, Muramatsu SI, Yamagata T. Gene Therapy in a Mouse Model of Niemann-Pick Disease Type C1. ***Hum Gene Ther.*** 2021; 32:589-598.
3. Kano K, Yamanaka G, Muramatsu K, Morichi S, Ishida Y, Takamatsu T, Suzuki S, Miyajima T, Nakagawa E, Nishino I, Kawashima H. Beta-propeller protein-associated neurodegeneration presenting Rett-like features: A case report and literature review. ***Am J Med Genet A.*** 2021; 185(2):579-583.
4. Kidokoro H, Yamamoto H, Kubota T, Motobayashi M, Miyamoto Y, Nakata T, Takano K, Shiba N, Okai Y, Tanaka M, Sakaguchi Y, Maki Y, Kawaguchi M, Suzuki T, Muramatsu K, Natsume J. High-amplitude fast activity in EEG: An early diagnostic marker in children with beta-propeller protein-associated neurodegeneration (BPAN). ***Clinical Neurophysiology.*** 2020; 131(9):2100-2104.
5. Saitsu H, Nishimura T, Muramatsu K (3名共筆頭著者), Kodera H, Kumada S, Sugai K, Kasai-Yoshida E, Sawaura N, Nishida H, Hoshino A, Ryujin F, Yoshioka S, Nishiyama K, Kondo Y, Tsurusaki Y, Nakashima M, Miyake N, Arakawa H, Kato M, Mizushima N, Matsumoto N. De novo mutations in the autophagy gene WDR45 cause static encephalopathy of childhood with neurodegeneration in adulthood. ***Nature Genetics.*** 2013; 45(4):445-9

オートファジー病の遺伝子治療

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オートファジーとは生命維持に必須の細胞成分分解機構である。オートファジー機構に関与する遺伝子は酵母の研究を発端に多数の重要な分子が同定されてきた。大隅先生のノーベル賞受賞もあり、オートファジーに関する分子生物学的な研究は、日本が世界をリードしている。一方、ヒトの生理機能への関与としては神経変性疾患だけではなく、心不全、がん、免疫異常、炎症性腸疾患や糖尿病など様々な病態において知られるようになっているが、まだ発展途上の段階である。

神経変性疾患ではオートファジー機能が直接関与する疾患を、オートファジー病と総称し、SENDA/BPAN（*WDR45*異常）とVici症候群（*EPG5*異常）が挙げられる。SENDA/BPANは小児期に知的障害やてんかんを呈し、成人期以降に急速に進行する運動機能障害と認知症を特徴とし、根治的治療法のない疾患である。脳内、特に黒質や淡蒼球に鉄が沈着する疾患で、Neurodegeneration with Brain Iron Accumulationの一型で、2013年に我々がオートファジー機能障害に基づく疾患であることを報告した。しかしながら、オートファジー機能障害と鉄代謝異常の関連など、まだ不明な点ばかりである

その後、動物モデルの解析も複数の研究グループにより実施され、高次機能障害は確認されているが、ヒトで生じる運動障害や脳内の鉄沈着はモデルの系統によって一定していない。原因遺伝子である*WDR45*はWIPI4をコードし、全身欠損でも生存可能であることから、WIPI1~WIPI4が相補的に機能している可能性が考えられている。WIPI4自体の機能もまだ明らかではなく、解析が進められている。

我々は、AMED等の支援の基に、患者由来細胞を用いた病態解明など、喫緊の課題である治療法開発を目指して前臨床試験に取り組んでいる。本シンポジウムではその成果についてお話したい。

略 歴

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研究分野　遺伝子治療、分子生物学、異染性白質ジストロフィー

学歴

1988～1992年　女子栄養大学
2005～2010年　日本医科大学（医学博士）

職歴

1992年～2003年　日本医科大学・第二生化学教室
各種ウイルスベクター（レトロウイルス、アデノウイルス、レンチウイルス、アデノ随伴ウイルスなど）の開発
2003年～2005年　スウェーデンLund大学（Dept. of Molecular Medicine and Gene Therapy）
造血幹細胞の増殖制御技術、新しい遺伝子導入法の開発
2005年～現在　生化学・分子生物学（分子遺伝学）
新規AAVベクターの検討、癌・遺伝病（特に異染性白質ジストロフィー）の遺伝子治療の開発

最近の関連出版物・論文

1. Miyake K, Miyake N and Shimada T. A new method for in vivo targeted gene transfer into oligodendrocytes using adenoviral and HIV vectors. Biomedical J. of Sci. & Tec. Res. 2021 Oct 39(3):31379-31385. doi:10.26717/BJSTR.2021.39.006306.
2. Matsumoto T, Miyake K, Miyake N, Iijima O, Adachi K, Narisawa S, Millán JL, Orimo H, Shimada T. Treatment with bone maturation and average lifespan of HPP model mice by AAV8-mediated neonatal gene therapy via single muscle injection. Mol. Ther. Methods Clin Dev. 2021 Jun 12;22:330-337. doi: 10.1016/j.omtm.2021.06.006.
3. Miyake N, Miyake K, Sakai A, Yamamoto M, Suzuki H, Shimada T. Treatment of adult metachromatic leukodystrophy model mice using intrathecal administration of type 9 AAV vector encoding arylsulfatase A. Sci Rep. 2021 Oct 15;11(1):20513. doi: 10.1038/s41598-021-99979-2.
4. Miyake N, Miyake K, Asakawa N, Yamamoto M, Shimada T. Long-term correction of biochemical and neurological abnormalities in MLD mice model by neonatal systemic injection of an AAV serotype 9 vector. Gene Ther. 2014 Apr;21(4):427-433. doi: 10.1038/gt.2014.17.
5. Miyake N, Miyake K, Yamamoto M, Hirai Y, Shimada T. Global gene transfer into the CNS across the BBB after neonatal systemic delivery of single-stranded AAV vectors. Brain Res. 2011 May 10;1389:19-26. doi: 10.1016/j.brainres.2011.03.014.

異染性白質ジストロフィーの遺伝子治療

三宅　紀子[1]、三宅　弘一[2]、酒井　真志人[1]、島田　隆[3]

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異染性白質ジストロフィー（Metachromatic Leukodystrophy:MLD）は、アリルスルファターゼA（Arylsulfatase A:ARSA）欠損により引き起こされる、常染色体劣性遺伝のライソゾーム病である。重篤な小児神経変性疾患であり、ARSAを介したスルファチドの機能的酵素分解の喪失は、ミクログリア、オリゴデンドロサイト、およびシュワン細胞のライソゾームにおける有毒なスルファチドの細胞内蓄積を引き起こし、中枢神経系および末梢神経系で広範な脱髄を引き起こす。その結果、中枢・末梢神経障害を引き起こす重度の神経症状を特徴としている。現在のところ、本疾患に対する有効な治療法はない。骨髄移植や酵素補充療法が報告されているが、神経障害の悪化を防ぐには治療効果が不十分である。神経変性を伴う疾患に対しては血液脳関門（Blood Brain Barea:BBB）の存在が大きな障害となり、有効な治療戦略が立てられていない。神経障害を治療するにはいかにBBBを通過するかと言うことが重要な鍵となってくる。近年、正常ARSA遺伝子を導入する遺伝子治療や、間葉系幹細胞、遺伝子・細胞併用療法などの開発が期待されている。レンチウイルスベクターを使った造血幹細胞遺伝子治療が発症を遅らせる効果があることが報告されているが、この方法は未発症患者にのみ有用なために、さらなる治療法の開発が必要である。我々はMLDの新規治療法の開発を目的にMLDモデルマウスを用いて、レトロウイルスベクターを用いたex vivo遺伝子治療、アデノ随伴ウイルス（Adeno-associated virus:AAV）ベクターを用いたin vivo遺伝子治療の研究を行っている。本シンポジウムではBBBを通過して中枢神経症状を改善する様々な遺伝子治療の方法を紹介するとともに、MLD遺伝子治療の今後の課題、展望について紹介したい。

CURRICULUM VITAE

Name Sandra P. Reyna, MD

Affiliation Novartis Gene Therapies

Field of Research Cardiovascular clinical genetics.

Professional Experience

Dr. Sandra P. Reyna received her M.D. degree from San Carlos University Medical School in Guatemala and subsequently pursued a career as a research physician in cardiovascular clinical genetics. She then trained in pediatrics at Primary Children's Hospital, Salt Lake City, Utah, and there continued her medical training in Neurology and Genetics. As an Assistant Professor of Neurology at the University of Utah, she led the Neurology Clinical Trials Unit as Director whilst keeping her appointment as Co-Director of the Pediatric Motor Disorders Research Program; and as Project Director of NeuroNEXT and StrokeNet, for the National Institute of Health awards.

Since 2005, Dr Reyna's academic career and research focus has been in neuromuscular disorders, with much of her time spent on Spinal Muscular Atrophy. Dr. Reyna led the SMA clinical trial network through Project Cure SMA, funded by CureSMA, which led to the CARNI-VAL trials and publications

After working at Massachusetts General Hospital from 2015 to 2016, she transitioned to her first industry position at Biogen. There she participated in the physician development program, gaining experience in Pharmacovigilance, Clinical Pharmacology and Clinical Development. She joined the SMA Clinical Research Development team and ultimately led all Nusinersen global trials with symptomatic and pre-symptomatic SMA affected children. She managed all medical aspects of the study, including medical monitoring, investigator interactions, subject recruitment and retention, event adjudication, data presentations, Global Steering Committee interactions and study data interpretation. She participated in the filing for Nusinersen and supported the launch of SPINRAZA. In February 2019, she transitioned to Bluebird Bio to work on gene therapy clinical trials for adrenoleukodystrophy. Throughout her time in industry, Dr. Reynahas stayed involved in multiple SMA and other movement disorders, initiatives and interest groups.

Currently she is Vice President of Medical Affairs, Global Therapeutic Area Head, where she is responsible for the Global Medical Affairs plan for SMA aligned with Novartis Gene Therapies' corporate strategy and tracking the execution of each tactic and in support of country launch teams.

Recent Related Publications (5 Papers)

- Chand DH, Mitchell S, Sun R, LaMarca N, Reyna SP, Sutter T. Safety of Onasemnogene Abeparvovec for Spinal Muscular Atrophy Patients Heavier than 8.5 kg in a Global Managed Access Program. Pediatr Neurol. 2022;132:27–32.
- Day JW, Mendell JR, Mercuri E, Finkel RS, Strauss KA, Kleyn A, Tauscher-Wisniewski S, Tukov FF, Reyna SP, Chand DH. Clinical Trial and Postmarketing Safety of Onasemnogene Abeparvovec Therapy. Drug Saf. 2021;44:1109–19.
- Chand DH, Zaidman C, Arya K, Millner R, Farrar MA, Mackie FE, Goedeker NL, Dharnidharka VR, Dandamudi R, Reyna SP. Thrombotic Microangiopathy Following Onasemnogene Abeparvovec for Spinal Muscular Atrophy: A Case Series. J Pediatr. 2021;231:265–8.
- Belter L, Jarecki J, Reyna SP, Cruz R, Jones CC, Schroth M, O'Toole CM, O'Brien S, Hall SA, Johnson NB, Paradis AD The Cure SMA Membership Surveys: Highlights of Key Demographic and Clinical Characteristics of Individuals with Spinal Muscular Atrophy. J Neuromuscul Dis. 2021;8:109–23.
- Elsheikh B, King W, Peng J, Swoboda KJ, Reyna SP, LaSalle B, Prior TW, Arnold WD, Kissel JT, Kolb SJ Outcome measures in a cohort of ambulatory adults with spinal muscular atrophy. Muscle Nerve. 2020;61:187–91.

Gene Therapy for Spinal Muscular Atrophy: Clinical and Real-World Experience and Clinical Program Update

Sandra P. Reyna, MD

Novartis Gene Therapies

First launched in the United States in 2019, gene therapy for spinal muscular atrophy (SMA) represents a significant milestone in the treatment of neuromuscular diseases. SMA is a debilitating, life-threatening, monogenic disease that leads to irreversible, progressive motor neuron loss as a result of mutations in the *survival motor neuron 1 (SMN1)* gene. This gene directs survival motor neuron (SMN) protein production critical for motor neuron development and maintenance. Onasemnogene abeparvovec (OA), a one-time, intravenously administered gene replacement therapy, delivers a functional copy of a human *SMN* gene to restore SMN protein expression. OA was designed to provide rapid and sustained expression of functional SMN protein, with patients in clinical trials experiencing early, clinically meaningful motor function benefits within the first month of treatment, profound improvement in survival, bulbar function (swallow, speech, and airway protection) and a durable response extending beyond 6 years. The efficacy and safety of OA have been demonstrated for presymptomatic infants (SPR1NT) and symptomatic patients with SMA type 1 (START, STRIVE-US, STRIVE-EU). As of March 2022, OA has been approved in >40 countries and >2,000 patients have been treated globally. OA is an important treatment option for patients and their caregivers who are unwilling or unable to maintain a long-term and perhaps lifelong alternatives. In later-onset SMA, there are no approved gene therapies providing constant expression of SMN protein via *SMN1* gene replacement. Thus, Novartis Gene Therapies is investigating in the safety and efficacy of intrathecal administration (IT) of OAV101 in a broad range of SMA patients, including those with later-onset disease. This seminar will provide the latest clinical program update and details on the evaluation of a potential -IT formulation of OA for older individuals with SMA. The seminar objectives include:

- Summarizing onasemnogene abeparvovec IV clinical and RWE experience
- Detailing two new and ongoing IV clinical programs
- Providing an overview of Novartis' IT clinical program, including the STEER and STRENGTH studies for older and later-onset SMA patients

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学歴
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職歴
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日本学術会議会員
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最近の関連出版物・論文

Tokuoka H, Imae R, Nakashima H, Manya H, Masuda C, Hoshino S, Kobayashi K, Lefeber DJ, Matsumoto R, Okada T, Endo T, Kanagawa M, Toda T. CDP-ribitol prodrug treatment ameliorates ISPD-deficient muscular dystrophy mouse model. ***Nat Commun***. 13:1847, 2022

Ujihara Y, Kanagawa M, Mohri S, Takatsu S, Kobayashi K, Toda T, Naruse K, Katanosaka Y. Elimination of fukutin reveals cellular and molecular pathomechanisms in muscular dystrophy-associated heart failure. ***Nat Commun***. 10:5754, 2019

SudoA, Kanagawa M, Kondo M, Ito C, Kobayashi K, Endo M, Minami Y, Aiba A, Toda T. Temporal requirement of dystroglycan glycosylation during brain development and rescue of severe cortical dysplasia via gene delivery in the fetal stage. ***Hum Mol Genet*** 27:1174-1185, 2018

Kanagawa M, Kobayashi K, Tajiri M, Manya H, Kuga A, Yamaguchi Y, Akasaka-Manya K, Furukawa JI, Mizuno M, Kawakami H, Shinohara Y, Wada Y, Endo T, Toda T. Identification of a Post-translational Modification with Ribitol-Phosphate and Its Defect in Muscular Dystrophy. ***Cell Rep*** 14:2209-2223, 2016

Taniguchi-Ikeda M, Kobayashi K, Kanagawa M, Yu CC, Mori K, Oda T, Kuga A, Kurahashi H, Akman HO, DiMauro S, Kaji R, Yokota T, Takeda S, Toda T. Pathogenic exon-trapping by SVA retrotransposon and rescue in Fukuyama muscular dystrophy. ***Nature*** 478:127-131, 2011

福山型筋ジストロフィーの治療戦略

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福山型筋ジストロフィー（FCMD）は本邦の小児期筋ジストロフィーの中ではデュシェンヌ型に次いで多く、先天性筋ジストロフィーに多小脳回などの脳形成障害を伴う常染色体性劣性遺伝疾患であり、90人に1人が保因者である。我々は原因遺伝子を同定し、遺伝子産物をフクチンと名付けた。フクチン遺伝子の変異によって発症し、ほとんどの患者は3'非翻訳領域にSVAレトロトランスポゾンの挿入変異を認める。

福山型は、muscle-eye-brain病(MEB)などと類似疾患とされる。我々は糖転移酵素POMGnT1の遺伝子がMEB原因遺伝子であることを明らかにした。FCMDやMEB、Walker-Warburg症候群、肢帯型2I型などに共通した病態として、αジストログリカンの糖鎖修飾異常が発見され、同様な糖鎖異常を発症要因とする疾患群はジストログリカン異常症と呼ばれる。我々は、さらにスプライス異常症という分子メカニズムと根本的治療法につながるアンチセンス核酸治療法を発見した。さらに1種類の核酸NS-035に最適化して、毒性安全性有効性試験を終了し、AMEDの支援を得て昨年医師主導治験を開始した。

またジストログリカン異常症の原因遺伝子のうち、フクチン、fukutin-related protein (FKRP)、ISPDの機能を明らかにし、糖鎖構造には、哺乳類で初めて存在が確認されたリビトールリン酸が含まれていることを発見した。ジストログリカン異常症の多くは、この修飾異常によって引き起こされる「リビトールリン酸異常症」といえる。さらにCDP-リビトール・テトラアセチル化誘導体による糖鎖修飾治療の有効性についても見出している。

本シンポジウムでは、FCMDの病態と遺伝子治療の可能性について概観する。

Symposium 9

Abstract & Curriculum Vitae

Genetic diseases

CURRICULUM VITAE

Name Toru Uchiyama

Affiliation National Center for Child Health and Development

Field of Research Gene Therapy for inborn errors of immunity

Education

1993-1999 Niigata University, School of Medicine, M. D.
2002-2005 Tohoku University, Graduate School of Medicine, Ph. D.

Professional Experience

1999-2000 Resident in Pediatrics, Niigata University School of Medicine, Japan
2001-2002 Medical staff in Pediatrics, Niigata Prefectural Central Hospital, Japan
2007-2010 Research Fellow in National Institutes of Health (NIH), USA
2011-2012 Assistant Professor in Pediatrics, Tohoku University School of Medicine, Japan
2013-(present) Chief, Department of Human Genetics, National Center for Child Health and Developemt (NCCHD), Japan
2020-(present) Director, Research and Development, Gene and Cell Therapy Promotion Center, NCCHD, Japan

Recent Related Publications (5 Papers)

1. Uchiyama T, Takahashi S, Nakabayashi K, Okamura K, Edasawa K, Yamada M, Watanabe N, Mochizuki E, Yasuda T, Miura A, Kato M, Tomizawa D, Otsu M, Ariga T, Onodera M. Nonconditioned ADA-SCID gene therapy reveals ADA requirement in the hematopoietic system and clonal dominance of vector-marked clones. *Mol Ther Methods Clin Dev.* doi: http://doi/org/10.1016/j.omtm.2021.10.003
2. Uchiyama T, Kawakami S, Masuda H, Yoshida K, Niizeki H, Mochizuki E, Edasawa K, Ishiguro A, Onodera M. A Distinct Feature of T Cell Subpopulations in a Patient with CHARGE Syndrome and Omenn Syndrome. *J Clin Immunol* 41: 233-237, 2021. doi: 10.1007/s10875-020-00875-7.
3. Ishikawa T, Okai M, Mochizuki E, Uchiyama T,Onodera M, Kawai T. BCG infections at high frequency in both AR-CGD and X-CGD patients following BCG vaccination. *Clin Infect Dis* doi: 10.1093/cid/ciaa1049.
4. Ishikawa T, Tamura E, Kasahara M, Uchida H, Higuchi M, Kobayashi H, Shimizu H, Ogawa E, Yotani N, Irie R, Kosaki R, Kosaki K, Uchiyama T, Onodera M, Kawai T. Severe Liver Disorder Following Liver Transplantation in STING-Associated Vasculopathy with Onset in Infancy. *J Clin Immunol*. 2021 Feb 5. doi: 10.1007/s10875-021-00977-w.
5. Igarashi Y, Uchiyama T, Minegishi T, Takahashi S, Watanabe N, Kawai T, Yamada M, Ariga T, Onodera M. Single cell-based vector tracing in patients with ADA-SCID treated with stem cell gene therapy. *Mol Ther Methods Clin Dev* 6: 8-16, 2017.

Progress of gene therapy for inborn errors of immunity

Toru Uchiyama

National Center for Child Health and Development

Transfer of therapeutic genes into hematopoietic stem cells and progenitor cells reconstitutes the functional immune system in patients with inborn errors of immunity (IEI), such as SCID-X1, ADA-SCID, Wiskott-Aldrich syndrome, and chronic granulomatous disease. A series of retroviral vector-mediated gene therapy trials confirmed this expectation, and most patients recovered from refractory infections. Whereas the use of retrovirus revealed the fundamental issue of vector integration, including the insertional mutagenesis, a lentiviral vector with a deletion of enhancer element has shown the improvements in the clinical manifestations without overexpression of proto-oncogene. The safety and efficacy feature now allows lentiviral gene therapy to be close to approval for IEI.
Gene editing using engineered nucleases shows the potential of targeted endogenous gene modifications, which could provide significant benefits to some IEI. Direct repair of the mutations could minimize the risk of insertional mutagenesis. Moreover, the transcription by the endogenous enhancer/promoter element on the chromosome enables a complete physiological expression of the genes. Modification of the mutant allele could also address the IEI with a dominant-negative form.These features allow genome editing to be potentially applicable to diseases that the gene addition strategy using viral vectors could not treat.Recently, clinical trials of CRISPR-based cell therapy have been launched for blood disorders, and their clinical efficacy also implies the promising results of these new strategies for IEI.

CURRICULUM VITAE

Name Hiroshi Kobayashi

Affiliation Division of Gene Therapy,
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The Jikei University School of Medicine

Field of Research Gene Therapy for Inborn Errors of Metabolism

Education

Ph.D. (Dr. of Medical Science), The Jikei university school of Medicine, Tokyo, Japan (2006)
M.D. Passed the Examination of National Board (1991)
B.A. Yamagata university, Yamagata-city, Japan

Professional Experience

1991-1993	Resident in Pediatrics, Jikei university school of Medicine, Tokyo, Japan
1994-2002, 2004-2007	Medical staff in Pediatrics Department of Pediatrics, Jikei university school of Medicine, Tokyo, Japan
2002-2004	Research Fellow, Department of Immunology and BMT, Childrens Hospital Los Angeles, CA, US.
2011-2021	Associate professor, Division of Gene Therapy, Research Center for Medical Sciences, Department of Pediatrics, Jikei university school of Medicine, Tokyo, Japan
2021-present	Professor, Division of Gene Therapy, Research Center for Medical Sciences, Department of Pediatrics, Jikei university school of Medicine, Tokyo, Japan

Recent Related Publications (5 Papers)

Koto Y, Sakai N., Lee Y, Kakee N., Matsuda J., Kobayashi H., et al. Prevalence of patients with lysosomal storage disorders and peroxisomal disorders: A nationwide survey in Japan Mol Genet Metab. 2021 Jul;133(3):277-288.
Morita M, Kaizawa T, Yoda T, Oyama T, Asakura R, Matsumoto S, Nagai Y, Watanabe Y, Watanabe S, Kobayashi H, Kawaguchi K, Yamamoto S, Shimozawa N, So T, Imanaka T. Bone marrow transplantation into Abcd1-deficient mice: Distribution of donor derived-cells and biological characterization of the brain of the recipient mice J Inherit Metab Dis. 2021 May;44(3):718-727.
Miwa S, Watabe AM, Shimada Y, Higuchi T, Kobayashi H, Fukuda T, et al. Efficient engraftment of genetically modified cells is necessary to ameliorate central nervous system involvement of murine model of mucopolysaccharidosis type II by hematopoietic stem cell targeted gene therapy. Mol Genet Metab. 2020;130(4):262-273.
Wada M, Shimada Y., Izuka S., IshiiN., Hiraki H., Tachibana T/. Maeda K., Saito M., Arakawa S., Ishimoto T.,Nakano T., Ida H., Ohashi T., Kobayashi H. Ex Vivo Gene Therapy Treats Bone Complications of Mucopolysaccharidosis Type II Mouse Models through Bone Remodeling Reactivation Mol Ther Methods Clin Dev. 2020 Sep 20;19:261-274.
Nojiri A, Anan I, Morimoto S, Kawai M, Sakuma T, Kobayashi M, (Kobayashi H) et al. Clinical findings of gadolinium-enhanced cardiac magnetic resonance in Fabry patients. Cardiol. 2020 Jan;75(1):27-33.

Research and Development of Gene and Cell therapy for lysosomal storage diseases

Hiroshi Kobayashi

Division of Gene Therapy, Research Center for Medical Sciences, The Jikei University School of Medicine

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Lysosomal storage disease is an inborn error of metabolism that results in the accumulation of substrates in organs due to dysfunction of enzymes and membrane proteins intrinsic to lysosomes. In lysosomal storage disease, enzymes secreted from normal cells are taken up through mannose 6-phosphate receptors on the surface of other cells, and adjacent enzyme-deficient cells also improve, so-called cross-collection mechanism. This mechanism allows enzyme replacement therapy (ERT) to work effectively, and many recombinant enzyme preparations are currently approved in Japan for more than 10 target diseases, and gene therapy has been developed in conjunction with ERT from an early stage. Currently, Clinical trials of gene therapy using viral vectors, mainly adeno-associated virus (AAV) or lentivirus, have already started mainly in overseas countries for lysosomal storage diseases including Gaucher disease, Fabry disease, metachromatic leukodystrophy (MLD), GM1/GM2 gangliosidosis, mucopolysaccharidosis type I, II, and III, Pompe disease (glycogenic type II), neural ceroid lipofuscinosis (NCL), and adrenoleukodystrophy (ALD) . In Japan, research and development of gene therapy is underway for diseases such as Niemann-Pick disease type C, aromatic L-amino acid decarboxylase (AADC) deficiency, GM2 gangliosidosis, and mucopolysaccharidosis type II. Adding gene therapy using viral vectors can be performed without taking into account genetic mutations or drug resistance, and the long-term expression of large amounts of enzyme proteins can improve tissues that have been difficult to treat in the past, such as the central nervous system and the bone system. However, long-term risk studies are yet to be conducted, and carcinogenicity due to insertional mutagenesis and shut-off, in which the efficacy declines over time, must be taken into consideration. On the other hand, clinical trials for editing gene therapy, which uses genome editing technology to repair disease-causing genes and return them to their normal sequence, have begun mainly in the United States. The status of research and development of these technologies and the efforts being made in Japan will be outlined.

CURRICULUM VITAE

Name Karin Kojima

Affiliation Department of Pediatrics, Jichi Medical University

Field of Research Pediatrics, pediatric neurology, gene therapy, human genetics, molecular genetics, neurodevelopmental disorder

Education

2001 Graduated from Faculty of Medicine, Jichi Medical University, Japan
2014 Graduated from Graduate School, Jichi Medical University, Japan

Professional Experience

2001-2003	Junior resident of internal medicine, Aomori Prefectural Central Hospital, Aomori, Japan
2003-2007	Medical director of internal medicine. National Health Insurance Hospital in Aomori, Japan
2007-2008	Senior resident of pediatrics,Jichi Children's Medical Center Tochigi, Tochigi, Japan
2008-2011	Medical director of Pediatric, Public health hospital, Nagasaki, Japan
2014-2017	Research associate, Department of Pediatrics, Jichi Medical University, Tochigi, Japan
2017-	Assistant Professor, Department of Pediatrics, Jichi Medical University, Tochigi, Japan
2019-2020	Postdoctoral associate, Research Scholar, Dr. Parchem lab, Center for Cell And Gene Therapy, Baylor College of Medicine, Houston, Texas, USA
2020-	Assistant Professor, Department of Pediatrics, Jichi Medical University, Tochigi, Japan
2021-	Manager in Pediatrics, Tochigi rehabilitation center hospital, Tochigi, Japan

Recent Related Publications (5 Papers)

1. Kojima K, Nakajima T, Taga N, Miyauchi A, Kato M, Matsumoto A, et al. Gene therapy improves motor and mental function of aromatic l-amino acid decarboxylase deficiency. Brain. 2019;142(2):322-33.
2. Keuls R, Kojima K, Lozzi B, Steele J, Chen Q, Gross S, et al. Mir-302 Regulates Glycolysis to Control Cell-Cycle during Neural Tube Closure. Int J Mol Sci. 2020;21(20):7534. soi: 10.3390/ijms21207534.
3. Onuki Y, Ono S, Nakajima T, Kojima K, Taga N, et al. Dopaminergic restoration of prefrontal cortico-putaminal network in gene therapy for aromatic l-amino acid decarboxylase deficiency. Brain Commun. 2021:3(3):fcab078. doi: 10.1093/braincomms/fcab078.
4. Kuwajima M, Kojima K, Osaka H, Hamada Y, Jimbo E, et al. Valine metabolites analysis in ECHS1 deficiency. Mol Genet Metab Rep. 2021;(29):100809. doi: 10.1016/j.ymgmr.2021.100809.
5. Kojima K, Shirai K, Kobayashi M, Miyauchi A, Saitsu H, Matsumoto N, et al. A patient with early myoclonic encephalopathy (EME) with a de novo KCNQ2 mutation. Brain Dev. 2018;40(1):69-73.

Long-term efficacy of gene therapy for AADC deficiency using AAV2-AADC vector

Karin Kojima

Department of Pediatrics, Jichi Medical University

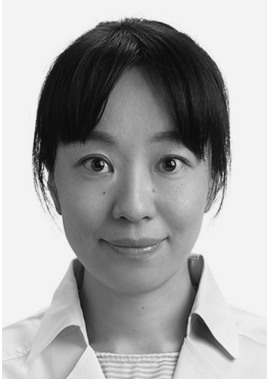
Aromatic L-amino acid decarboxylase (AADC) deficiency (OMIM #608643) is an autosomal recessive neurotransmitter disorder caused by defects in the DDC gene, which encodes AADC. AADC catalyzes the formation of neurotransmitters from L-DOPA and 5-hydroxytryptophan to dopamine and serotonin, respectively. The main phenotype of AADC deficiency is movement disorder, including loss of voluntary movements, hypotonia, intermittent oculogyric crisis (OGC) and limb dystonia. Patients also present with autonomic dysfunction, intellectual disability and emotional instability. Patients with AADC deficiency are classified as severe type (80%: bedridden and fully dependent), mild (5%: ambulatory without assistance, intellectual disability) and moderate (15%: between severe and mild).

Since 2015, we have been performing gene therapy for AADC deficiency as a clinical study using an AADC-expressing AAV2 vector. Today, we report on the long-term course after gene therapy.

Eight patients with AADC deficiency (7 severe type and 1 moderate type) were subjected to gene therapy by injecting the AAV2 vector carrying the DDC gene into the putamen on both sides by stereotactic brain surgery.

During the maximum 6 and a half years after gene therapy, improvement of symptoms continues in all cases, to varying degrees. Before treatment, seven severe type patients had no head control and had frequent dystonia attacks with full assistance. However, 6 of severe patients are able to control their heads and to use walkers. All dystonia attacks disappeared. One moderate type patient was assisted walking before gene therapy, however, now she goes to school on her own. She gains intellectual development, which has reached a normal level. As a result of PET image analysis using FMT, which is an AADC tracer, FMT accumulation in the putamen is expressed at the same level as after 5 years and 6 months after treatment, and the effect of gene therapy is sustained for a long period of time. Resting fMRI activity was compared before and after surgery, and the functional connectivity of the basal ganglia centered on the putamen was improved after treatment.Although there was a difference in efficacy depending on the severity of the gene mutation site and the age at the time of treatment, gene therapy improved clinical symptoms in all cases, and the effect of gene therapy was maintained even after 6 years. It is considered that the recovery of dopamine in the putamen by this treatment promoted the functional recovery of the basal ganglia network. We are conducting a doctor-led clinical trial of gene therapy for AADC deficiency.

CURRICULUM VITAE



Name Reiko Arakawa

Affiliation Department of Genomic Medicine,
National Center for Global Health and Medicine
Medical Genomics Center,
National Center for Global Health and Medicine

Field of Research Medical genetics, Neuromuscular disorders

Education

2007-2011 Affiliated Field of Medical Genetics, Division of Biomedical Engineering and Science, Graduate Course of Medicine, Graduate School of Tokyo Women's Medical University, Tokyo, Japan

Professional Experience

2011-2018 Assistant professor of Institute of Medical Genetics, Tokyo Women's Medical University
2019-2022 Department of Genomic Medicine, National Center for Global Health and Medicine

Recent Related Publications (5 Papers)

1. Effective Valproic Acid Treatment in Motor Function is Caused by Possible Mechanism of Elevated Survival Motor Neuron Protein Related with Splicing Factor Gene Expression in Spinal Muscular Atrophy. Takano K, Arakawa R, et al. Tokyo Women's Medical University Journal.2022
2. Analysis of spinal muscular atrophy-like patients by targeted resequencing. Hosokawa S, Arakawa R, et al. Brain Dev. 42.148-156.2020
3. Identification of a peripheral blood cell population capable of monitoring the level of survival motor neuron protein. Otsuki N, Arakawa R, et al. PLoS One. e0201764.2018
4. Relationships between long-term observations of motor milestones and genotype analysis results in childhood-onset Japanese spinal muscular atrophy patients. Kaneko K, Arakawa R, et al. Brain Dev. 39.763-773.2017
5. Imaging flow cytometry analysis to identify differences of SMN protein expression in spinal muscular atrophy patients. Arakawa R, et al. Pediatric Neurology.61.70-75.2016

Implementation of gene therapy for spinal muscular atrophy using an adeno-associated virus vector

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Spinal muscular atrophy (SMA) is a hereditary neuromuscular disease caused by a deficiency of the survival motor neuron (SMN) protein. Due to progressive muscular atrophy and respiratory distress, severe type I disease often requires ventilator management by the age of two years. In recent years, three new drugs for SMA have been launched. Although the mechanisms of action of these three drugs are divergent, they supplement the function of the SMN protein. One such drug is "Zolgensma® Intravenous Infusion" (hereinafter referred to as "Zolgensma"), which was the first gene therapy drug to be launched in Japan using an adeno-associated virus (AAV) vector.
Zolgensma is a gene therapy drug that uses the AAV9 vector to supplement the function of the *SMN1* gene and was endorsed by medical insurance companies in May, 2020. It is intended for SMA patients under two years of age and has been administered to 53 patients in Japan as of February 2022. The *SMN* gene introduced by Zolgensma does not integrate into the patient's chromosome and remains as an episome in the nucleus of the cell. Since it remains stable for a long period of time in end-division cells such as motor neurons, it is administered intravenously only once.
Zolgensma is a breakthrough drug that allows patients with type I SMA to obtain self-reliance without the need for mechanical ventilation. For an optimal therapeutic effect, administration in the early postnatal period is necessary and is indicated even before the onset of symptoms. With the availability of therapeutic agents, early diagnosis of SMA is required and some local governments have included SMA in newborn mass screening, from when, presymptomatic administration of Zolgensma can start.

Symposium 10

Abstract & Curriculum Vitae

Cancer Gene Therapy 2

CURRICULUM VITAE

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Field of Research Cancer gene therapy, Molecular oncology, Cancer immunology

Education

1987 Shinshu University, School of Medicine, Japan
1995 Ph.D. degree in Medicine, Shinshu University

Professional Experience

1987-1989 Trainee, Department of Neurology, School of Medicine, Shinshu University
1989-1992 Resident, Medical Oncology, National Cancer Center Hospital
1992-1994 Chief resident, Medical Oncology, National Cancer Center Hospital
1994-1997 Researcher, Genetics Division, National Cancer Center Research Institute (NCCRI)
1997-1999 Researcher, Howard Hughes Medical Institute, University of Michigan, USA
1999-2010 Head, Section for Studies on Host-Immune Response, NCCRI

2010-2017 Chief, Division of Gene and Immune Medicine, NCCRI
2017-present Chief, Department of Immune Medicine, NCCRI
2018-present Deputy Director, NCCRI

Recent Related Publications (5 Papers)

1. Yamamoto Y, Nagasato M, Rin Y, Henmi M, Ishikawa Y, Yachida S, Ohki E, Hiraoka N, Tagawa M, Aoki K: Strong antitumor efficacy of a pancreatic tumor-targeting oncolytic adenovirus for neuroendocrine tumors. ***Cancer Med*** 6: 2385-2397, 2017.
2. Hirata A, Hashimoto H, Shibasaki C, Narumi K, Aoki K. Intratumoral IFN-α gene delivery reduces tumor-infiltrating regulatory T cells through the down-regulation of tumor CCL17 expression. ***Cancer Gene Ther*** 26:334-343, 2019.
3. Narumi K, Miyakawa R, Ueda R, Hashimoto H, Yoshida T, Aoki K. Local Administration of GITR agonistic antibody induces an antitumor immunity. ***Sci. Rep.*** Article number: 5562, 2019. doi:10.1038/s41598-019-41724-x.
4. Kudo-saito C, Miyamoto T, Imazeki H, Shouji H, Aoki K, Boku N. IL33 is a key driver of treatment resistance of cancer. ***Cancer Res.*** 2020 80: 1981-1990, 2020.
5. Arakawa A, Ichikawa H, Kubo T, Motoi N, Kumamoto T, Nakajima M, Yonemori K, Noguchi E, Sunami K, Kuroda T, Kiyokawa T, Yamada K, Yanaihara N, Takahashi K, Okamoto A, Hirabayashi S, Hasegawa D, Manabe A, Ono K, Matsuoka M, Arai Y, Togashi Y, Shibata T, Nishikawa H, Aoki K, Kohno T, Ogawa C. Two cases of mother-to-"infant" cancer transmission in mothers with cervical cancer: potential risk of vaginal delivery and strong response of child to immune checkpoint inhibition therapy. ***NEJM*** 384:42-50, 2021.

Exploration of novel targets for the development of lung cancer immunotherapy based on the immune tumor microenvironment

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The lung cancers are the leading cause of cancer deaths in Japan and worldwide. Although the identification of immune checkpoint blockade (ICB) is changing the approaches to cancer treatment including non-small cell lung cancer (NSCLC), resistance to immune checkpoint blockades remains an issue in clinical oncology; 20% of NSCLC patients respond to anti PD-1/PD-L1 therapy. The identification of novel target for the development of immune therapy and gene and cell therapy is awaited for the patients with NSCLC.

Since the responsiveness of immune therapy is mainly determined by the immune status of tumor microenvironment (TME), it is promising to discriminate the immunological subtype from the viewpoint of tumor infiltrating lymphocytes (TILs). To examine the immunological characteristics of TME, we constructed the integrated data base of TIL profiling, RNA-seq, whole exsome seq and clinico-pathological findings using 282 fresh resected tissues of lung cancer. The multicolor flow cytometry differentiated 30 cell types of TILs, and to understand the characteristics of TILs in NSCLC, we first compared the percentage and number of each immune cell type in tumor specimens with those in normal adjacent lung tissues (NATs). NSCLC tissues showed the composition of $CD4^+$ T cells, $CD8^+$ T cells, B cells and effector Tregs was increased, and conversely that of myeloid cells including macrophage, monocytic myeloid-derived suppressor cells and NK cells was decreased in NSCLC tissues than NATs.

The unsupervised clustering of TIL showed that adenocarcinoma (LUAD) as well as squamous cell carcinoma (LUSQ) were divided into 3 distinct immune subtypes (cold-, myeloid cell-, CD8 T cell-dominant subtype), respectively. The patient prognosis was significantly correlated with the immune subtypes, and, especially, the $CD8^+$ T cell-subtype showed the better patient outcome compared to other 2 subtypes.

The RNA-seq analysis showed that the expression of IFN-g and granzyme B and scores of type I IFN and IFN-g signatures were elevated in $CD8^+$ T cell-subtype, indicating that the immune reaction was induced in $CD8^+$ T cell-subtype in LUAD and LUSQ. The immunogram showed the immunologically disturbed steps on the cancer immunity cycles in LUAD and LUSQ. Gene enrichment analysis showed that specific signaling pathways were significantly activated or suppressed in respective immune subtypes common to LUAD and LUSQ.

It may be possible to change the immunosuppressive TME to an anti-tumorigenic state by regulating the immune subtype-specific pathways and manipulating responsible genomic alterations, which could enhance the antitumor immune response by immune therapy, leading to a personalized immune therapy system.

CURRICULUM VITAE

Name Takafumi Nakamura

Affiliation Tottori University Faculty of Medicine

Field of Research His research interests include 1) oncolytic virotherapy, 2) viral vector development, 3) cancer immunotherapy, 4) viral entry and fusion for infection, and 5) tumor biology. He has published over 50 articles including major academic journals such as Nature Biotechnology, Science Translational Medicine, Molecular Therapy, Cancer Research and Clinical Cancer Research, and has registered over 15 patents. Recently, he has been focusing on translating his next-generation oncolytic vaccinia viral vectors into clinic.

Education
He graduated School of Life Science (1997) and Graduate School of Medical Sciences (2001) at Tottori University, and was received his PhD from Tottori University (2001).

Professional Experience
He joined Professor Stephen J. Russell laboratory in molecular medicine program at Mayo Clinic,Rochester, MN, USA as a postdoctoral research fellow (2002 - 2004) and research associate (2004 - 2005). He worked as Sakigake Researcher (2006 - 2009) and Project Associate Professor (2009 - 2012) at The University of Tokyo, Japan. In 2012, he was appointed as an Associate Professor in the Division of Molecular Medicine, Graduate School of Medical Sciences, Tottori University, Japan.

Recent Related Publications (5 Papers)
1. Horita K, Kurosaki H, Nakatake M, Kuwano N, Oishi T, Itamochi H, Sato S, Kono H, Ito M, Hasegawa K, Harada T, Nakamura T. LncRNA UCA1-mediated Cdc42 signaling promotes oncolytic vaccinia virus cell-to-cell spread in ovarian cancer. Mol Ther Oncolytics 13: 35–48, 2019.
2. Nakatake M, Kurosaki H, Kuwano N, Horita K, Ito M, Kono H, Okamura T, Hasegawa K, Yasutomi Y, Nakamura T. Partial deletion of glycoprotein B5R enhances vaccinia virus neutralization escape while preserving oncolytic function. Mol Ther Oncolytics 14: 159-171, 2019.
3. Nakao S, Arai Y, Tasaki M, Yamashita M, Murakami R, Kawase T, Amino N, Nakatake M, Kurosaki H, Mori M, Takeuchi M, Nakamura T. Intratumoral Expression of IL-7 and IL-12 Using an Oncolytic Virus Increases Systemic Sensitivity to Immune Checkpoint Blockade. Science Translational Medicine 12: eaax7992, 2020.
4. Nakatake M, Kuwano N, Kaitsurumaru E, Kurosaki H, Nakamura T. Fusogenic oncolytic vaccinia virus enhances systemic antitumor immune response by modulating the tumor microenvironment. Molecular Therapy 29: 1782-1793, 2021.
5. Kurosaki H, Nakatake M, Sakamoto T, Kuwano N, Yamane M, Ishii K, Fujiwara Y, Nakamura T. Anti-Tumor Effects of MAPK-Dependent Tumor-Selective Oncolytic Vaccinia Virus Armed with CD/UPRT against Pancreatic Ductal Adenocarcinoma in Mice. Cells 10: 985, 2021.

Fusogenic oncolytic vaccinia virus enhances systemic antitumor immune response by modulating the tumor microenvironment

Takafumi Nakamura

Tottori University Faculty of Medicine

Vaccinia virus, once widely used for smallpox vaccine, has been genetically engineered and used as an oncolytic viral vector for cancer virotherapy. The clinical benefits of oncolytic virotherapy are limited due to tumor heterogeneity and the complexity of tumor microenvironment. To address questions, we have found fusogenic vaccinia virus (FUVAC) exerts higher antitumor effects by inducing stronger antitumor immunity than non-fusogenic conventional vaccinia virus (MDRVV). Using a bilaterally tumor-bearing syngeneic mouse model, unilateral administration of FUVAC more efficiently enhanced CD8+ T cell infiltration and inhibited tumor growth in not only treated tumors but also untreated tumors than MDRVV. Interestingly, FUVAC reduced tumor-associated immune suppressive cells such as regulatory T cells, myeloid-derived suppressor cells and tumor-associated macrophages in the injected tumor. In accordance with the change for the better, the anticancer effects of FUVAC in both injected and non-injected tumors were completely suppressed by depletion of CD8+ T cells. Furthermore, the simultaneous expression of immune-modulating genes in FUVAC augmented the antitumor activity and achieved complete response in both treated and untreated tumors. Single-cell analysis of tumor microenvironment revealed new insight to therapeutic response of the armed FUVAC. Thus, FUVAC would be better therapeutic platform as a next-generation oncolytic vaccinia virus and be suitable for a novel immune modulator for overcoming oncolytic virus-resistant tumors.

CURRICULUM VITAE

Name Koji Tamada

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Field of Research Cancer Immunology

Education

1986-1992	Kyushu University, Department of Medicine, Japan
1994-1998	Graduate Student, Medical Institute of Bioregulation, Kyushu University, Japan

Professional Experience

1992-1993	Residency, Spinal Injuries Center, Japan
1993-1994	Residency, Kyushu University Hospital, Japan
1998-2002	Post-doctoral fellow, Mayo Clinic College of Medicine, US
2002-2004	Research Associate, Department of Immunology Mayo Clinic College of Medicine, US
2004-2007	Assistant Professor, Johns Hopkins University School of Medicine, US
2007-2009	Assistant Professor, University of Maryland School of Medicine, US
2009-2011	Associate Professor, University of Maryland School of Medicine, US
2011-present	Professor and Chairman, Department of Immunology Yamaguchi University of Graduate School of Medicine, Japan
2016-present	Director, Yamaguchi University Science Research Center, Japan
2016-present	Adjunct Professor, The Institute of Medical Science, University of Tokyo, Japan

Recent Related Publications (5 Papers)

1. Tokunaga Y, Sasaki T, Goto S, Adachi K, Sakoda Y, Tamada K. Enhanced Antitumor Responses of Tumor Antigen-Specific TCR T Cells Genetically Engineered to Produce IL7 and CCL19, Mol Cancer Ther. 2022 Jan;21(1):138-148.
2. Mori J, Adachi K, Sakoda Y, Sasaki T, Goto S, Matsumoto H, Nagashima Y, Matsuyama H, Tamada K. Anti-tumor efficacy of human anti-c-met CAR-T cells against papillary renal cell carcinoma in an orthotopic model. Cancer Sci. 2021 Apr;112(4):1417-1428.
3. Goto S, Sakoda Y, Adachi K, Sekido Y, Yano S, Eto M, Tamada K. Enhanced anti-tumor efficacy of IL-7/CCL19-producing human CAR-T cells in orthotopic and patient-derived xenograft tumor models. Cancer Immunol Immunother. 2021 Sep;70(9):2503-2515.
4. Nakajima, M., Sakoda, Y., Adachi, K., Nagano, H., Tamada, K. Improved survival of CAR-T and tumor-specific T cells caused by anti-PD-1 scFv-producing CAR-T cells. Cancer Science. 2019 Oct;110(10):3079-3088.
5. Adachi K, Kano Y, Nagai T, Okuyama N, Sakoda Y, Tamada K. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. Nature Biotechnology. 2018 Apr;36(4):346-351.

Novel technologies of CAR-T cell therapy for solid cancers

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Gene-modified T cells utilizing artificial chimeric antigen receptor (CAR) have been rapidly developed and translated into clinic in recent years. Outstanding clinical effects in hematological malignancies have been demonstrated, resulting in regulatory approval of anti-CD19 and anti-BCMA CAR-T cell therapies for refractory or relapsing B cell acute lymphoblastic leukemia/ lymphoma and multiple myeloma, respectively. On the other hand, CAR-T cell therapy in solid tumors has yet to be fully developed, as only a few exceptional cases have been reported to demonstrate clinical efficacy. Potential hurdles for the effects of CAR-T cell therapy in solid tumors include heterogeneity of tumor-associated targets, insufficient migration and infiltration of CAR-T and endogenous immune cells into tumor tissues, and immunosuppressive nature of tumor microenvironment. Various modifications and improvement of CAR-T cells to overcome these hurdles have been actively investigated by worldwide researchers.

To achieve this goal, our group has developed novel CAR technology which enables CAR-T cells to simultaneously produce interleukin-7 (IL-7) and CCL19, aiming at efficient accumulation, expansion, and survival of immune cells inside solid tumor tissues. Treatment of pre-established solid tumors with IL-7/CCL19-producing CAR-T cells achieved a potent inhibition of tumor growth followed by prolonged mouse survival and long-term memory response specific to tumor. IL-7/CCL19-producing CAR-T cell therapy was associated with massive accumulation of immune cells including both the transferred CAR-T cells and endogenous T cells and DC in the tumor tissues. Host tumor-reactive T cells specific to endogenous tumor antigens were found to play an important role in the IL-7/CCL19-producing CAR-T cell therapy. In addition, combination of IL-7/CCL19-producing CAR-T cells with anti-PD-1 mAb demonstrated synergistic effects. Thus, our studies propose a novel CAR platform technology to improve the anti-tumor effects against solid tumors.

CURRICULUM VITAE

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Recent Related Publications (5 Papers)
1. Zhang M, Kiyono T, Aoki K, Goshima N, Kobayashi S, Hiranuma K, Shiraishi K, Saya H, Nakahara T. Development of an in vitro carcinogenesis model of HPV induced cervical adenocarcinoma. Cancer Sci. 2022 Mar;113(3):904-915. doi: 10.1111/cas.15246.
2. Kato Y, Tabata H, Sato K, Nakamura M, Saito I, Nakanishi T. Adenovirus vectors expressing eight multiplex guide RNAs of CRISPR/Cas9 efficiently disrupted diverse Hepatitis B Virus gene derived from heterogeneous patient, Int J Mol Sci. 2021; 22(19)10570. doi: 10.3390/ijms221910570.
3. Nakanishi T, Maekawa A, Suzuki M, Tabata H, Sato K, Mori M, Saito I. Construction of adenovirus vectors simultaneously expressing four multiplex, double nicking guide RNAs of CRISPR/Cas9 and in vivo genome editing, Sci Rep.2021;11(1)3961. doi: 10.1038/s41598-021-83259-0.
4. Hirose S, Murakami N, Takahashi K, Kuno I, Takayanagi D, Asami Y, Matsuda M, Shimada Y, Yamano S, Sunami K, Yoshida K, Honda T, Nakahara T, Watanabe T, Komatsu M, Hamamoto R, Kato MK, Matsumoto K, Okuma K, Kuroda T, Okamoto A, Itami J, Kohno T, Kato T, Shiraishi K, Yoshida H. Genomic alterations in STK11 can predict clinical outcomes in cervical cancer patients. Gynecol Oncol. 2020; 156(1):203-210. DOI: 10.1016/j.ygyno.2019.10.022
5. Murakami I, Egawa N, Griffin H, Yin W, Kranjec C, Nakahara T, Kiyono T, *Doorbar J. Roles for E1-independent replication and E6-mediated p53 degradation during low-risk and high-risk human papillomavirus genome maintenance. PLoS Pathog. 2019;15(5):e1007755.

Development of HPV genome-targeted gene therapy for HPV induced cancers by an all-in-one adenovirus vector expressing multicopy guide RNAs and Cas9 nickase

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Oncogenic human papillomavirus (HPV) infection is responsible for almost 5% of all cancer worldwide. Two major HPV associated cancers are cervical cancer and oropharyngeal cancer. HPV16 is the most prevalent HPV type in these cancers followed by HPV18. It is well established that neoplastic progression of HPV-associated cancers and their precursor lesions depends on the expression of viral oncogenes, E6 and E7. However, no specific treatment targeting HPV oncogenes has been developed. HPV is an ideal target for the gene therapy with genome-editing technology because HPV contains double-stranded circular DNA genome and its presence is necessary for the development and maintenance of the cancers. In this research, we aimed to develop a novel therapy for HPV-associated malignancy by introducing CRISPR/Cas9-generated DNA breaks in HPV genomes using a recombinant adenovirus vector.

A major obstacle for clinical application of CRISPR/Cas9 gene editing technology is its off-target effects. A double-nicking technology is inducing double stranded DNA breaks by introducing two nicks on both DNA strands using two gRNAs and Cas9 nickase which contains endonuclease activity to single stranded DNA. It is shown that a double nicking technology can reduce off-target effects to a negligible level. We developed an adenovirus vector expressing multiplex gRNAs based on cosmid amplification in a novel polygonal structure. In order to eliminate HPV16 genome from cancer cells completely, we aimed to develop a novel adenovirus vector simultaneously expressing Cas9 nickase and 4 double nicking gRNA pairs (8 gRNAs) targeting HPV16 oncogenes to introduce multiple DNA breaks. First, the efficacy of gRNAs to introduce a DNA break in a cell free system as well as in HPV16 positive cervical cancer cells (SiHa) was examined and 16 possible double nicking gRNA pairs were selected from initial 96 candidates. Second, the combinations of multiple double nicking gRNA pairs were introduced to SiHa cells together with Cas9 nickase expression and the supressive effects on cell growth was evaluated. An all-in-one recombinant adenovirus expressing the most effective 8 gRNAs and Cas9 nickase was successfully generated. This all-in-one adenovirus vector strongly suppressed proliferation of HPV16 positive cervical cancer, its precursor and oropharyngeal cancer cells. Importantly, a little to no cytotoxicity was observed in HPV negative, normal epithelial cells. Intratumoral administration of the adenovirus vector suppressed tumor growth in a mouse xenograft model with cervical cancer cells. Furthermore, analysis for possible off-target effects by amplicon-sequencing indicated that the off-target effects of Cas9 nickase was less than 1/1000 of that of the wild type Cas9 in cervical cancer cells with the same gRNA set. Taken all together, these results indicated that the adenovirus vector we developed is safe and effective and is ready for further development to practical application.

Symposium 11

Abstract & Curriculum Vitae

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平成26年4月1日–	大阪大学大学院医学系研究科免疫再生制御学講座　特任准教授
平成28年7月1日–	大阪大学大学院医学系研究科先進幹細胞治療学講座　特任教授
平成30年4月1日–	大阪大学大学院医学系研究科最先端再生医療学共同研究講座　特任教授
令和3年7月1日–	大阪大学大学院医学系研究科心臓血管外科学教授

最近の関連出版物・論文

1. Miyagawa S, Toda K, Nakamura T, et al. Building a bridge to recovery: the pathophysiology of LVAD-induced reverse modeling in heart failure. *Surg Today*. 2016;46(2):149-154.
2. Miyagawa S, Sawa Y. Building a new strategy for treating heart failure using Induced Pluripotent Stem Cells. *J Cardiol*. 2018;72(6):445-448.
3. Miyagawa S, Kawamura T, Ito E, et al. Evaluation of the Efficacy and Safety of a Clinical Grade Human Induced Pluripotent Stem Cell-Derived Cardiomyocyte Patch: A Pre-Clinical Study. *bioRxiv*. 2021:2021.2004.2007.438744.
4. Miyagawa S, Domae K, Yoshikawa Y, et al. Phase I Clinical Trial of Autologous Stem Cell-Sheet Transplantation Therapy for Treating Cardiomyopathy. *J Am Heart Assoc*. 2017;6(4).
5. 宮川 繁. 重症心不全に対する再生治療：大阪大学から世界に発信する再生医療の実際. *Pediatric Cardiology and Cardiac Surgery*. 2021;37(2):73-77.

重症心不全に対する心筋組織移植を用いたトランスレーショナルリサーチ

宮川　繁

大阪大学大学院医学系研究科心臓血管外科

これまで重症心不全の克服を目指し、重症心不全に対して、再生医療技術を用いて人工組織を作成し移植する新しい治療法の開発を行っている。今回、我々は、重症心不全に対する細胞シートを用いたトランスレーショナルリサーチについて報告する。

我々は、新しい心不全の治療法として、細胞シートを用いた組織移植法を開発した。自己筋芽細胞シートを小大動物心不全モデルに移植したところ、心機能の改善、生存率の向上がみられた。筋芽細胞シートの再生効果は、筋芽細胞より分泌される多種のサイトカインによる血管新生、幹細胞の集積によるものであることが判明した。その結果を踏まえ、LVADを装着したDCMに対する臨床試験を4例に行い、うち2例は心機能の回復を認め、LVADより離脱した。また、LVADを装着していないICM、DCM患者に自己筋芽細胞シートを移植した。これらの患者の一部において、reverse remodeling効果を認めた。現在、筋芽細胞シート（商品名：ハートシート）は虚血性心筋症に対して保険収載された。

近年、我々は、iPS細胞由来心筋細胞を用いたシートを作成し、同組織の安全性、有効性の検証を行っている。特に、移植したiPS細胞由来心筋細胞のレシピエント心筋との同期的挙動、及び筋芽細胞シートと比較した優位性を検証するとともに、心筋細胞の大量培養法や安全性の検証システムを構築し、医師主導型治験下にて虚血性心筋症患者に対してFirst in human試験を行った。

今後、細胞シート治療の重症心不全に対する有効性が臨床的に証明されることにより、心不全治療の新たな扉を開くことが期待される。

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学歴

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2020年　熊本大学大学院　再春館・先進老化医学共同研究講座教授（併任）

最近の関連出版物・論文

Sato M. et al. The lncRNA *Caren* antagonizes heart failure by inactivating DNA damage response and activating mitochondrial biogenesis. ***Nat Commun*** 12:2529, 2021
Hirata T. et al. Associations of Cardiovascular Biomarkers and Plasma Albumin with Exceptional Survival to the Highest Ages. ***Nat Commun*** 11:3820, 2020
Tian Z. et al. ANGPTL2 activity in cardiac pathologies accelerates heart failure by perturbing cardiac function and energy metabolism. ***Nat Commun*** 7:13016, 2016
Oike Y. et al. ANGPTL2: a new causal player in accelerating heart disease development in the aging (Review). ***Circ J*** 81:1379-1385, 2017

ミトコンドリア機能維持を基盤とした心不全治療戦略

尾池　雄一

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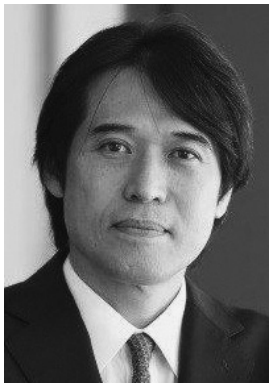
高血圧、糖尿病などの生活習慣病患者の増加や高齢化に伴い、心不全患者数は世界的に増加している。特に、高血圧、糖尿病、加齢は左室拡張機能障害をもたらすため、左室駆出率低下が認められない拡張不全を主体とする心不全(HFpEF)患者数が急増している。標準治療が確立している左室駆出率が低下した収縮不全を主体とする心不全(HFrEF)に対して、HFpEF治療薬は、SGLT2阻害薬など治療効果を示す薬が出現してきたが、未だ十分ではない。我々は、収縮機能のみならず拡張機能にもエネルギー供給は重要であることに着目し、エネルギー産生の鍵となるミトコンドリアの機能維持の観点から心不全の分子病態機構を解明し、その成果に立脚した新たな治療戦略開発に挑んでいる。

本講演では、心筋細胞の細胞質に豊富に存在する新規long noncodingRNAである*Caren* (for cardiomyocyte-enriched noncoding transcript) (Nat Commun 12:2529, 2021) の心筋細胞におけるミトコンドリア機能制御機構と、その変容による心不全病態との連関について、さらにその介入による心不全治療薬開発の可能性について議論したい。

略 歴



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最近の関連出版物・論文

1. Tamai K et al, PDGFR α -positive cells in bone marrow are mobilized by high mobility group box 1 (HMGB1) to regenerate injured epithelia. *Proc. Natl. Acad. Sci U. S. A. 108*, 6609-6614 (2011).
2. Iinuma et a, Transplanted bone marrow-derived circulating PDGFRa+ cells restore type VII collagen in recessive dystrophic epidermolysis bullosa mouse skin graft. *J. Immunol. 94*, 1996-2003 (2015).
3. Aikawa E et al, Systemic high-mobility group box 1 administration suppresses skin inflammation by inducing an accumulation of PDGFRa(+) mesenchymal cells from bone marrow *Sci. Rep. 5*, 11008 (2015).
4. Ho YT et al, Chromatin accessibility identifies diversity in mesenchymal stem cells from different tissue origins. *Sci Rep. 8* 17765 (2018)
5. Has C et al, Consensus re-classification of inherited epidermolysis bullosa and other disorders with skin fragility. *Br J Dermatol. 183,* 614-627 (2020)

栄養障害型表皮水疱症に対する再生誘導医薬開発

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栄養障害型表皮水疱症はVII型コラーゲン（Col7）の遺伝的機能不全により発症する遺伝性水疱性皮膚疾患である。患者皮膚には生直後から一生涯、日常生活の軽微な外力で全身熱傷様の水疱、びらん、潰瘍が生じる。栄養障害型表皮水疱症の治療法は局所治療のみで、手指の癒着、食道狭窄、瘢痕癌といった合併症の進行を抑制し得る全身性の治療は全くない。我々は、栄養障害型表皮水疱症の剥離表皮内壊死細胞から放出される核内シャペロン蛋白HMGB1が骨髄内間葉系幹細胞を血中へと動員していること、血中動員された間葉系幹細胞（間葉球）は壊死組織に集積し、炎症や線維化を抑制し、損傷皮膚の再生を促進することを見出した。そこで我々は、化学合成したHMGB1ペプチドを表皮水疱症モデルマウス（Col7低形成マウス）へ投与し、手指や小腸の瘢痕癒着の有意な抑制効果、生存期間の著明な延長効果を確認した。シングルセルRNA/ATACシークエンス解析により、HMGB1ペプチド投与で皮膚や小腸に間葉系幹細胞が増加し、炎症性マクロファージが減少することが示された。さらにMSC系譜追跡マウスを用いた解析では、Col７ノックアウトマウス皮膚に集積した間葉球は、間葉-上皮転換により表皮幹細胞を再生していることが明らかとなった。これらの基礎研究を背景として、我々は健常成人男性48名を対象としたHMGB1のプラセボ対照2重盲検第I相医師主導治験を実施し、HMGB1ペプチド投与の安全性と間葉球誘導効果を確認した。次いで、我々は8名の栄養障害型表皮水疱症患者を対象に第II相医師主導治験を実施した。その結果、HMGB1ペプチド投与により全身皮膚の病変面積は有意に減少し、その効果は1年以上持続した。現在我々は、栄養障害型表皮水疱症に対する再生誘導医薬HMGB1ペプチドの薬事承認取得に向けて、PMDAと最終臨床試験内容について協議中である。

Plenary Session

Abstract

A leucine residue in the N terminal degron of AAV AAP is one of the amino acids essential for its degradation

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Recombinant adeno-associated (AAV) vectors are widely used as a tool for gene delivery with several FDA approved vectors available in the market. There are however several limitations to its use, one being its production. The assembly of AAV viral vector capsids requires the involvement of VP capsid proteins, assembly-activating protein (AAP) and several other host cellular factors ultimately forming a capsid structure with only the VP proteins. We have observed that AAP quickly degrades during the assembly process but the mechanisms behind its degradation remains unknown. Here we present evidence that the N terminus hydrophobic region of AAP contains a degradation motif that facilitates its degradation and a leucine residue within the motif is functionally critical . We analyzed the HR region because it shares characteristics similar to that of known degrons. To determine if HR is the degron, we made GFP constructs fused to the HR region of AAP (AAPxHR-FLAG-GFP, x=serotype). The HR's derived from all AAP serotypes from 1-12 showed effective degradation of GFP protein except for AAP4 and AAP11. This serotype dependent degradation led us to identifying amino acid residues within the AAP HR's that is shared in each of the two groups: degradation resistant AAP's (AAP4 and AAP11) and the AAP's prone to degradation (AAP1, 2, 3, 5, 6, 7, 8, 9, 10 and 12) and investigate its significance in the degradation activity. Alignment of the HR region of AAP's between the two groups revealed amino acid at position 21 to have a P in AAP4 and 11 whereas a L, K or Q in the degradation prone AAP's (note: AAP positions are those corresponding to those of AAP2). Amino acid at position 25 has a T in AAP4 and 11 and L in the remaining AAP's. To analyze these two positions, we created mutants in the HR region of AAP9 thereby constructing Q21P and L25T mutants of AAP9 fused to FLAG-GFP and compared their GFP expression against AAP9HR-FLAG-GFP. AAP9HR and AAP9HRQ21P fusions showed a substantial decreased in GFP expression whereas AAP9HRL25T fusion to GFP retained GFP expression albeit at a level lower than the control FLAG-GFP. This indicated that the leucine (L) residue at position 25 in AAP9's HR plays a critical role in the degron activity. To summarize, our data suggests that the HR region of AAP at the N-terminus acts as a degron with leucine residue at position 25 critical for its degron function. Further studies are underway to understand the significance of AAP degron in capsid assembly and the pathways that lead to AAP degradation.

ゲノム編集iPS細胞を用いた悪性神経膠腫に対する遺伝子幹細胞療法の開発

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背景：悪性神経膠腫（グリオーマ）は、極めて浸潤性の高い予後不良な脳腫瘍であることから、有効な治療法の開発が急務である。我々は、これまで細胞vehicleとしての神経幹細胞(NSC)及び間葉系幹細胞(MSC)の脳内遊走能を比較した上で、iPS細胞由来NSCを治療遺伝子（自殺遺伝子）の細胞vehicleとして用いる新たな治療法を研究してきた。さらにiPS細胞における自殺遺伝子の恒常安定発現をゲノム編集技術CRISPR/Cas9により達成し、有効な抗腫瘍効果を生み出す治療用NSCを作製することに成功したため、報告する。
方法：脳切片培養法により、ヒトiPS由来NSC、胎児由来NSC、脂肪組織及び骨髄由来MSCのグリオーマ細胞に対する遊走能を定量評価した。また、レンチウイルスベクターによるiPS細胞への遺伝子導入では、位置効果による自殺遺伝子yCD-UPRTの不活性化が生じたために、CRISPR/Cas9を用いて、house keeping 遺伝子領域やsafe harbor領域にyCD-UPRTを挿入したヒトiPS細胞を作製した。その後、ヒトグリオーマ幹細胞モデルに対する治療効果を評価した。
結果：脳切片培養法を用いたtimelapse撮影によりiPS由来NSCが最も優れたグリオーマ細胞に対する遊走・指向性を示すことを明らかにした。さらに、house keeping遺伝子領域にyCD-UPRTを挿入したヒトiPS細胞は、問題なくNSCに誘導可能で、挿入遺伝子の恒常安定発現を達成した。その結果、ヒトグリオーマ幹細胞モデルに対して顕著な生存期間の延長を示した。
結語：ゲノム編集によりyCD-UPRTを恒常安定発現するiPS細胞を作製することで、安全面及び品質の面でも優れた治療用NSCの供給を可能とした。

Antitumor effect of GAIA-102 on refractory tumors and its underlying mechanism

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[Background] Natural killer (NK) cells, a subpopulation of cytotoxic lymphocytes, play a role in the innate immune system that kills infected, foreign, or transformed cells, including tumor cells. NK-cells based immunotherapy has been attempted for decades. However, their therapeutic effect is limited partly due to the low infiltrating ability into solid tumors. Previously, we established a method to generate highly activated NK-like cells from peripheral blood mononuclear cells (PBMCs), named GAIA-102, and found that they can aggressively infiltrate into spheroids of various types of tumors. Hence, we hypothesized that GAIA-102 has high potency against solid tumors. In this study, we aimed to verify this hypothesis and to clarify the underlying mechanism. For this purpose, we used the CT26 colorectal carcinoma cells-inoculated mouse, which is known as a refractory solid tumor model.

[Methods] For evaluation of the establishment of acquired immunity, CT26 was intraperitoneally inoculated into BALB/c mice as previously described (Kasagi Y et al., Cancer Res. 2016) (first challenge). After 3 days, the formation of CT26 nodules was confirmed and GAIA-102 was administrated with IL-2 (10000 IU/shot) 9 times to the mice. One hundred days after tumor inoculation, the surviving mice were further subcutaneously inoculated with 4T1 and CT26 cells into the left and right flank, respectively (second challenge). These tumor cells were injected into untreated mice as a control with the same procedure. To evaluate the response of T cells, 3 days after CT26 intraperitoneal inoculation, GAIA-102 was administrated 3 times. After 7 days, CT26 tumor nodules were sampled, and analyzed the number of infiltrated CD4+ and CD8+ T cells by flow cytometry.

[Results] In the first challenge, after 100 days from tumor inoculation, 90% of GAIA-102-administrated mice survived (n=10), whereases all GAIA-102-untreated mice died during this period (n=10). In the second challenge, 4T1 tumors were formed in all mice cured by GAIA-102, while no CT26 tumors were formed in these mice (n=9). However, in unchallenged mice, both tumors were formed in all cases (n=12). Evaluation of T cells response showed that the number of infiltrated CD8+ and CD4+ T cells into CT26 nodules in the GAIA-102-treated mice was approximately 200 and 400-fold greater than that of untreated ones, respectively.

[Conclusion and discussion] In this study, we showed that CT26-specific acquired immunity was established in the mice that were cured by GAIA-102. Moreover, GAIA-102 had activity in attracting CD4+/CD8+ T cells into CT26 nodules. Our data suggest that GAIA-102 plays a role in enhancing the establishment of acquired immunity. Additionally, we were attempting to evaluate the effect of the GAIA-102 on cytotoxic T lymphocytes activity, especially, focusing on the regulation of cancer cells' MHC I expression and the synergetic therapeutic effect of PD-1 blockade.

A single-dose CD117 antibody-drug conjugate allows for efficient engraftment of gene-modified CD34+ cells in a non-human primate model for lentiviral gene therapy

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Hematopoietic stem cell (HSC) gene therapy is curative for multiple genetic diseases; however, it is limited by morbidity and mortality from chemotherapy-based conditioning. Therefore, we developed an antibody-drug conjugate (ADC) targeting CD117 (c-KIT) to specifically deplete both HSCs and progenitor cells. In our preliminary rhesus study, 0.2 mg/kg ADC conditioning resulted in >99% bone marrow depletion, detectable engraftment of gene-modified cells (vector copy number (VCN) ~0.01), and minimal toxicities (ASH 2019). In this study, we investigated escalating doses of ADC to determine the optimal dose to enable engraftment of gene-modified CD34+ HSCs in rhesus macaques.

We administered single-dose CD117-ADC at 0.3 mg/kg (ZL13 and ZJ62) and 0.4 mg/kg (H635 and H96G), compared with myeloablative busulfan (Bu 5.5 mg/kg x 4 days) (12U018 and 12U020). Mobilized rhesus CD34+ cells (ADC 3.8±1.9e7 vs. Bu 2.9±0.2e7, n.s.) were transduced with a lentiviral vector encoding *BCL11A*-targeting shmiRNA and a truncated human erythropoietin receptor for stable fetal hemoglobin (HbF) induction (Sci Transl Med. 2021) as a therapeutic model for hemoglobin disorders. These cells (*in vitro* VCN 10.1±3.8 vs. 10.2±7.3, n.s.) were transplanted into autologous animals 6 or 10 days after ADC conditioning (0.3 or 0.4 mg/kg, respectively) or 1 day after Bu conditioning. Granulocyte (Gr >500/ml, day 6-9 vs. day 8-9), reticulocyte (>50,000/ml, day 10-14 vs. day 11), and platelet (>30,000/ml, day 2-8 vs. no reduction) recoveries were similar between ADC and Bu conditionings, respectively. Only ADC conditioning resulted in a reduction of platelet counts as well as a transient rebound in all major lineages. Two months post-transplant, efficient gene marking (VCN in Gr 0.43 in ZJ62 and 0.26±0.14 in 0.4 mg/kg ADC vs. 0.47±0.11 in Bu, n.s.) was observed at a plateau level in 3 of 4 animals in ADC conditioning. Robust and durable HbF induction was detected by both HbF+ percentages (F-cell 7.2% and 7.6±1.6% vs. 11.6±4.1%, n.s.) and HPLC-quantitated HbF amounts (5.7% and 6.5±3.9% vs. 9.3±4.0%, n.s.) in these animals for 0.5-1.5 years. In ZL13 (1 of 2 animals in 0.3 mg/kg ADC), lower gene marking (VCN in Gr 0.03) was obtained, along with low HbF induction (F-cell 2.5% and HbF amounts 1.7%), suggesting that 0.3 mg/kg ADC is marginal, and 0.4 mg/kg ADC is sufficient for robust engraftment of gene-modified cells. Importantly, CD117-ADC conditioning resulted in minimal toxicities, unlike Bu conditioning.

In summary, we demonstrated that a single dose of CD117-ADC allows for efficient engraftment of gene-modified CD34+ HSCs and robust HbF induction in a rhesus gene therapy model, achieving a similar level as myeloablative Bu conditioning. This targeted approach for safer conditioning could improve the risk-benefit profile in HSC gene therapy.

Disclosures: K.L., R.P., N.Y., P.B., K.B., and L.O. are employees of Magenta Therapeutics.

アデノ随伴ウイルスベクターの分析と品質管理の現状と展望

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アデノ随伴ウイルス（AAV）ベクターはカプシド改変などの創薬から大量製造技術にいたるまで研究開発が盛んに進められており、発展が期待される。一方、有効性・安全性の観点から重要となるのがウイルスベクターの分析と品質管理であり、演者らは、AAVベクターを主な対象として分析・品質管理技術の開発を多面的に取り組んできた。本講演ではAAVベクターの分析・品質管理において重要となる各手法について、演者らの研究成果の紹介を含めて報告する。純度分析で重要となる項目として完全粒子と空粒子の定量があるが、AAVベクターにおいては2015年頃から超遠心分析による評価が利用されはじめた。ただし、実際の試料には完全粒子と空粒子に加えてマイナーピークが観測され、各ピークの帰属が課題であった。演者らは、遠心沈降パターンの多波長モニタリングと数値解析を組み合わせることで試料に含まれる、沈降係数が異なる成分の吸収スペクトルを特定する手法を開発し、完全粒子と空粒子を含め観測される各ピークの構成的特徴の特定を可能とした。また、各粒子の吸光スペクトル決定が可能となった結果、吸光度測定による純度検定を高い信頼性で行うことが可能となった。また、カプシドを構成するVPについては、キャピラリー電気泳動ならびに液体クロマトグラフィー質量分析により、構成比および一次構造の決定を可能としている。他にも品質管理に重要となる項目についても特徴と限界を含めて紹介する。

[参考文献]

[1] Maruno T, et al., Comprehensive Size Distribution and Composition Analysis of Adeno-Associated Virus Vector by Multiwavelength Sedimentation Velocity Analytical Ultracentrifugation. J. Pharm. Sci. 110, 3375-3384 (2021).
[2] Oyama H, et al., Characterization of Adeno-Associated Virus Capsid Proteins with Two Types of VP3-Related Components by Capillary Gel Electrophoresis and Mass Spectrometry. Hum. Gene Ther. 32, 1403-1416 (2021).

HER2陽性の再発・進行骨・軟部肉腫及び婦人科悪性腫瘍を対象とする非ウイルス遺伝子改変HER2 CAR-T細胞の臨床第Ⅰ相医師主導治験

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[背景] CAR-T細胞療法は、固形がんに対しては期待されたほどの臨床効果が示されていない。その原因として、免疫抑制の強い腫瘍微小環境におけるCAR-T細胞の免疫疲弊や持続性の低下が考えられている。我々が開発してきた野生型*piggyBac*トランスポゾンを用いた非ウイルス遺伝子改変CAR-T細胞は、分化早期の増殖能が高く、免疫疲弊が生じていない幹細胞様T細胞が多く含まれるため、固形がん治療においてより高い有効性が期待できる。今回、*piggyBac*法により抗HER2 CAR遺伝子を導入したCAR-T細胞を主成分とする細胞加工製品（BP2301）を開発し、臨床第Ⅰ相医師主導治験を計画したためその概要を報告する。
[目的] 再発・難治性HER2陽性骨・軟部肉腫および婦人科腫瘍患者に対する、治験製品BP2301（HER2 CAR-T細胞製剤）投与の忍容性検討並びに安全性の評価及び有効性の探索的検討を行う。
[方法] 本治験は第Ⅰ相の非盲検・用量漸増試験で、信州大学医学部附属病院の単施設で実施する。BP2301の製造も同施設で行う。標準治療が不応・不耐もしくは標準治療後に再発又は進行したHER2陽性の骨・軟部肉腫および婦人科悪性腫瘍患者に、BP2301を開始用量 8.3×10^5 cells/kg、最大用量 2.7×10^6 cells/kg（最大 1.6×10^8 cells/body）で投与する。主要評価項目は、各用量における用量制限毒性発現割合、副次評価項目は、有害事象の発生状況およびCAR-T細胞療法の抗腫瘍効果とした。探索的評価項目は、CAR-T細胞の体内動態および血中サイトカイン濃度とした。また、BP2301の長期安全性を前向きに5年間観察する付随研究も併せて実施する。
[結論]　本試験ではBP2301の臨床安全性及び有効性を検証し、再生医療等製品としての承認を取得し、有効な治療法がない患者が恩恵を受けることを最終目的としている。

Oral Session

Abstract

EPHファミリーたんぱくに多重特異性を有するリガンド型CAR-T細胞療法の開発

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【目的】遺伝子改変キメラ抗原受容体T細胞（CAR-T細胞）開発において、複数の標的抗原を同時に認識、殺傷することが可能な多重特異性CAR-T細胞の開発が注目されている。われわれは、EPHファミリーに多重特異性をもつ自然リガンドを抗原認識部位としたCAR-T細胞を開発し、EPHA2またはEPHB4発現腫瘍に対する抗腫瘍効果について評価を行った。
【方法】EPHB4の自然リガンドであるEphrin B2について、ヒトEPHファミリーであるEPHA2、EPHB4との結合性をフローサイトメトリー法で評価した。Ephrin B2細胞外ドメインを抗原認識部位にもつ第2世代CAR-T細胞（EPHB4-CAR-T細胞）を、ピギーバックトランスポゾン法を用いて作製し、EPHA2陽性、EPHB4陽性神経芽腫細胞株に対する抗腫瘍効果を評価した。また、マウスEPHファミリーたんぱくと、ヒトEphrin B2との結合性をフローサイトメトリーで評価した。マウス正常組織との交差反応性について、免疫不全マウスにEPHB4-CAR-T細胞を単回投与し、血液生化学検査、各臓器の病理学的検査を行った。
【結果】Ephrin B2はEPHA2、EPHB4など複数のEPHファミリーたんぱくに結合性を示した。ヒト健常人アフェレーシス由来末梢血単核球を用いてEPHB4-CAR-T細胞を製造したところ、CAR陽性率48.8 ± 10.4%であった。このEPHB4-CAR-T細胞は、EPHA2陽性SK-N-AS細胞、EPHB4陽性SH-SY-5Y細胞に対して強く持続的な抗腫瘍効果を示した。また、EPHB4-CAR-T細胞はマウスEPHA2、EPHB4たんぱくとも交差反応性を示したことから、免疫不全マウスに対してEPHB4-CAR-T細胞を単回投与することでOff tumor毒性を評価したところ、血液生化学検査、凝固機能検査、病理学的検査に明らかな異常所見を認めなかった。
【考察】EPHファミリーは種々の腫瘍細胞で高発現することから、EPHファミリーに対する多重特異性CAR-T細胞は、腫瘍内における標的抗原発現の不均一性や、抗原発現低下による免疫逃避を克服しうる可能性がある。

RetroNectin® 誘導Tリンパ球を用いたNK拡大培養法のCAR-NKへの応用と機能解析

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キメラ抗原受容体発現T細胞 (CAR-T) 療法は、急性リンパ性白血病を筆頭に血液腫瘍に対して高い有効性が示されている反面、サイトカイン放出症候群や神経障害といった副作用が報告されている。また、自家移植が必要であるために高い製造コストが課題となっている。

NK細胞は、自然免疫において中心的な役割を持った細胞傷害性リンパ球であり、GVHDを誘導することがないため他家移植が可能である点、サイトカイン放出症候群や神経障害を起こすリスクが低いと報告されていることから高い安全性を示す可能性を持つ点、においてキメラ抗原受容体発現NK細胞 (CAR-NK) としての利用が期待されている細胞である。

過去に我々は、RetroNectin® 誘導Tリンパ球（RN-T）をフィーダー細胞として用いた、独自のNK細胞培養技術を確立した。本検討では、このNK細胞培養技術を用いてCAR-NK細胞を製造するための検討、並びに機能面での解析を進めた。

まず、前述のNK細胞培養技術を用いて、ウイルスベクターによる遺伝子導入を試みた。その結果、遺伝子導入のタイミングとしては、培養7-10日目において高い遺伝子導入効率が認められた。また、RetroNectin® を用いた遺伝子導入法により、レトロウイルスベクターによる遺伝子導入効率だけでなく、NK細胞に感染しにくいと報告されている、レンチウイルスベクターによる遺伝子導入効率を増加させることが示された。次いで、CARのシグナルドメインとして、28ζと4-1BBζとを比較したところ、4-1BBζの細胞傷害活性の方が長期間持続することが示された。
以上のことから、RetroNectin® を用いた遺伝子導入法は、CAR-NK細胞製造への応用が期待される。

Expression of CAR targets on solid tumors by armed oncolytic virus has synergetic effect on CAR-T cell therapy

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Chimeric antigen receptor (CAR) T cell therapy showed limitation in solid tumors compared to hematological malignancies due to the heterogeneous surface antigen expression patterns in solid tumors, which reduce the effective antitumor response of CAR-T cell therapy. Furthermore, tumor cells may escape from CAR-T by partial or complete loss of target antigen expression.
To overcome the limitation of CAR-T therapy for solid cancer, we developed a combination therapy of CAR-T and an oncolytic virus that induces the expression of antigens in tumors. Here we engineered an attenuated oncolytic herpes simplex virus-1 (HSV-1) by deletion of the neurovirulence viral genes ICP 34.5 and replacing it by insertion of mesothelin (MSLN) gene (HSV-MSLN) to selectively deliver MSLN to malignant cells. Our virus showed *in vivo* safety even after using a high dose (1×10^7 pfu) in tumor-bearing mice and a strong antitumor effect. *In vitro*, HSV-MSLN induced cell cytotoxicity and expressed MSLN in the Pan02 tumor cell line in a MOI-dependent manner. HSV-MSLN-infected Pan02 cell line induced activation and expression of IFN-γ in MSLN-CAR T cell. Our findings may reveal a mechanism that allows the combination of OVs and CAR-T to trigger and improve CAR-T cell antitumor response in solid tumors *in vivo*.

羊膜間葉系幹細胞による筋ジストロフィーモデルを用いた細胞治療の有効性

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【背景と目的】Duchenne型筋ジストロフィー(DMD)は進行性で重症の筋萎縮と筋力低下を示し、呼吸循環不全に至る重篤な疾患である。DMDの病態進行には、ジストロフィン欠損に加えて慢性炎症が深く関与しているが、ステロイド療法による副作用の観点から、安全性が高くて効果的な新たな抗炎症療法の開発が望まれる。我々はこれまでに、骨髄や歯髄に由来する間葉系幹細胞を用いたDMDモデル動物における有効性を報告している。本研究では、MSCの社会実装を目指して、非侵襲的に採取・大量調製が可能な羊膜由来MSC(AMSC)に注目し、AMSCの免疫制御作用を利用したDMDに対する炎症制御療法の可能性を検討した。
【方法】AMSCsとマクロファージ共培養による炎症制御作用を解析した。炎症反応が著しい幼若期のDMDモデル*mdx*マウスにヒト由来AMSCsを静脈内反復投与し、病理解析、握力および自発運動機能、心機能解析を行った。
【結果】AMSCsによるM2マクロファージの炎症制御活性の促進を*in vitro*系で明らかにした。そこで、*mdx*マウスへAMSCsの投与を行った結果、投与マウスはDMDマーカーである血中CK値が低下し、骨格筋におけるIL-6値の減少などマイオカインの発現変動、炎症細胞浸潤の限局、中心核線維の減少、さらにM2マクロファージの集積増加を示し、AMSCsによる炎症抑制効果が示唆された。また、AMSCs投与マウスは投与10ヶ月後においても、非投与群と比べて強い握力を示し、1日あたりの走行距離および持続走行距離が1.5倍増加した。さらに、心機能解析により、AMSCs投与マウスにおける左室内径短縮率%FSの回復を認めた。以上の結果から、AMSCs投与による骨格筋機能および心機能が長期間維持されることが示唆された。
【結語】幼若期よりAMSCsの全身的反復投与を行ったDMDマウスにおいて、炎症病変の緩和や運動機能の改善が認められ、病態進行の遅延および有効性が示唆された。今後、DMDに対する新たな細胞療法として期待できる。

脳虚血再灌流障害における羊膜由来間葉系幹細胞移植による脳保護効果の検討

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[背景と目的] 羊膜由来間葉系幹細胞（AMSC）は、羊膜組織より大量に採取可能で、増殖能も高いとされており、これらを用いた新たな幹細胞治療への取り組みが注目されている。そこで我々は、ラット一過性局所脳虚血モデルを用いてヒトAMSC移植における脳保護効果を検討した。
[方法] 雄性SDラットの中大脳動脈を90分間閉塞し、再灌流直後にヒトAMSC（1 × 10^6 個）を尾静脈より投与した。対照群としてPBSも同様に投与した。虚血再灌流後において、動物に対し神経徴候、ロタロッド試験、Y-maze試験を施行し長期的に評価した。梗塞体積はTTC染色法より測定し、虚血側脳における炎症性蛋白の発現、神経細胞死の評価を分子生物学的手法にて行った。
[結果] 細胞治療群では、虚血再灌流後3日における姿勢異常および片麻痺の有意な改善を認め（p<0.05）、ロタロッド試験では再灌流後3日、7日、28日において対照群に比べて騎乗時間が延長した（p<0.01）。また、再灌流後28日に施行したY-maze試験では、細胞治療群で交替行動率が有意に上昇した（p<0.01）。対照群に比べて細胞治療群で梗塞体積は有意に縮小していた（p<0.05、p<0.01）。免疫組織染色法では、細胞治療群において虚血境界領域におけるIba-1発現やTNF-α発現の有意な抑制を認め（p<0.05、p<0.05）、変性神経細胞数は有意に減少していた（p<0.01）。ELISA法による虚血側脳のIL-1およびIL-6濃度も細胞治療により有意に低下していた（p<0.05、p<0.05）。
[結論] 脳虚血再灌流モデルにおいて、AMSC静脈投与により抗炎症作用を介した脳保護効果が示され、長期的な運動機能や認知機能の改善が促進された。この新たな幹細胞治療が脳虚血後の脳保護効果に寄与する可能性が示唆された。

A cryopreservation method to maintain the viability and cytotoxicity of highly activated natural killer-like cells

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Background: Natural killer (NK) cells have an important role in defense against tumors. They can recognize and lyse a wide range of tumor cells while showing little cytotoxicity to healthy cells. Although clinical trials of NK cells-based immunotherapy showed success in blood cancers, it failed in solid tumors. We previously developed a method to produce highly activated NK-like cells from peripheral blood mononuclear cells (PBMCs), which have the potential to cure solid tumors. In clinical point of view, cellular products need to be cryopreserved to make them an off-the-shelf medicine for availability in hospital. Although several studies demonstrated cryopreservation of NK cells, they failed to maintain cytotoxicity. Similarly, we previously performed cryopreservation of the activated NK-like cells, however, their cytotoxicity was dramatically decreased after thawing (Saito et al., Hum Gene Ther Methods, 2013). In this study, we aimed to develop a new cryopreservation method to maintain not only the viability but also cytotoxicity of the activated NK-like cells. For this purpose, we focused on the effects of dilution solutions in thawing process, and attempted to identify the solution composition that can maintain the cell viability and cytotoxicity of the cryopreserved activated NK-like cells.
Methods: After removing CD3+ and CD34+ cells from PBMCs, the cells were cultured with KBM 501 (KOHJIN BIO) medium for 14 days to produce the activated NK-like cells. They were cryopreserved using STEM-CELLBANKER® EX and stored at -80℃ for more than 2 days. Immediately after thawing at 37℃, cells were incubated with various balanced salt solutions at room temperature for 3-3.5 h. Then, the cell viability and cytotoxicity were assessed by flow cytometry. For cytotoxicity assay, the NK-like cells were co-cultured with K562 cells at 37℃ for 2 h in RPMI medium (10% FBS).
Results: By comparing the effects of incubation with various balanced salt solutions on the cryopreserved NK-like cells after thawing, we obtained following results: (1) Incubation with the solutions containing glucose or calcium decreased viability; (2) Incubation with the solutions containing potassium and acetate maintained the cytotoxic activity as well as cell viability; (3) For the protective effects on the viability and cytotoxicity, the solutions should be pH > 6.4 and 247-484 mOsm.
Conclusion: Our results showed that incubation with a certain solution after thawing can maintain the viability and cytotoxicity of the cryopreserved NK-like cells. This simple procedure provided a new practical cryopreservation method for the activated NK-like cells. However, the underlying mechanism of the protective effects on the viability and cytotoxicity is still unclear, and we are studying this issue.

iPS細胞由来ミクログリアを用いたアルツハイマー型認知症治療法の開発

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高齢者に頻繁にみられるアルツハイマー型認知症は、正常に代謝できないアミロイドβ(Aβ)等のタンパク質が脳内に蓄積して神経機能障害を引き起こすことが発症の原因であると考えられている。この所謂「アミロイドカスケード仮説」は、提唱された1980年代から現在に至るまで幅広く支持され、世界中で多くの研究が実施されてきたが、未だ根本的な治療方法はみつかっておらず、中にはAβの蓄積によって生じる老人斑の消失を認めたにも関わらず、認知機能の低下は抑制できなかったとの報告もある。これまでの多くの治療法開発の失敗においても尚、Aβの蓄積は疾患発症の最上流の発症要因であり、発症に至る20年前からの蓄積をいかに抑制するかが治療の鍵とされている。一方で、Aβの蓄積は発症の要因ではあっても認知機能低下の直接的な原因ではなく、老人斑周囲に集積する脳内免疫を司るミクログリアの過剰活性による神経回路の破壊が原因だとする考え方も支持されてきた。ミクログリアは細胞外環境に蓄積するAβを貪食により除去する機能を有する一方、Aβに刺激され、活性化したM1型ミクログリアの炎症反応誘導が周囲の神経細胞の細胞死を引き起こすため、諸刃の剣として病態に深く関与していると考えられている。

本研究ではiPS細胞を介して、Aβの除去機能を補強し、かつ炎症反応を過剰に誘発しないミクログリアの作成ができれば、アルツハイマー型認知症の画期的な治療法となり得るとの着想に基づき、遺伝子機能改変ミクログリアの作成に取り組んでいる。炎症誘導型M1型と、もう一方の抗炎症性作用を有するM2型の変遷はTLR4受容体により調節されていることが知られており、TLR4シグナルの抑制によりM1型誘導を阻害し、かつAβの貪食に関わる事が報告されている複数の受容体の強制発現により貪食機能を強化した細胞の作成を試みる。本発表では、上記細胞設計に基づいた細胞作成の進捗と機能評価の内容を報告する。

患者由来肉腫細胞におけるがん治療用ヘルペスウイルスの効果の検討

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【目的】第三世代がん治療用ヘルペスウイルスG47Δはあらゆる固形がんに対して有効であり、本邦初のウイルス療法薬として2021年に悪性神経膠腫に対して承認された(デリタクト®注; 一般名teserpaturev)。肉腫は既存治療の有効性が低いためウイルス療法が有効であれば治療体系のブレイクスルーとなる可能性があるが、希少疾患ゆえに研究材料を得難いことなどがハードルとなり十分な検討はなされていない。本研究は、患者由来肉腫細胞を用いてウイルス療法の効果を検討した。
【方法】G47Δと同等の機能を持つ実験用ウイルスであるT-01を用いた。国立がん研究センター研究所で作成された患者由来肉腫細胞(平滑筋肉腫、未分化多形肉腫、明細胞肉腫、CIC-DUX4肉腫、横紋筋肉腫)を対象に、ウイルスの複製能比較試験、in vitroの殺細胞効果、マウス皮下腫瘍モデルを用いた腫瘍縮小効果を検討した。
【結果】複製能比較試験では、いずれの肉腫細胞においてもT-01は良好な複製能を示した。in vitroの殺細胞効果の検討では、T-01をMOI 0.1で感染させると4日目にはほぼ全ての肉腫細胞が死滅した。横紋筋肉腫と明細胞肉腫の2種で、マウス皮下腫瘍モデルの検討も行った。腫瘍径が5 mmとなった時点でウイルスを腫瘍内投与したところ、T-01投与群では腫瘍の完全消失を認めた(day 45時点で横紋筋肉腫は5/8匹、明細胞肉腫は5/10匹が消失)。
【結論】がん治療用ウイルスT-01は、複数の患者由来肉腫細胞に対して有意な抗腫瘍効果を示した。本研究結果は、肉腫に対するウイルス療法の臨床試験へと進む大きな根拠となることが期待される。また、再投与でも有効な可能性が高いことから、ウイルス療法は選択肢の少ない肉腫治療におけるブレイクスルーとなることが期待される。

The mechanism of novel oncolytic adenovirus serotype 35-mediated anti-tumor effects

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Oncolytic adenoviruses (Ads) (OAds) are getting much more attention due to not only the efficient tumor cell lysis activity but also efficient activation of anti-tumor immunity. Although almost all OAds are based on human Ad serotype 5 (Ad5) (OAd5), OAd5 has two concerns, which potentially attenuate the anti-tumor effects of OAd5. First, more than 80% of adults have anti-Ad5 neutralizing antibodies by natural exposure to Ad5. Second, the expression of the primary infection receptor, coxsackievirus-adenovirus receptor, is often declined on malignant tumor cells. To overcome these drawbacks, we developed a novel OAd fully composed of human Ad serotype 35 (Ad35) (OAd35). Only 20% or fewer adults have pre-existing neutralizing antibodies against Ad35. OAd35 uses CD46, which is a complement regulatory factor, as an infection receptor. Several studies reported that expression levels of CD46 are up-regulated on malignant tumor cells. Previously, we found that although OAd35 showed efficient *in vitro* tumor cell lysis activities on a wide variety of tumor cells, negligible levels of genome copy numbers of OAd35 were found in the tumors following intratumoral administration, in spite of the efficient tumor growth suppression of OAd35. These data suggested that other mechanisms different from virus replication in the tumor were involved in the anti-tumor effects of OAd35. Since previous studies reported that Ad35 activated natural killer (NK) cells more efficiently than Ad5, we examined the involvements of NK cells on the OAd35-mediated anti-tumor effects. Intratumoral administration of OAd35 in nude mice bearing H1299 xenograft tumors resulted in significant increases in the infiltration of activated NK cells to the tumor. The increased infiltrations of activated NK cells were not observed by OAd5 injection. OAd35 also induced the infiltration of activated NK cells to B16 mouse melanoma tumor in immunocompetent C57BL6/J mice and showed significant anti-tumor effects. Furthermore, depletion of NK cells by pre-administration of anti-GM1 antibody significantly restored the tumor growth following intratumoral administration of OAd35. The genome copy numbers of OAd35 in the tumors were increased by more than 75-fold in the NK cell-depleted nude mice on day 21 after OAd35 administration. These data indicated that NK cells cleared the OAd35-infected tumor cells, leading to efficient tumor growth suppression. In conclusion, OAd35, which induces the anti-tumor effects *via* infiltration of activated NK cell in the tumors, is expected to become an alternative cancer immunotherapy agent.

Immunization with adenovirus-p53-transduced dendritic cell vaccine enhances the antitumor efficacy of p53-armed oncolytic virotherapy in colorectal cancer

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Background: Dendritic cell (DC) vaccine therapy is an immunotherapy that induces tumor antigen-specific antitumor effects using tumor antigen-presenting DC. As the tumor suppressor p53 is the most frequently mutated gene and mutant p53 protein is overexpressed in various cancers, mutant or normal p53 epitopes have been expected to function as tumor-associated antigens. Vaccine therapy using DC transduced by a non-replicative adenovirus expressing wild-type p53 (Ad-p53) has been shown to induce p53-specific cytotoxic T cells. We have developed tumor-specific replication-competent oncolytic adenovirus OBP-702 that expresses the tumor suppressor *p53* gene and induces cytopathic activity against telomerase-positive tumor cells. In this study, we investigated the combined effects of OBP-702 and vaccine therapy with Ad-p53-DC in murine colorectal cancer models.
Methods: To obtain Ad-p53-DC, bone marrow-derived cells isolated from the femur of C57BL6/J mice were stimulated with IL-4 and GM-CSF for 5 days, and then infected with Ad-p53 for 2 days. Expression of DC surface markers (CD11c, CD86, CD103, MHC-class II) was analyzed by flow cytometry. In animal experiments, Ad-p53-DCs were administered subcutaneously every week for 4 times before tumor inoculation. Then, subcutaneous tumors were developed using murine colorectal cancer cell lines CT26 (p53 wild type) and MC38 (p53 mutant type). OBP-702 was administered intratumorally every week for 2 times. Tumor growth was compared among 4 groups;1) control group, 2) Ad-p53-DC treatment group, 3) OBP-702 treatment group, 4) combination treatment group. Tumor immune microenvironment was analyzed by flow cytometry and immunohistochemistry. To evaluate systemic antitumor immunity, CD8-positive T cells derived from splenocytes were used for conducting a cytotoxic T lymphocyte (CTL) assay, in which cytotoxicity was evaluated by analyzing the amount of extracellular lactate dehydrogenase (LDH) released from tumor cells.
Results: Ad-p53-DCs exhibited a significantly higher proportion of cells with DC maturation markers (CD86, CD103, MHC-class II) in CD11c-positive DCs compared to non-infected DCs. In murine CT26 and MC38 tumor models, combination of Ad-p53-DC and OBP-702 significantly suppressed tumor growth compared to OBP-702 monotherapy, whereas Ad-p53-DC monotherapy did not suppress tumor growth compared to control group. Flow cytometric analysis and immunohistochemistry revealed that combination therapy-treated tumors contained a significantly higher proportion of CD45-positive hematopoietic cells, containing CD8-positive T cells, CD11c-positive DCs, and F4/80-positive macrophages, compared to the other groups. CTL assay further demonstrated that combination therapy significantly increased the cytotoxic activity of CD8-positive T cells in the spleens compared to the other groups.
Conclusion: Our data suggest that immunization with Ad-p53-DC vaccine enhances the antitumor efficacy of p53-armed oncolytic adenovirus OBP-702 in murine colorectal cancer models probably via induction of p53-specific antitumor immune response.

増殖型レトロウイルスベクターを用いたイヌ悪性腫瘍に対するがん自殺遺伝子療法

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増殖型レトロウイルスベクター (RRV: Retroviral Replicating Vector) は、癌細胞特異的に感染・増殖する性質を持つ。自殺遺伝子として酵母由来のシトシン脱アミノ化酵素 (yCD) を搭載したRRVは、様々なヒト癌細胞を用いた担癌マウスモデルにおいて高い感染伝播効率と優れた治療効果を示すことが明らかとなっている。そこで、近年著しく増加しているイヌの癌においてもRRVが有効ではないかと考えた。本研究では、イヌ悪性腫瘍モデルにおいて2種のRRV（マウス由来のAMLVとテナガザル由来のGALV）を用いた自殺遺伝子療法の有効性を検討した。

GFP発現RRVを用いた検討では、両RRVともイヌ正常細胞においては感染伝播を認めなかったが、今回用いた10種類のイヌ腫瘍細胞株においてはいずれも両RRVの効率的な感染伝播を認め、特に線維肉腫細胞においては感染10日後までに90%以上の感染効率を得た。次に自殺遺伝子yCD発現RRVを用いた検討では、正常細胞においてはRRVによる殺細胞効果は認めなかったが、イヌ腫瘍細胞株ではいずれもRRV感染伝播効率と薬物前駆体5-FCに依存した殺細胞効果を認めた。さらにイヌ皮下腫瘍マウスモデルにおいて、ルシフェラーゼ発現RRVを腫瘍内投与後に生体イメージングによって経時的に観察したところ、両RRVは腫瘍内でも効率よく増殖伝播した。イヌ線維肉腫細胞を用いた皮下腫瘍マウスモデルを用いた治療実験においては、RRV治療群ではいずれも有意な抗腫瘍効果が認められた。

これらのデータは、RRVを用いたがん自殺遺伝子療法がヒトのみならず、イヌのがん治療においても有用であり、獣医学分野でも応用可能であることを示している。

Development of a novel CAR-T cell therapy targeting insulin-like growth factor I receptor for lung adenocarcinoma

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Introduction
Insulin-like growth factor I receptor (IGF1R) is observed on the tumor cell membrane with high expression among the various types of tumors. Although some kinds of IGF1R-targeted drugs have been developed against lung adenocarcinoma, few clinical effects against lung adenocarcinoma were shown. On the other hand, high expression of IGF1R might be related to short recurrence-free survival and the mechanisms of resistance of epidermal growth factor receptor tyrosine kinase inhibitor. We have developed the ligand-based CAR-T cell therapy targeting IGF1R using the *piggyBac* transposon system. Herein, we demonstrated the efficacy of IGF1R CAR-T against lung adenocarcinoma *in vitro*.
Methods
The expression of IGF1R on the tumor cell membrane was evaluated using flow cytometry in 16 types of lung adenocarcinoma cell lines. Then, co-culture experiments were performed with IGF1R CARTs and activated T cells (ATC) against the cell lines with high expression of IGF1R. IGF1R CAR-Ts were generated by a non-viral gene modification called piggyBac transposon method. Co-culture experiments were performed at an effector/target (E/T ratio) of 4, 2, 1, 0.5, and 0.25. The antitumor efficacy of the co-culture experiments was evaluated on day 4. IFN-γ and IL-2 released by CAR-T cells in the co-culture experiments were evaluated by ELISA.
Results
Thirteen cell lines (81%) expressed IGF1R, while seven cell lines (44%) expressed over 90%. IGF1R expression in H1355, H1568, and A549 was 100%, 99%, 95%, respectively. IGF1R CAR was expressed on an average of 40% of T-cells on day 14. In the co-culture experiment against these tumor cell lines, it was shown that IGF1R CAR-T cells could suppress the increase of every tumor cell line more than that of control. Moreover, cytokine release of IGF1R CAT-T cells was higher than that of control.
Conclusion
In this study, IGF1R CAR-T using the *piggyBac* transposon system demonstrated well anti-tumor effect against lung adenocarcinoma *in vitro*. *In vivo* tests are going to be planned in near the future.

STING Activator 2'3'-cGAMP Enhances the Anti-tumor Efficacy of C-REV, an HSV1 based Oncolytic Virus

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Recent researches have demonstrated oncolytic virus (OV) therapy to be capable of remodeling the immunosuppressive microenvironment of immune cold tumors, making it a promising strategy to evoke an anti-tumor immunity. Inflammations caused by viral infections activate host viral defense mechanisms including the STING-IFNs pathway to eradicate viruses. Here, we show that although the STING activator, 2'3'-cGAMP was expected to increase anti-viral immunity, the combination therapy of C-REV with 2'3'-cGAMP significantly enhanced anti-tumor immunity. C-REV treatment followed by 2'3'-cGAMP treatment showed synergistic anti-tumor immune responses not only in treated tumors but also in the non-treated distal tumors. Due to the mostly defective STING pathway of tumor cells which prevents them from inducing IFNs, 2'3'-cGAMP treatment permitted efficient viral replication inside tumor cells. Although 2'3'-cGAMP single treatment had little effect on the recruitment of immune cells to tumors, combination therapy efficiently induced the proliferative KLRG1-high PD1-low CD8+ T cell population and increased the number of activated dendritic cells (DCs). Moreover, tumor-draining lymph nodes under the combination therapy contained significantly high numbers of CD44+ CD8+ T cells which exhibited strong tumor antigen-specific cytotoxicity. Finally, combination therapy-treated mice that eradicated primary tumors were resistant to secondary tumor rechallenge experiments. Taken together, our results strongly suggest that C-REV combination therapy with 2'3'-cGAMP enhances systemic anti-tumor immunity.

レオウイルス感染腫瘍細胞由来細胞外小胞による抗腫瘍効果に関する検討

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【背景・目的】近年、細胞外小胞 (Extracellular Vesicle; EV)が注目を集めている。EVは産生細胞由来のタンパク質や核酸を含んでおり、細胞間の情報伝達を担っている。さらに近年、ウイルス感染細胞由来EVがウイルスゲノムやウイルスタンパク質だけでなく、ウイルス粒子そのものを内封していることも報告されている。腫瘍溶解性ウイルスが感染した腫瘍細胞もEVを産生すると考えられるが、腫瘍溶解性ウイルス感染腫瘍細胞由来EVが腫瘍細胞や担癌生体にどのような効果を示すかは、ほとんど明らかになっていない。そこで本研究では、腫瘍溶解性ウイルスであるレオウイルスが感染した腫瘍細胞が分泌するEVの抗腫瘍効果を中心に、その特性を検討した。
【方法】レオウイルスをB16細胞にMOI1で感染させ、培養上清よりレオウイルス感染B16細胞由来EV(Reo-EV)を超遠心法により回収した。Reo-EVを各種癌細胞に作用させ、殺細胞効果を検討するとともに、B16担癌マウスに尾静脈内投与し、腫瘍体積、腫瘍におけるレオウイルスの増殖や腫瘍細胞のアポトーシスについて解析した。
【結果】レオウイルスをB16細胞にMOI1で作用させたところ、明らかな細胞死は観察されなかったものの、培養上清より感染能を有したReo-EVが回収された。Reo-EVは、各種癌細胞に対し、レオウイルスと同程度の殺細胞効果を示した。Reo-EVは静脈内投与後、皮下腫瘍の成長を有意に抑制し、腫瘍内ではウイルスタンパクやウイルスゲノム、カスパーゼ3の活性化が検出された。
【結論】レオウイルス感染後、腫瘍細胞の溶解が起こる前に子孫ウイルスがEVと相互作用した状態で放出されていることが示唆された。さらに、Reo-EVはレオウイルスと同程度の抗腫瘍効果を有することが示された。

Gliomaに対する第2世代REIC/Dkk-3遺伝子発現アデノウイルスとbevacizumabの併用療法

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【はじめに】当科ではReduced Expression in Immortalized Cells/Dickkopf-3 (REIC/Dkk-3)発現アデノウイルス（Ad-REIC）のgliomaに対する抗腫瘍効果の検討を行ってきた。現在、第2世代Ad-REIC（Ad-SGE-REIC）が開発されている。今回、我々はAd-SGE-REICと抗VEGF抗体であるbevacizumabの併用による抗腫瘍効果について検討を行った。
【方法】Glioma細胞株（U87ΔEGFR、U251、MGG8、MGG23）を使用しcytotoxicityを評価した。アデノウイルスはAd-SGE-REICを用いた。腫瘍細胞のmigrationはdouble chamber assay法にて評価した。また、ヌードマウスの右脳にU87ΔEGFRを移植し、4群（control群、bevacizumab群、Ad-SGE-REIC（SGE）群、併用群）に分けた。移植後5日目より週2回bevacizumabを腹腔内に、また、7日目に腫瘍内にAd-SGE-REICを投与し、各群の生存期間を比較した。
【結果】Cytotoxicityを調べたところ、いずれの細胞株においても、SGE群と併用群で腫瘍細胞に対して高い治療効果を示した。Double chamber assayでは、bevacizumabによる浸潤誘導、SGE群における抗遊走効果が示された。併用群では、bevacizumab誘導性浸潤を抑えることができた。Tube formation assayにてangiogenesisを調べたところ、併用群において著明に抑制されていた。U87ΔEGFRのnuclear beta-cateninの発現を調べたところ、bevacizumab群、SGE群において有意に低下しており、併用群ではさらに低下していた。In vivoでは、併用群において有意な生存期間の延長が認められた。
【結語】Ad-SGE-REICとbevacizumabを併用することにより、gliomaに対し高い抗腫瘍効果を示す可能性が示唆された。現在、Ad-SGE-REICを用いた再発glioma症例に対する治験を行っている。

Metabolic reprogramming by anti-mitochondrial agent promotes sensitivity to oncolytic adenoviruses in non-glycolytic pancreatic cancer

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers. Despite recent advances in multi-agent chemotherapy for the treatment of PDAC, the overall 5-year survival rate is still less than 10%. Oncolytic virotherapy has recently emerged as a novel therapy to induce tumor-specific lytic cell death. We recently demonstrated the antitumor effect of telomerase-specific replication-competent oncolytic adenoviruses OBP-301 and p53-armed OBP-702 in human PDAC cells. However, the sensitivity to oncolytic adenoviruses has been suggested to be modulated by metabolic phenotypes of cancer cells. In this study, we assessed the sensitivity to OBP-301 and OBP-702 in human PDAC cells with different metabolic phenotypes and the therapeutic potentials of combination therapy with oncolytic adenoviruses and metabolic agent against virotherapy-resistant PDAC cells.

Methods: Two glycolytic human PDAC cells (MIA PaCa-2, PK-45H) and two non-glycolytic human PDAC cells (PK-59, Capan-2) were used in this study. The sensitivity to oncolytic adenoviruses was analyzed by the XTT assay. The virus replication and p53 induction were analyzed in virus-infected PDAC cells by real-time RT-PCR and western blot analysis. The effect of metabolic reprogramming from non-glycolytic phenotypes to glycolytic phenotypes in virus sensitivity of PDAC cells was analyzed by exposure to hypoxia and low glucose or by treatment with anti-mitochondrial agent (CPI-613). Subcutaneous PDAC tumor models with glycolytic MIA PaCa-2 cells and non-glycolytic PK-59 cells were used to evaluate the antitumor effect of monotherapy with oncolytic adenoviruses or combination therapy with OBP-702 and CPI-613.

Results: Glycolytic PDAC cells were sensitive to OBP-301 and OBP-702, whereas non-glycolytic PDAC cells were less sensitive. Virus-mediated expression of E1A and p53 was significantly higher in glycolytic PDAC cells than non-glycolytic PDAC cells. Exposure to hypoxia and low glucose or administration of CPI-613 induced the metabolic reprogramming from non-glycolytic phenotypes to glycolytic phenotypes in non-glycolytic PDAC cells, resulting in the increased sensitivity to oncolytic adenoviruses. In contrast, glycolytic PDAC cells maintained the glycolytic phenotypes after exposure to hypoxia and low glucose or administration of CPI-613. In vivo experiments demonstrated that glycolytic MIA PaCa-2 tumors were more sensitive to oncolytic adenoviruses compared to non-glycolytic PK-59 tumors. Moreover, combination therapy with OBP-702 and CPI-613 significantly suppressed the tumor growth compared to monotherapy in non-glycolytic PK-59 tumor models.

Conclusion: Our results suggest that non-glycolytic PDAC cells are less sensitive to oncolytic adenoviruses than glycolytic PDAC cells. Combination of anti-mitochondrial agent CPI-613 would be a promising antitumor strategy to promote the antitumor efficacy of oncolytic adenoviruses against virotherapy-resistant PDAC cells with non-glycolytic phenotypes via induction of metabolic reprogramming.

Novel armed oncolytic HSV exhibits strong antitumor effects that lead to complete tumor regression

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We have developed the new armed oncolytic virus (OV) carrying three cytokines. The cytokines were selected among various combination, and IL7+CCL19+IL21 or IL15+ CCL19+IL21 were considered as the best two combination for more potent antitumor effects. The backbone of this armed OV is a natural mutant of HSV-1, C-REV (canerpaturev, formally known as HF10) with which more than 170 patients were treated in the clinical trials for melanoma, pancreatic, breast, and head and neck cancers. The armed cytokines are expected to increase adaptive antitumor immunity, especially T cells and dendritic cells. Intratumoral administration of cytokine armed-C-REVs led to dose-dependent antitumor effect in SCC-VII and Pan02 murine tumor models. In the SCC-VII model, Three of six mice treated with IL-7-CCL19-IL-21 armed-C-REV achieved complete tumor regression, and two of six mice treated with IL-15-CCL19-IL-21 armed-C-REV achieved complete tumor regression, whereas only one of six mice achieved complete tumor regression after treatment with C-REV. Next, we conducted bilateral tumor models to examine whether localized activation of immune responses by cytokine armed-C-REVs affects systemic immunity. The growth of the contralateral tumor was suppressed as much as the cytokine armed-C-REV injected tumor, suggesting that the antitumor response in the contralateral tumors is due to immune responses caused by tumor-infiltrating lymphocytes. Infiltration of cytotoxic T cells and cDC-1 were increased in the injected tumor and the contralateral tumor, whereas T-regulatory was decreased in the tumor. Furthermore, no increase in blood cytokine levels was observed after cytokine armed-C-REV treatment. From these results, cytokine-armed C-REVs are highly safe oncolytic viruses with a strong antitumor effect.

Retroviral replicating vector-mediated prodrug activator gene therapy for lung cancer

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Background: Despite the recent development of tyrosine kinase inhibitors and immunotherapy, lung cancer (LC) remains one of the most difficult malignancies to treat, and new therapeutics and treatment strategies are required. Retroviral replicating vectors (RRV) are capable of highly efficient replication and transduction in cancer cells. Prodrug activator gene therapy with Toca 511 (*vocimagene amiretrorepvec*), an RRV encoding yeast cytosine deaminase (yCD), which converts the prodrug 5-fluorocytosine (5-FC) to the anticancer drug 5-fluorouracil (5-FU) within Toca 511-infected cells, has been shown to achieve significantly enhanced survival benefit in a variety of preclinical tumor models. In an international randomized Phase 2/3 clinical trial for patients with recurrent high-grade glioma, Toca 511 and 5-FC treatment did not meet its endpoints overall, but demonstrated highly promising evidence of survival benefit in pre-specified patient subgroups. Additionally, a Phase1b clinical trial for patients with advanced solid tumors and lymphoma suggested possible activity in these heavily pretreated patients. Therefore, we evaluated the therapeutic activity of RRV-mediated prodrug activator gene therapy in human LC-derived cell lines and in a preclinical model for lung cancer.
Methods: We first quantitated the replication kinetics of RRV in human lung cancer cell lines (A549, H226 and SBC-3) and mouse LC cell line (Ex-3LL). Human and mouse LC cell lines were infected with RRV expressing the GFP marker gene (RRV-GFP) at a multiplicity of infection (MOI) of 0.01, and analyzed by flow cytometry. Next, we evaluated in vitro cytotoxicity in these cell lines by MTS assay after exposure to different concentrations of 5-FC after transduction with Toca 511. Finally, we evaluated in vivo therapeutic efficacy of Toca 511/5-FC prodrug activator gene therapy in a subcutaneous tumor model and an orthotopic pleural dissemination model.
Results: In both human and mouse LC lines, RRV-GFP infected at MOI of 0.01 showed rapid viral replication resulting in high levels of transduction, as indicated by the percentages of GFP-positive cells reaching nearly 90% over time. In MTS assays, the viability of Toca 511-transduced cells was reduced by approximately 80% after 3 days of exposure to 0.1mM 5-FC prodrug, as compared to control cells. Furthermore, in subcutaneous tumor models, the Toca 511/5-FC treated group showed significant tumor growth inhibition compared to control groups ($p<0.0001$) with 80% showing complete tumor regression. In the orthotopic pleural dissemination model, the Toca 511/5-FC treatment group showed prolonged overall survival ($p=0.0138$) compared to the control group.
Conclusion: These in vitro and in vivo data suggest that RRVs are highly efficient vehicles for delivering therapeutic genes such as yCD to lung cancer cells, and Toca 511/ 5-FC treatment has potential as a novel therapeutic strategy for human lung cancer.

Retroviral replicating vector-mediated gene therapy activates anti-tumor immune responses in an immunocompetent murine pancreatic cancer model

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Background: Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal cancers. Even with modern multidisciplinary treatment regimens, its clinical outcome remains poor. Thus, novel therapeutic strategies need to be developed. Prodrug activator gene therapy with Toca 511, a tumor-selective retroviral replicating vector encoding an optimized yeast cytosine deaminase (yCD) prodrug activator gene, is a new strategy currently under clinical evaluation for high-grade glioma. Toca 511 exerts direct anti-tumor effects through intratumoral conversion of the prodrug 5-fluorocytosine (5-FC) to the active drug 5-fluorouracil (5-FU) by its encoded yCD enzyme. Recent studies have demonstrated that 'bystander' effects of Toca 511/5-FC treatment can also induce anti-tumor immunity by eliminating immunosuppressive cells within the tumor. Because many patients with PDAC develop metastatic lesions, activation of systemic anti-tumor immunity may improve outcomes. Therefore, in this study, we evaluated therapeutic efficacy and anti-tumor immune responses activated by Toca 511/5-FC prodrug activator gene therapy in an immunocompetent murine PDAC model.
Methods: We established bilateral subcutaneous tumor models by inoculation of the syngeneic murine PDAC cell line Pan02 in both flanks of syngeneic C57BL/6J mice. Tumors inoculated into the ipsilateral flank were initially transduced with Toca 511, while contralateral flank tumors were not initially transduced. Subsequently, the treatment group received intraperitoneal injections of 5-FC, and PBS was administered to the control group. Tumor size was measured twice a week, and qPCR of tumor tissue was performed to quantitate vector integration. For CTL assays, CD8+ T effector cells were isolated from splenocytes and cocultured with Pan02 target cells at effector-to-target cell ratios ranging from 0:1 to 30:1, and cytotoxicity was determined. Immunopathologic analyses of RRV-uninfected contralateral tumors was also performed by immunohistochemical staining with mouse anti-CD4, -CD8 and -FOXP3 antibodies. The number of positive cells per was counted in five randomly selected high-power fields in both the center and periphery of the tumor.
Results: We first evaluated whether initially untransduced contralateral tumors might subsequently become infected over time. However, qPCR analysis showed little or no Toca 511 infection in contralateral tumors. Nonetheless, in addition to complete suppression of Toca 511-infected ipsilateral tumor growth following 5-FC prodrug treatment, partial inhibition of uninfected contralateral tumor growth was also observed. In CTL assays, the 5-FC treatment group demonstrated higher CD8+ T effector cell-mediated cytotoxicity against Pan02 target cells than the control group. Immunohistochemical analysis also showed significantly higher $CD8^+$ and $CD4^+$ T cell infiltration in the 5-FC treatment group compared to the control group.
Conclusion: These data suggest that Toca 511/5-FC treatment can induce systemic anti-tumor immune responses in this syngeneic immunocompetent PDAC model, and that induction of tumor-specific CD8+ T effector cells was associated with therapeutic efficacy and tumor growth inhibition even of Toca511-uninfected contralateral lesions.

Tropism of Adeno-Associated Virus toward Neural Stem Cells in Gerbil Hippocampus

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[Background] Tropism of adeno-associated virus (AAV) vectors is determined by the amino acid sequence of viral capsids.
[Objective] The purpose of this study is to determine the tropism of natural serotypes of AAV, including AAV2, 5, and rh10, toward neural stem cells of the hippocampus.
[Material and Methods] Four-week-old male gerbils were administered with 1.5×10^{10} viral genomes of AAV solution (AAV2, 5, or rh10) carrying green fluorescent protein (GFP) or phosphate-buffered saline (2 µl) into the right dentate gyrus using stereotaxic apparatus (n = 3, each). One week later, the animals were decapitated under deep anesthesia, and the brains were sliced into 10 µm-coronal sections for immunohistochemical analysis.
[Results] The brain sections showed that the GFP-expressing area of the AAVrh10 group was the largest followed by AAV5 and AAV2 (AAV2: 0.3 ± 0.2 mm^2, AAV5: 2.2 ± 0 mm^2, AAVrh10: 4.7 ± 0.2 mm^2, $p<0.001$, significant in sham vs AAV2, AAV2 vs AAV5, and AAV5 vs AAVrh10). Next, the immunohistochemical analysis showed that the number of SOX2/GFP double-positive cells in the dentate gyrus was significantly higher in AAV5 compared to AAVrh10 (AAV5: 73.3 ± 4.8 /slice, AAVrh10: 46.3 ± 10.5 /slice, $p<0.05$). In contrast, the number of NeuN/GFP double-positive cells in the dentate gyrus was significantly higher in AAVrh10 compared to AAV5 (AAV-5: 13.6 ± 5.9 /slice, AAV-rh10: 143.4 ± 19.9 /slice; $p<0.001$). Few double-positive cells were found in the AAV2 group in this study.
[Conclusions] AAV5 showed higher transduction efficiency toward neural stem cells of dentate gyrus compared to AAVrh10. In contrast, AAVrh10 showed higher transduction efficiency toward mature neurons compared to AAV5. Overall, this knowledge will be useful for the choice of viral serotype to undergo safe and effective gene transduction.

Systemic therapy for Fabry disease using AAV vector

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[Objective**]** Fabry disease is an X-linked lysosomal storage disease caused by deficiency of α-galactosidase A (α-GLA). Enzyme replacement therapy and molecular chaperone therapy are available, although they need to be administrated permanently because of their short half-time. The aim of this study is to assess efficacy of adeno-associated virus (AAV)-mediated gene therapy for Fabry disease.
[Methods**]** We confirmed the efficacy of AAV9 vector containing human α-GLA gene by intravenous injection into 6-weeks-old male α-GLA knockout (KO) mice. Plasma α-GLA activity was analyzed by fluorescence-enzymatic activity assay 3 and 8 weeks after injection. Animals were killed 8 weeks after injection for α-GLA activity assay and quantitative PCR of brain, heart, liver, and kidney (n = 7-8, each group).
[Results**]** The AAV-treated mice showed significantly higher α-GLA activity in the heart and liver, compared to the wild type mice (brain: [wild type] 62.62 ± 4.18 nmol/h/mg protein, [vehicle] undetectable, [AAV9] 6.41 ± 1.59 nmol/h/mg protein; heart: [wild type] 2.37 ± 0.17 nmol/h/mg protein, [vehicle] undetectable, [AAV9] 9.75 ± 3.65 nmol/h/mg protein; liver: [wild type] 26.57 ± 0.78 nmol/h/mg protein, [vehicle] 1.80 ± 0.29 nmol/h/mg protein, [AAV9] 46.77 ± 5.06 nmol/h/mg protein; kidney: [wild type] 16.71 ± 1.38 nmol/h/mg protein, [vehicle] 1.55 ± 1.29 nmol/h/mg protein, [AAV9] 6.63 ± 1.58 nmol/h/mg protein). The vector genome copy numbers per diploid genomes significantly increased in the all four organs of AAV-treated mice (brain: [wild type] 0.05 ± 0.01, [vehicle] 0.13 ± 0.01, [AAV9] 0.67 ± 0.17 ; heart: [wild type] 0.03 ± 0.01, [vehicle] 0.02 ± 0.00, [AAV9] 2.30 ± 0.74 ; liver: [wild type] 0.04 ± 0.00, [vehicle] 0.08 ± 0.01, [AAV9] 186.75 ± 82.89 ; kidney: [wild type] 0.01 ± 0.00, [vehicle] 0.04 ± 0.00, [AAV9] 1.25 ± 0.53).
[Conclusions**]** Intravenous injection of AAV vector will be a promising gene therapy for patients with Fabry disease. Because the α-GLA activity expressed by the delivered gene was insufficient in the brain and kidney of α-GLAKO mice, we need further experiment to find the appropriate serotype or administration route of AAV vector.

マウス脳内神経細胞及び血管内皮細胞に効率よく遺伝子導入が可能な新規AAV2キャプシドCereAAVの開発

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アデノ随伴ウイルスベクター（AAV）は、分裂及び非分裂細胞にも効率よく遺伝子導入が可能であり、非病原性で安全性も高い面から、様々な疾患を対象として多くの前臨床及び臨床試験が実施されている。AAV9は中枢神経系を含む脳を対象とした遺伝子導入によく用いられる血清型であるが、異なる血清型としてAAV2変異体であるAAV2-BR1は、全身投与により血管内皮細胞に効率よく導入されることが報告されている。しかしながら、脳血液関門を越えて中枢神経系に効率的に遺伝子導入できるAAV2変異体はまだ報告がない。そこで、我々はAAV2血清型を用いて、脳を標的とした新規AAVベクターの開発を試みた。

ウイルス粒子表面にランダムペプチドを露出させたウイルスライブラリーを用いた定向進化法により、マウス脳に効率よく遺伝子導入可能なAAV2キャプシド変異体(AAV2mt)を同定した。AAV2mtをマウスに静脈内投与し(1x10^12 vg)、4週間後のAcGFP遺伝子発現を解析したところ、AAV2mt投与群ではAAV2及びAAV9投与群よりも脳全体で顕著なAcGFP発現が検出され、特に海馬及び視床において発現が高かった。また、このAcGFP発現は、マウス脳内の神経細胞や微小血管内皮細胞に認められたが、その他の細胞には認められなかった。マウス脳内のAAV2mtゲノム量は、AAV2及びAAV9と比較して、それぞれ250倍及び50倍と高かったが、肝臓のAAV2mtゲノム量は、AAV2よりも顕著に低かった。これらの結果から、最も効率よく脳に遺伝子導入ができ、肝臓への遺伝子導入が低いAAV2mtをCereAAV™と名付け、現在、マーモセットにおいてCereAAV™の遺伝子導入評価を実施している。以上の結果より、CereAAV™は、脳を対象とした遺伝子導入ベクターとして非常に有用なツールとなることが期待される。

リゾリン脂質アシル転移酵素LPCAT4/LPLAT10の肝臓特異的な高発現による、2型糖尿病モデルマウスに対する治療効果の検討

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近年、糖尿病などの生活習慣病の病態解明において、脂質を構成する脂肪酸の多様性が注目されている。生体膜の主要な脂質であるリン脂質は2本の脂肪酸を有し、脂肪酸の組み合わせにより多種類のリン脂質が存在する。リン脂質の種類の変化は、肥満や非アルコール性脂肪性肝疾患などの発症・進展に関与することが報告されている。そこで、糖代謝の中心臓器である肝臓においてリン脂質の種類を変化させることは、糖尿病に対する新たな治療につながると考えた。本研究では、リン脂質の多様性を形成する酵素「リゾリン脂質アシル転移酵素」のひとつであり、肝臓において発現量が少ないlysophosphatidylcholine acyltransferase 4（LPCAT4）/lysophospholipid acyltransferase 10（LPLAT10）に注目した。そして、LPCAT4/LPLAT10を糖尿病モデルマウスの肝臓で高発現させ、2型糖尿病に対する影響を検討した。

LPCAT4/LPLAT10を高発現させるベクターとして、以前に開発した、肝障害性が低く、長期に渡って目的遺伝子を発現可能な改良型アデノウイルス（adenovirus; Ad）ベクター（Ad-E4-122aT）を選択し、LPCAT4/LPLAT10を搭載したAdベクター（Ad-LPCAT4/LPLAT10）を作製した。作製したAdベクターを糖尿病モデルマウスに静脈内投与し、2週間後の肝臓におけるリン脂質の分子種を液体クロマトグラフィー質量分析法により測定したところ、Ad-LPCAT4/LPLAT10投与群では多価不飽和脂肪酸を有するリン脂質が増加していた。2型糖尿病に対する影響を調べるため、糖負荷試験を行ったところ、Ad-LPCAT4/LPLAT10群はコントロール群と比較して、血糖値の上昇が緩やかであるとともに、インスリン分泌量の増加が示された。したがって、LPCAT4/LPLAT10の肝臓における高発現は、食後高血糖を抑制することが示された。以上より、LPCAT4/LPLAT10は糖代謝異常の改善に寄与することから、2型糖尿病に対する新しい治療ターゲットとなる可能性が見出された。

Successful Re-administration of Adeno-associated Virus Vectors to Change the Serotypes in Mice

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[Background] Intravenous administration of adeno-associated virus (AAV) vector is a promising gene therapy approach for monogenic diseases. However, re-administration of the same AAV serotype is impossible due to the induction of anti-AAV neutralizing antibodies (Nabs) after initial AAV treatment.
[Aim] To examine the feasibility of re-administration of AAV vectors to change the serotypes.
[Method] We intravenously administrated AAV3B, AAV5, or AAV8 vectors harboring human factor IX targeting liver to C57BL/6 mice (0.5×10^{11}/kg), and then assessed the emergence of Nabs in serum. The mice then received the same dose of secondary AAV vectors expressing the secretory type of NanoLuc. The success of secondary transduction into the liver was assessed by serum NanoLuc activity.
[Results] We confirmed that re-administration of the same serotype was not possible for all serotypes. The highest neutralizing activity Nab in serum was induced by AAV5 treatment compared with other serotypes. However, the Nabs elicited by the first AAV5 treatment did not abolish the transduction with AAV3B and AAV8 (0/12). Secondary administration in all mice treated by AAV5 was succeeded with AAV3B or AAV8. Conversely, the re-administration of AAV5 could be succeeded in all mice treated with AAV3B and AAV8. The effective secondary administration of AAV3B and AAV8 was also observed in most mice treated with AAV8 and AAV3B, respectively. However, few mice developed Nabs cross-reactive with the other serotype (2/12, AAV3B Nab in AAV8-treated mice; 1/12, AAV8 Nab in AAV3B-treated mice).
[Conclusion] Secondary systemic administration of AAVs targeting liver transduction was successfully achieved by switching AAV serotypes in mice. Considering the re-administration of AAV vectors to patients who have failed to respond to initial gene therapy or who have lost therapeutic effect over time, it is crucial to develop different AAV preparations targeting for one disease.

アデノ随伴ウイルス受容体AAVRを利用した温和なAAV精製法の開発

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アデノ随伴ウイルス（AAV）ベクターは安全性が高く、遺伝子治療用医薬品として広く利用されている。AAV には様々な血清型が存在し、大部分は AAV 受容体（AAVR；KIAA0319L）との結合を介して細胞へ侵入して感染する。AAVR との相互作用により AAV の分離が可能となれば、感染能を有する高純度な AAV を精製・分析できる。そこで著者らは、AAVR をゲルに固定化したアフィニティクロマトグラフィカラム（以下、AVR カラム）を開発した。AVR カラムを使用し、中性条件で AAV をカラムに吸着させたのち、pH を下げて AAV を溶出することで、AAVの吸脱着を確認した。カプシドのアミノ酸に変異を導入したAAV変異体の分析を試みたところ、各種変異体の溶出時間と感染能とに相関がみられたことから、AAVR を介した感染性を評価できることが示唆された。さらに、培養上清や細胞溶解液中の AAV の検出にも成功し、精製することなく培養中のAAV生産量のモニタリングを可能にした。

AVRカラムは、一般的なアフィニティクロマトグラフィー同様、pH中性条件でカラムと結合し、pHを下げてAAVを溶出（酸溶出）する。しかしながら、酸性下においてAAVは品質が悪化し、感染能が低下することが知られている。そこで、より温和な条件でAAVを溶出する方法を検討した。

検討により、リガンドであるAAVRとAAVはカルシウムイオンにより親和性が向上することがわかった。そこで、親和性を弱めることを目的に、種々の中性緩衝液（pH6.0-9.0）によるAAV精製法を検討した。結果、キレート剤もしくは高濃度の金属塩を含む緩衝液により、AAVの中性条件での溶出を確認した。さらに、精製したAAVは酸溶出したAAVと比較して高い感染能を有していた。本技術を用いることで、一般的に利用されていた酸溶出によるAAVの変性を抑制でき、高純度、高タイターなAAVの製造に有用と考えられる。

アデノ随伴ウイルス（AAV）ベクター粒子の自動判別システムの開発

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ヒト・霊長類をホストとするAAVは増殖および非増殖細胞に感染可能な非病原性ウイルスであり、その組換えベクターであるAAVベクターのウイルスゲノム上に搭載された目的遺伝子の高い伝達効率から安全かつ有効な遺伝子治療用ベクターとしていくつかの疾患に応用されている。一方、AAVベクターの中和抗体の存在や臓器指向性の不完全さによる副作用などにより単回大量投与による治験での死亡例の発生などから、AAVベクターの安全性および有効性についての改善が必要である。また、その作製過程において、キャプシド内部に完全長の目的遺伝子を包括した完全体粒子のほか、DNAを含まない空の外殻である中空粒子、さらには粒子の一部が損傷した破損粒子あるいは、断片化した目的遺伝子が包括された中間体などが産出される。このためAAVベクターの安全性および有効性に対する品質向上のためには、高品質ベクター製造評価を目的としたQuality by Designとして、上述の種々のウイルス粒子群を識別できる品質管理システムの構築が必須となる。現在、これらの粒子混合物の検出には分析用超遠心装置を用いた超遠心分析（AUC）が行われているが、必要サンプル量が多いなどの課題が存在している。一方で、透過型電子顕微鏡および極低温電子顕微鏡を用いたAAV粒子の検出では比較的少量のサンプル量で解析可能などの長所があるが、検出には目視による確認が主に行われており、煩雑さや検出エラーなどの課題が残されている。そこで、高性能および自動化されたウイルス判別を支援するシステムの構築を人工知能（AI）を用いて行った。このシステムの構築によって、ベクターの各製造過程における各AAV粒子の生物学的活性評価や生成機構の推定にもつながることが期待される。

遺伝子治療用レンチウイルスベクターの構造最適化

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レンチウイルスベクターは、初代培養細胞や幹細胞、神経細胞などの非分裂細胞を含む、ほぼすべての哺乳類細胞に遺伝子導入が可能なため、分子生物学研究ツールとして幅広く利用されている。さらに、導入遺伝子は、染色体に組み込まれ長期間安定発現することから、癌、免疫不全症、代謝疾患、先天性疾患などの遺伝子治療への応用が進められている。

我々はこれまで、安全性を配慮してHIV由来配列を可能な限り削減した、Tat 非依存性の第三世代型レンチウイルスベクタープラスミド (pLVpro) と、ベクターの大量製造を実現するための、高力価ウイルス産生系の開発を進めてきた。臨床用途にレンチウイルスベクターを用いる場合、染色体に挿入され遺伝子発現が長期間持続するため、より安定かつ機能的なベクター構造の選択が重要となる。今回は、遺伝子治療の中でも、TCR/CAR-T細胞療法等で、レンチウイルスベクターが盛んに利用されるT細胞をターゲットとし、ベクター構造の最適化を実施した。

内部プロモーターや目的遺伝子の異なる、複数のレンチウイルスベクターを作製したところ、それらの組み合わせによりベクター産生量は大きく異なった。さらに、内部プロモーターの種類や目的遺伝子によりプロウイルス中でのスプライシングの発生頻度に差が生じることを確認した。

また、WPRE配列は、目的遺伝子の発現を促進させる目的でレンチウイルスベクターに広く利用される機能的配列の一種だが、細胞種により目的遺伝子の発現の低下が認められた。加えて、薬剤耐性遺伝子などの複数遺伝子を発現させる場合には、WPRE配列の搭載位置が、目的遺伝子の発現に大きく影響することも明らかとなった。

以上より、効果的な遺伝子治療法の確立には、細胞種・目的遺伝子ごとに、内部プロモーターの選択やWPRE配列の挿入の有無など、レンチウイルスベクター構造の最適化を行うことが非常に重要である。

中枢神経移行型酵素を搭載したAAVによる肝臓を標的としたムコ多糖症II型への遺伝子治療

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【背景】ムコ多糖症II型（MPS II）はイズロン酸-2-スルファターゼ（IDS）の欠損を原因とするX連鎖性潜性（劣性）遺伝病であり、中枢神経病変を含む様々な症状を全身性に呈する。近年、AAVを用いた遺伝子治療はMPS IIを含む様々な疾患に対して開発が行われているが、高い有効性とウイルス力価低減の両立が重要な課題となっている。この両者を実現し得る方法として我々は、治療標的臓器を酵素分泌能に優れる肝臓とし、発現させるIDSを血液脳関門を透過可能な抗トランスフェリン受容体抗体融合IDS（TfR-IDS）とする方法を着想した。そこで本研究では、TfR-IDS搭載AAVを作成し、MPS IIモデルマウスに投与することでその治療効果の評価を行った。
【方法】マウスアルブミンプロモーターとTfR-IDSを搭載するAAV9を作成し、8週齢のMPS IIマウスへ静脈投与した（1 x 10^12 vg/kg）。投与後、経時的に血中IDS活性を測定し、治療6ヶ月経過した時点で各種臓器を採取し、IDS酵素活性並びに蓄積基質であるグリコサミノグリカン（GAG）量の評価を行った。
【結果・考察】投与1ヶ月後の血中IDS活性は野生型よりも約18倍高く、6ヶ月後では約6倍の活性が認められた。治療したマウスの肝臓や脾臓では野生型を上回るIDS活性が検出され、殆どの個体でGAG蓄積の正常化も認められた。さらに、大脳などの中枢神経においてもIDS酵素活性の上昇ならびに蓄積するGAG量の大幅な減少が認められた。我々は以前、CMVプロモーター下でIDSを発現するAAV9を静脈投与することで本研究と同様の中枢神経への効果が得られることを確認したが、その際に必要なAAV力価は今回の3倍量だった。以上より、TfR-IDS搭載AAVを用いた肝臓を標的とした遺伝子治療は、MPS IIに対する有望な治療となり得ると考えられる。

Preclinical Safety and Efficacy Validation of CD4LVFOXP3 Cells as an Innovative Treg-like Cell-based Gene Therapy for IPEX Syndrome

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FOXP3 is an essential transcription factor for regulatory T cell (Treg) function, and a key regulator of immune tolerance. Genetic or acquired defects in Treg play a key role in immune mediated diseases including Immune dysregulation, Polyendocrinopathy, Enteropathy, X-linked (IPEX) syndrome. We have optimized a protocol for the generation of Treg-like cells from CD4+ T cells of healthy donors or IPEX patients, by lentiviral-mediated gene transfer of *FOXP3* ($CD4^{LVFOXP3}$) which acquire stable functional regulatory properties. The $CD4^{LVFOXP3}$, despite being generated from effector T cells, are phenotypically stable in the presence of rapamycin, which is a widely used treatment for IPEX syndrome. Indeed, $CD4^{LVFOXP3}$ show similar stability to that of expanded Tregs in the presence of rapamycin. Furthermore, autologous $CD4^{LVFOXP3}$ generated in GMP grade conditions are functionally stable after cryopreservation and showed dose-dependent xeno-GvHD protection. In vivo studies also showed that $CD4^{LVFOXP3}$ do not inhibit response to pathogens or tumor clearance.

To facilitate pre-clinical safety and efficacy assessments, we have developed a humanized-mouse model in which the FOXP3 gene is knocked-out (KO) using CRISPR/Cas9 in human HSPCs, then transplanted into immunodeficient mice. The use of multiple sgRNAs targeting the FOXP3 locus significantly improved targeting when compared to a single sgRNA. The immune-deficient mice transplanted with FOXP3-KO HSPCs developed lymphoproliferation 10-12 weeks after transplant, which was controlled by $CD4^{LVFOXP3}$.

These data complete the IND-enabling studies supporting the clinical use of $CD4^{LVFOXP3}$ in a Phase 1 trial to treat IPEX syndrome and future clinical use in other immune-mediated diseases caused by insufficient or dysfunctional $FOXP3^{+}$ Tregs.

アデノウイルスベクターを用いたⅡ型くる病モデルラットの遺伝子治療およびゲノム編集治療

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[背景・目的]
Ⅱ型くる病はビタミンD受容体（VDR）遺伝子の変異が引き起こす遺伝性疾患である。主症状として骨形成異常と脱毛症があるが、後者については治療法が存在しない。本研究ではⅡ型くる病疾患モデルラット（VDR-KOラット）[1]にラットVDR cDNA発現アデノウイルスベクター(VDR-adeno)を用いた遺伝子治療を行うことで、脱毛症に対する治療効果を検証した。本研究の最終目標は、ゲノム編集治療技術を用いた半永久的なⅡ型くる病の治療の実現である。
[方法]
生後7週齢の雌ラットを用い、①VDR-KOラット:VDR-adeno投与群、②VDR-KOラット:VDR-adeno非投与群③野生型ラットの3群比較を行うことで、VDR-adenoの治療効果を評価した。まず、バリカンで各ラットの背部の毛を剃った後、約10^{10} pfu/匹のVDR-adenoを皮内投与した。投与から2、10、23日後に各ラットから皮膚を回収し、発毛および脱毛状況を観察するとともに、HE染色により毛包の性状を、また、免疫染色およびウェスタンブロッティングによりVDRの発現を調べた。一方、ヒトケラチノサイト由来HaCaT細胞にCas9発現アデノウイルスベクター、VDR遺伝子をターゲットとするガイドRNAを発現するアデノウイルスベクターを共感染させ、ゲノム編集効率（VDR遺伝子欠損）を評価した。
[結果・結論]
VDR-adeno投与群では非投与群に比べ、投与箇所及びその周辺での発毛促進及び脱毛抑制、嚢胞形成の抑制、皮膚でのVDRの顕著な発現が認められた。また、HaCaT細胞では33%と高効率でゲノム編集が起こっていることが確認された。今回得られた結果より、Ⅱ型くる病の遺伝子治療、ゲノム編集治療の可能性が拓けたと考えられる。
[1] Nishikawa et al. Sci Rep. 10:5677 (2020)

中枢神経移行型酵素を搭載したAAVによるGM1ガングリオシドーシスへの遺伝子治療

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【背景】
ライソゾーム病の一つであるGM1ガングリオシドーシスは、βガラクトシダーゼ(βgal)をコードするGLB1の遺伝子変異により基質が神経細胞、骨細胞に蓄積し中枢神経症状、骨症状を呈する。現在有効な治療法はなく、乳児型では発症から数年で死亡する。
AAVを用いた遺伝子治療はマウスに対しては中枢神経へも有効性が示されてきたが、ヒトでの再現性が課題となっている。近年BBB透過性を高めるべく、トランスフェリン受容体(TfR)を利用した輸送方法が検討されている。抗TfR抗体を酵素と融合した酵素製剤はヒトに対し良好な結果を示している。そこで我々はAAVにより抗TfR抗体融合βgalを肝臓から全身に供給することでGM1ガングリオシドーシスの中枢神経症状に対する遺伝子治療の検討を行なった。

【方法】
マウスアルブミンプロモーターとTfR-βgalを搭載するAAV9を作成し、10週齢のGM1マウスへ静脈投与した。投与後経時的に血中酵素活性を測定し、治療後３３週で各種臓器を採取し、酵素活性並びに蓄積基質であるGM1ガングリオシド（GM1）量の評価を行った。

【結果・考察】
様々なdosedで検討を行った結果、high dose群ではTfR高発現の影響でHBG低下等貧血の副作用が認められた。Low dose (5E+12 vg/kg)投与後６週の血中βgal活性は野生型よりも約4倍高く、投与後33週でも活性が維持されており、肝臓や脾臓では野生型を上回るβgal活性が検出された。さらに、大脳などの中枢神経においてもβgal酵素活性の上昇を認め、蓄積するGM1量は健常なマウスと同等まで低下していた。以上より、TfR融合βgalを発現するAAVを用いた遺伝子治療は、GM1ガングリオシドーシスの中枢神経症状に対して有用な治療となり得ると考えられる。

低侵襲かつ高効率な栄養障害型表皮水疱症遺伝子治療法開発

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劣性栄養障害型表皮水疱症（recessive dystrophic epidermolysis bullosa, RDEB）は、皮膚基底膜を真皮に接合するための接着分子であるVII型コラーゲンの遺伝的な機能破綻により生じる遺伝性皮膚疾患である。RDEB患者はわずかな外力で皮膚に全身熱傷様の水疱、びらん、潰瘍を形成し、経過とともに手指の棍棒状癒着や瘢痕癌を高率に合併する。RDEBの治療法として自家培養表皮シート移植が保険適応となっているが、VII型コラーゲン遺伝子の変異部位が自然復帰したリバータントモザイク皮膚を使用しない場合、その効果は不十分であり、新たな治療法の開発が望まれている。そこで本研究では、自己間葉系幹細胞を用いたRDEBに対するex vivo遺伝子治療の開発を行った。

本研究において明らかとなったことは以下の通りである。
i) RDEB患者の水疱内溶液から間葉系幹細胞を採取可能であること、ii) 採取した自己間葉系幹細胞にレンチウイルスベクターを用いてVII型コラーゲン遺伝子を高効率に導入可能であること、iii) 遺伝子導入細胞の投与経路として、水疱内投与が他の投与経路(皮内投与)と比較して、VII型コラーゲンタンパク質の基底膜領域への沈着効率が高いこと。
以上の点が明らかとなった。

以上をまとめると、本研究は水疱内溶液から得られる自己間葉系幹細胞に対してVII型コラーゲン遺伝子を導入し、水疱内へ遺伝子導入細胞を投与することによる、低侵襲かつ高効率なRDEBに対するex vivo遺伝子治療法を提案するものである。

低ホスファターゼ症に対する遺伝子治療薬（ARU-2801）の有効性及び安全性の検討

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[背景] 低ホスファターゼ症（HPP) は組織非特異型アルカリホスファターゼ（TNALP) の欠損により引き起こされる遺伝性骨疾患で、血清ALP活性の低下を認め、全身骨の低石灰化、体重増加不良、けいれんなどの症状を呈する。治療法としてはTNALPの酵素補充療法が行われているが、週３－６回の一生にわたる投与が必要である。今回我々は1回の筋肉内投与により治療が可能な遺伝子治療薬（TNALP発現8型AAVベクター; ARU-2801）の有効性及び安全性の検討を行った。
[目的] 低ホスファターゼ症の新規遺伝子治療薬の開発を目的にARU-2801の有効性と安全性を齧歯類及び哺乳類を用いて検証する。
[方法] ARU-2801の有効性を検証するために*Alpl*遺伝子が欠損したHPPモデルマウスの新生仔にARU-2801（$1.0x10^{11}$、$3.0x10^{11}$、$1.0x10^{12}$ vg) を1回の筋肉内投与を行い、血清中のALP活性、生存期間、骨形成の改善などを18ヶ月にわたり経過を追い検討した。また、安全性に関してはHPP マウス及び霊長類を用いて、生化学的検査、腫瘍形成、異所性石灰化などを検討した。
[結果] 未治療のHPPマウスは全例1ヶ月以内に死亡したのに対し、$3.0x10^{11}$及び $1.0x10^{12}$ vg投与群では長期にわたり10 U/ml前後のALP活性を認めると共に生存期間の延長を認めた。$1.0x10^{12}$ vg投与群のHPPマウスはWild typeマウスと同等の体重増加、骨形成、骨密度を認め、肝機能、腎機能などの生化学検査は異常なく、腫瘍形成や異所性石灰化なども認めなかった。また、霊長類を用いた試験においても（1.0 $x10^{13}$ vgの1回の筋肉内投与）長期にわたる持続的なALP活性を認め、生化学的検査に異常はなく、腫瘍形成、異所性石灰化などの異常所見も認めなかった。
[結論]1回の筋肉内投与によるARU-2801の低ホスファターゼ症に対する治療はHPPマウス及び霊長類で有効かつ安全であることが示唆された。本治療はHPP患者にとって延命効果はもちろんのこと、ADL、QOLの改善につながる治療であると考えられた。

腫瘍溶解性単純ヘルペスウイルスの精製および保存方法が収量および機能性に及ぼす影響について

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背景：遺伝子工学の進歩により、癌治療や遺伝子治療のための多種多様な単純ヘルペスウイルス（HSV）ベクターの作製が可能になった。しかし、遺伝子組換えHSVの高効率な製造は依然として困難であり、その臨床的な可能性が制限されている。この問題に対処するため、我々はこれまでに無毒化HSVベクターであるJΔNI5の生産および精製に関する至適条件について報告した。本研究では、腫瘍溶解性HSV（oHSV）の精製および保存条件を詳細に検証し、ベクターの収量および機能性を最大にすることを目指した。
方法：野生型HSVのKOSおよびoHSVをVero細胞上で生産し、異なる遠心分離条件および異なるタイプのフィルターで清澄化した。清澄化された上清は、様々な遠心力で高速遠心分離することにより濃縮された。次に、HSVストックの安定性を評価するために、ウイルスストックを異なる条件下で保存した。ウイルス力価について、物理的力価は定量PCR法で、生物学的力価は標準プラークアッセイ法で測定した。さらに、それらの抗腫瘍活性をin vitro細胞毒性試験およびマウス異種移植モデルで比較検討した。
結果：遠心分離条件を最適化することにより、KOSおよびoHSVの物理的・生物学的力価を有意に増加させることができた。また、上清oHSVを4℃で長期保存しても、それらの有意な低下は認めなかった。一方、精製oHSVの生物学的力価は4℃での長期保存により劇的に低下し、ベクターのformulationがoHSVの安定性に影響を及ぼしていることが示唆された。また、精製oHSVの生物学的力価は凍結融解を繰り返すことで著明に低下した。さらに、最適な保存条件でなければ、in vitroおよびin vivoでのoHSVの抗腫瘍活性が低下することが判明した。
結論：本結果は、高力価・高品質のHSVベクター調製のための適切な精製・保存方法の重要性を示し、実験および前臨床試験で使用するHSVベクターの精製・保存のための至適プロトコールの確立に貢献すると考えられる。

Inter-subunit associations of AAV icosahedron inform AAP requirement for capsid assembly

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Capsid assembly in adeno-associated viruses (AAV) requires VP1, 2, 3, assembly-activating protein (AAP) and host cellular factors. Although AAP is required for maximal capsid assembly in all AAV serotypes, AAV4, 5, 11 and rh32.33 are capable of AAP-independent capsid assembly. Mechanisms dictating AAP requirement for capsid remain poorly understood despite our growing knowledge of AAV biology. Here we report that the strength of associations of the subunits of the AAV icosahedron is a key factor in determining AAP requirement. In our AAV capsid mutagenesis studies, we have identified a set of the same serotype-derived AAP-dependent and independent variants for the following serotypes, AAV4, 5, 11 and 12. We compared the predicted disorder between the variants (within each serotype) using Predictor of Natural Disordered Regions (PONDR). The disorder near the mutated region always tends to be higher in the AAP-independent variant. Because the mutated regions lie either on the 2-fold axis or 5-fold axis, we found that AAP-independent variants tend to have slightly more disorder either close to the 5-fold axis or the 2-fold axis. This could confer enhanced flexibility in these regions, promoting VP subunit associations and improving functional VP oligomerization and subsequent capsid assembly. We tested this hypothesis by determining the Association Energies (AE) and Buried Surface Areas (BSA) of these AAV variants. Using SWISS MODEL, we modelled both AAP-independent and dependent VP variants of each serotype against a reference template. Upon obtaining these models, we applied Viperdb's energyPlot function to determine the AE and BSA of each variant. We find that AAP-independent variants have tighter associations predominantly along the axis where the mutations reside (i.e., 2-fold axis for AAV4, 11, and 12; and 5-fold axis for AAV5), indicating greater inter-subunit associations in the AAP-independent variants. Tighter associations were indicated by relatively lower association energy (higher tendency to associate) and higher buried surface areas in AAP-independent variants. In the AAV icosahedron, VP subunit association is the tightest at the 3-fold axis compared to the 2-fold and 5-fold axes. Thus, VP proteins with increased propensity to associate at the 2-fold or 5-fold axis would have a tendency to oligomerize rather than be monomers, thus protecting them from degradation. Conversely, VP proteins with relatively lower propensity to associate at the 2-fold and 5-fold axis would need non-VP factors for stabilization and therefore depend on AAP for capsid assembly. Together, our findings indicate a model where AAP aids inter-subunit associations especially at weaker axis such as the 2-fold axis; whereas AAP-independent AAV variants have enhanced VP subunit associations at the VP-VP interface, thereby capable of AAP-independent capsid assembly.

Identification and characterization of an AAV capsid that efficiently transduces the pancreas with limited off-target consequences in non-human primates following retrograde pancreatic duct injection

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Type 1 diabetes (T1D) is a chronic metabolic disease caused by destruction of insulin-producing beta cells in the pancreas. Recent success of in-vivo reprogramming of non-beta cells into insulin-producing beta-like cells in rodent models has opened an avenue to the development of novel in-vivo gene therapy approaches to cure of T1D. One of the promising approaches is to express key transcription factors with reprogramming capability by viral vector-mediated gene delivery. For this purpose, adeno-associated virus (AAV) vectors have been explored; however, clinically relevant effective and safe approaches have yet to be established. To seek establishment of AAV vector-mediated in vivo gene therapy for T1D, we have recently established a real-time image-guided retrograde pancreatic duct (PD) injection method using non-human primates (NHPs) that can safely deliver therapeutic agents to the pancreas. We have shown that the procedure can be implemented safely with only a transient elevation of serum amylase and lipase that does not result in clinical symptoms. We have also shown that AAV9 vector transduces predominantly acinar cells and occasionally islet cells. Here we perform for the first time a high-throughput screening of AAV capsids in rhesus macaques using our AAV DNA/RNA barcoding approach and identify AAV-KP1 as an AAV capsid that outperforms other AAV capsids and transduces pancreatic islet cells in NHP substantially better than AAV9 following PD injection. In this study, we injected rhesus macaques via the PD route with a DNA/RNA-barcoded AAV library containing 45 different AAV capsids (major AAV serotypes and various capsid mutants) and harvested tissues including the pancreas 6 weeks post-injection. The harvested pancreata were enzymatically digested, and endocrine cells and duct cells were flow-sorted using cell type-specific monoclonal antibodies. The AAV Barcode-Seq analysis revealed that the AAV-KP1 vector can efficiently transduce pancreatic cells more than an order of magnitude better than AAV9. To validate the AAV Barcode-Seq analysis, we then infused 8.4 x 10^{12} to 1.4 x 10^{13} vg of AAV9 and AAVKP1-CAG-tdTomato vector into the PD in two and four rhesus macaques, respectively, and assessed pancreatic transduction 4 weeks post-injection. Immunofluorescence microscopic analysis confirmed that AAV-KP1 vector transduces the pancreas substantially better than AAV9 with preference toward islet cells while the degree of islet tropism varied among the animals. Importantly, although all AAV capsids could leak into the bloodstream following PD injection, AAV-KP1 vector concentrations in the blood circulation were substantially lower than those of AAV9, leading to a very limited vector dissemination to non-target organs including the liver. These observations indicate that retrograde PD injection of AAV-KP1 vector is a promising approach for AAV vector-mediated in-vivo gene delivery to pancreatic islets with minimal off-target effects. Further studies are warranted to support clinical translation of AAV-KP1 vector-mediated gene therapy for T1D.

AAVKP1 Shows a Peculiar Biological Phenotype Effective for Localized In Vivo Gene Delivery While Detargeting the Liver

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Local administration of adeno-associated virus (AAV) has the potential to enhance target organ transduction with a smaller vector dose while avoiding off-target gene delivery. However, it is now widely appreciated that robust AAV capsid vectors such as those derived from AAV9 can efficiently transduce the liver and other organs following local vector delivery due to the inadvertent leakage into the systemic circulation. Here, we report that AAVKP1, a novel AAV capsid identified by directed evolution in human pancreatic islets in vitro, has a peculiar biological phenotype that allows effective hepatic gene delivery when injected systemically while detargeting the liver when delivered locally. This biological attribute is unique among other liver-tropic AAV capsids including AAV8 and AAV9. In mouse experiments, we comprehensively characterized transduction efficiencies of 47 AAV capsids in various organs following intravenous vector injection using AAV DNA/RNA Barcode-Seq. A set of AAV capsids including AAV8, AAVrh8, AAVrh10, AAVAnc80 and AAVKP1 transduced the liver effectively at levels equal to or higher than that of AAV9, demonstrating the liver-tropic nature of AAVKP1. To compare liver transduction efficiencies of various AAV capsids following local gene delivery, we took advantage of our ongoing project using rhesus macaques in which we deliver AAV vectors directly into the pancreatic duct (PD) and assessed off-target gene delivery to non-pancreatic organs. In this PD injection study, we used AAV DNA/RNA-barcoded library that covers more than 40 serotypes and mutants and blood samples were collected at various time points up to 72hr after injection for pharmacokinetic profiling of each AAV capsid. The animals were euthanized 4 weeks after injection and multiple tissues were harvested for downstream analyses. The results showed that the blood vector concentrations after injection as an indicator of vector spillover are highly variable among AAV capsids. Among a set of the capsids that showed robust local transduction within the pancreas, we identified AAVKP1 as an AAV capsid whose blood concentrations are substantially lower than those of AAV9 at any time points. Consistent with its low blood concentrations, AAVKP1 transduced the liver 100 times less efficiently than AAV9 although both AAVKP1 and AAV9 are liver tropic capsids when intravenously administered. On the other hand, another liver tropic capsid, AAV8 showed several times higher blood concentrations and accordingly led to higher liver transduction than AAV9. These data demonstrate that the degree of vector spillover after local administration of AAV is capsid dependent and varies significantly among different AAV capsids. The vector pharmacokinetic profile is one of the important determinants of vector bio-distribution. Importantly, our study has shown that AAVKP1 is an attractive vehicle for effective local gene delivery while substantially limiting off-target vector spillover particularly in the liver.

Screening human immortalized cell lines for efficient production of recombinant adeno-associated virus vector

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Background: Recombinant adeno-associated virus (rAAV) vector holds great promise in gene therapy. However, the clinical application can be hindered by manufacturing costs, productivity and scalability. Traditionally, rAAV production has been achieved by transient transfection of human embryonic kidney (HEK) 293 cells or HEK-derived cells with AAV packaging plasmids and pAAV expression vector. It has been shown that host cell proteins play a crucial role in AAV DNA replication and capsid assembly but little is known of what cell characteristics is suitable for rAAV vector production. To address these questions, herein we performed screening to identify suitable human cell source for efficient rAAV vector production.
Methods: To determine the efficiency of AAV replication, human immortalized cell lines derived from various tissues were infected with AAV1 or AAV2 and adenovirus and the viral yield in supernatant and cell was quantitated by qPCR 3 days after infection. For generation of rAAV host cells, selected human immortalized cell lines were stably transfected with adenovirus E1 gene and the E1 expression was confirmed by immunostaing and Westernblot analysis. The efficacy of rAAV vector production with E1-transfected human immortalized cell was evaluated by transfection of pAAV-AcGFP, pHelper and pRepCap plasmids.
Results: Screening human immortalized cell lines revealed that several cell lines showed the efficient AAV1 and AAV2 replication compared to others and the efficiency was similar or superior to conventional rAAV host HEK293 cells. Notably, the viral yield of AAV2 in supernatant was higher than HEK293 cells, which is advantageous for rAAV2 vector production. Furthermore, we also generated human immortalized cell lines stably expressing E1 gene to assese the productivity of rAAV. We show that infectious rAAV can be efficiently produced by triple transfection to the human immortalized cells stably expressing E1 gene.
Conclusion: Taken together, our results suggested that cellular property can greatly influence rAAV production and human immortalized cell lines isolated in this study are promising cell source to generate novel rAAV host cell lines.

接着性HEK293EB細胞を用いた固定床バイオリアクターによるAAV9ベクター生産法の検討

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[目的] アデノ随伴ウイルス（AAV）は、ヒトの疾患とは関連がなく、自己複製が不可能で、他のウイルスより免疫原性が低く、さまざまな細胞種に感染する可能性を持つため、その組換えベクター（rAAV）は遺伝子治療で注目されている。臨床試験や治療に用いるrAAV量の増加に対する需要を満たすためのスケーラブルな大容量テクノロジーが必要となっている。しかし。これまでのバイオリアクターは浮遊細胞に特化されており、付着性のHEK293細胞に適応できるものは皆無であった。
[方法] 使い捨ての固定床バイオリアクターであるiCELLis Nanoバイオリアクター（PALL Corporation）を使用して、無血清培地でのAAV9ベクター生産のための細胞（HEK293EB細胞：恒常的に E1 遺伝子領域と Bcl-xL 遺伝子を発現）増殖条件、一過性トランスフェクション条件等を検討した。
[結果] 固定床として0.8m^2のバイオリアクターを用いた場合、10%FCS含有培養液を用いて、1.5--2.5 × 10^5細胞 / cm^2をサポートできた。トランスフェクションに用いるDNA濃度、培養液pH、収穫日、などの培養およびトランスフェクションパラメーターを検討した。無血清培地内でのトランスフェクション後2- 3 日までは細胞の増殖を認めた。プロセスに培地の変更を追加しないことで、rAAV9を宿主細胞タンパク質の汚染が少ない状態で培養培地内へ分泌させ、固定床1平方メートルあたり6-10 × 10^{13} vector genomeのウイルス粒子を得た。
[結語] これらの結果は、iCELLis NanoはAAV遺伝子治療製品の臨床生産のための有望な技術であるが、さらなるブラッシュアップが必要と思われる。

アデノ随伴ウイルス（AAV）ベクターの抽出法開発

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【背景・目的】
アデノ随伴ウイルス（AAV）ベクターを用いた遺伝子治療は、上市や後期治験に進んでいるものもあり、ベクターの必要量は年々増加しており、ラージスケールで純度の高いAAVベクターを再現よく精製する手法の開発が課題である。AAVベクターを産生細胞から回収する手法としては、産生細胞を溶解・破壊して抽出する方法が一般的であるが、同時に抽出される細胞由来の核酸等がAAVと結合すると、下流工程での頑健性低下の原因や最終製品への混入の恐れがある。本研究では、細胞溶解・破壊を抑えてAAVベクターを産生細胞から簡便に高効率で抽出する溶液を開発し、性能評価を行った。

【方法】
浮遊化HEK293細胞へトランスフェクション試薬を用いてAAV生産用プラスミドを共導入し、蛍光タンパク質ZsGreen1を発現するアデノ随伴ウイルス2型（AAV2）を産生させた。産生細胞を界面活性剤添加で細胞溶解する条件（以下、従来法）と、今回開発した抽出溶液（以下、AAV抽出溶液）を添加する条件でそれぞれAAV2抽出を行った。抽出液のベクターゲノム数をAAVpro® Titration Kit、感染粒子数をHEK293T細胞への感染実験により測定した。また、混入核酸量はPicoGreen®アッセイにより測定した。

【結果】
AAV抽出溶液で抽出されたAAV2のベクターゲノム数および感染粒子数は、従来法と比較してほぼ同等であった。また、PicoGreen®アッセイによって測定された混入核酸量は従来法と比較して1/2程度に抑制されていた。更にAAV抽出溶液で抽出されたAAVベクターは従来法と同様、清澄化後AAVアフィニティー用担体で精製可能であることを確認した。

【結論】
混入核酸量を低減してAAVベクターが抽出可能な溶液を開発できた。

mRNA医薬開発用合成試薬の開発

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新型コロナウイルスに対するmRNAワクチンが海外企業2社によって迅速に開発・実用化され、mRNAが新たな創薬モダリティとして注目されている。mRNAは、配列さえ分かればあらゆるタンパク質に対して迅速に設計可能で、ゲノムへの挿入リスクもない。今後は感染症ワクチンとしてだけでなく、がんワクチンや遺伝性疾患医薬としても増々開発が加速されると予想される。

そこで当社では、mRNA医薬開発を加速するため、mRNAの鋳型DNAを簡便・迅速に作製する試薬と、鋳型DNAからmRNAを高効率に合成する試薬を開発した。mRNAの鋳型DNAを作製する試薬では、予め線状化されたベクターに、T7 promoter、転写開始配列、5'-UTR、3'-UTR、Poly(A)配列を含み、発現させたい遺伝子のコーディング配列をクローニングするだけで、ヒトやマウスといった哺乳類細胞で使用可能なmRNA合成用のプラスミド鋳型を構築することを可能とした。一方、mRNAの合成試薬では、反応条件の最適化により、収量を当社の従来法と比べ約6倍増加させることに成功した。例えば、Cap構造およびシュードウリジンを含む約1.9 kbのfirefly luciferase mRNAを合成した場合には、20 µlの反応系で通常180 µg以上のmRNAが得られた。また、その合成したmRNAを培養細胞に導入したところ、他社が販売しているmRNAより高い発光量が確認された。このことは、本試薬を用いて合成したmRNAから、目的タンパク質が効率的に翻訳できていることを示している。これらの結果より、本試薬は直ちに評価できるmRNAを簡便・迅速に大量調製することを可能とし、mRNA医薬開発の促進に寄与できると期待される。本発表では、mRNA合成のノウハウとアプリケーションデータについても併せて紹介する。

Non-viral gene-modified CAR-T cells targeting IGF1 receptors against solid tumors

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Insulin-like growth factor 1 receptor (IGF1R) is a receptor tyrosine kinase that belongs to the insulin receptor family and is recognized to be overexpressed on various malignant tumors. Hence, IGF1R has been researched extensively as the ideal target molecule for the clinical development of monoclonal antibodies and tyrosine kinase inhibitors. However, there are yet to be products that have been approved. Although Huang and colleagues reported the non-clinical study of single-chain variable fragment (scFv)-based IGF1R-specific chimeric antigen receptor (CAR) T-cells against sarcomas, there has been no clinical study of IGF1R CAR-T cells worldwide. We are developing IGF1R CAR-T cells using wild-type piggyBac-based gene-modification and ligand-based CAR design technologies. The antigen-binding domain of IGF1R CAR was selected out of the 5 types of insulin-like growth factor 1 (IGF1)-related candidate proteins, based on CAR expression, manufacturing efficiency, and cytotoxic activity of CAR-T cells. The IGF1R CAR-T cells expressed approximately 40% CAR and exhibited significant anti-tumor effects against endometrial cancer cell line (ARK1), cervical cancer cell line (Hela), ovarian cancer cell line (RMG1), triple-negative breast cancer cell line (Mx-1), and lung adenocarcinoma cell lines (H1568 and H1355), as well as acute myeloid leukemia cell line (THP-1) upon co-culture experiments. Currently, in vivo anti-tumor effects are being evaluated. Our ligand-based IGF1R CAR-T cells can be a promising drug candidate entering clinical development due to the ability to target a broad range of tumors and the lack of competitive products with the same target.

Anti-tumor effect of recombinant Bifidobacterium displaying Wilms' tumor 1 combined with anti-PD-1 therapy against renal cell carcinoma in mice

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[Bacground and Objectives] In recent years, cancer immunotherapy using immune checkpoint inhibitors has been used with great success in treatment of various kinds of cancers. However, monotherapy of immune checkpoint inhibitors showed the objective response rate of approximately 20% in advanced renal cell carcinoma. Therefore, combination of immunotherapy will be a promising modality to improve the response in cancer patients. Some strains of commensal *Bifidobacterium* are reported to enhance the efficacy of immune checkpoint inhibitor in patients. In this study, we investigated that the therapeutic efficacy of oral vaccination of *Bifidobacterium longum* displayed Wilms' tumor 1 (WT1) protein (*B. longum* 420) combined with anti-PD-1 therapy against renal cell carcinoma in mice.

[Materials and Methods] One million cells of Renca, a WT1 protein and PD-L1-postive murine renal cell carcinoma cell line, were subcutaneously inoculated into Balb/c mice. One week after tumor inoculation, 1×10^9 colony forming units of pasteurized *B. longum* 420, *B. longum* 2012 (negative control), or PBS were orally vaccinated 5 times a week for following weeks. Intraperitoneal injections of anti-programmed death-1 (PD-1) antibody were combined with oral vaccination twice a week for following weeks. After the treatment, tumors and spleens were collected to analyze the WT1-specific immune responses and tumor infiltrating lymphocytes (TILs).

[Results] In Renca mice, anti-PD-1 monotherapy showed no significant anti-tumor effect compared with control. On the other hand, the combination therapy of *B. longum* 420 and anti-PD-1 antibody significantly decreased tumor growth compared with control and significantly prolonged overall survival of Balb/c mice ($p<0.05$, respectively). Flow cytometric analysis showed that the number of tumor-infiltrating CD107a-positive CD8T cells were increased substantially in TILs when mice were treated with the combination therapy. The oral vaccination of *B. longum* 420 significantly increased the WT1-specific CD8T cells secreting tumor necrosis factor-α(TNF-α) compared with other control treatment in the spleen culture ($p<0.05$).

[Conclusions] In this study, we demonstrated that oral vaccination of *B. longum* 420 induced the WT1-specific effector CD8T cells and enhanced the therapeutic efficacy of anti-PD-1 therapy by recruiting the tumor-infiltrating CD8T cells. Our findings suggested that *B. longum* 420 possibly broke the immune tolerance in the immune checkpoint therapy by induction of effector CD8T cells in tumor environments. These results indicated that *B. longum* 420 may be a new therapeutic candidate for advanced renal cell carcinoma in combination with immune checkpoint inhibitors.

Biomarkers predicting the antitumor effect of p53-armed telomerase specific oncolytic adenovirus

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Background:
Personalized medicine using biomarkers has been adopted for various types of cancer in clinical practice. Original telomerase-specific oncolytic adenovirus (OBP-301) is currently undergoing clinical trials in Japan and overseas. We created OBP-702, a "next-generation" oncolytic adenovirus armed with p53 gene, a tumor suppressor gene, and have advanced development of OBP-702 for clinical application. Here, we aimed at investigating biomarkers predicting the antitumor effect of OBP-702.

Methods:
A total of 47 human cancer cell lines, 34 types of gastrointestinal cancer and 13 types of non-gastrointestinal cancer, were used for the study. Cytotoxic assays were performed to calculate IC50 values of three viruses, OBP-702, OBP-301 and Ad-p53, on these 47 cell lines. We divided the antitumor mechanisms of OBP-702 into three elements: "virus infection", "virus replication", and "p53 status", and explored biomarkers in each element.

Results:
OBP-702 showed potent cytotoxic effects on all 47 cell lines. While these effects of OBP-702 were stronger than OBP-301 and Adp53 on all cell lines, there was a strong correlation on IC50 values of OBP-702 and OBP-301 (|r [the correlation coefficient]|=0.69), and a moderate correlation between OBP-702 and Ad-p53 (|r|=0.58). As a biomarker on virus infection, the expression of Coxsackie-Adenovirus Receptor (CAR) on each cell line was moderately correlated with the IC50 value of OBP-702 (|r|=0.44). Regarding virus replication, the expression of Ki-67 on each cell line was weakly correlated with IC50 value of OBP-301 (|r|=0.313). And as for p53 status, p53 mutation was found in 13 of 47 cell lines, on which Ad-p53 showed significantly stronger cytotoxic activity than p53 wild-type cell lines.

Conclusion:
CAR, Ki-67, and p53 status can be biomarkers predicting OBP-702 effects.

Combination of fusogenic oncolytic vaccinia virus and HDAC inhibitor treatment synergistically induces anti-cancer effect through enhancing viral cell-cell fusion

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Oncolytic virus is an effective anti-cancer agent by inducing viral oncolysis and anti-tumor immunity. One of the candidates, vaccinia virus is well known as a smallpox vaccine, and now widely engineered as an oncolytic agent. We have previously developed MAPK-dependent recombinant vaccinia virus (MDRVV), which achieved tumor specific viral replication by deletion of two viral growth factors, VGF and O1L. Moreover, our recent report demonstrated a novel fusogenc oncolytic vaccinia virus (FUVAC) having cell-cell fusion, which is isolated from non-fusogenic MDRVV. Its fusion phenotype induced efficient oncolysis while causing the immunogenic cell death and decreasing tumor-associated immune suppressive cells. This local modulation of tumor immune microenvironment elicited cytotoxic T lymphocytes systemically. Thus, FUVAC enhanced oncolytic potential due to its fusion phenotype.

However, FUVAC couldn't induce the sufficient cell-cell fusion in every tumor cells. For example, murine tumor cells tended to have lower fusion efficacy because of their lower viral tropism. Therapeutic function of FUVAC was strongly inhibited in those cells, compared with human cancer cells having higher viral tropism. In order to reinforce the viral tropism, histone deacetylase (HDAC) inhibitors were frequently combined with oncolytic virotherapy. HDAC inhibitors mainly inhibit the expression of type 1 interferon genes relating to the virus defense response. Therefore, HDAC inhibitors are considered to assist oncolytic viral activity. In this study, fusogenic vaccinia virus was combined with HDAC inhibitor: Tricostatin A (TSA) treatment to enhance its fusion phenotype against the fusion-resistant tumor cells.

TSA-treated murine tumors (CT26 and B16-F10) were clearly increased cell-cell fusion after the FUVAC infection, but not MDRVV infection. TSA dose-dependently enhanced cellular fusion and cytopathic effect of FUVAC. On the other hand, viral growth curve showed TSA equally increased viral replication of both non-fusiogenic MDRVV and FUVAC regardless of their fusion difference. Cellular whole mRNA was recovered from mock- or TSA-treated CT26 and B16-F10 cells and analyzed by RNA-sequencing. Gene ontology analysis revealed up-regulation of components of the extracellular space and region after the TSA treatment in both tumor cells. These suggests TSA modulates cellular environment to improve cell-cell fusion. The combination effect of FUVAC and TSA was also examined in syngeneic murine tumor model. Mice were bilaterally transplanted CT26 tumors and treated with TSA before the virus injection into one flank. Noninvasive bioluminescence imaging showed viral Fluc luminescence was increased by TSA pre-treatment, especially in the FUVAC injection. In comparison to the FUVAC or TSA alone, these combination therapy tended to increase the anti-tumor effect and prolong the survival of mice.

Our study showed that HDAC inhibitor TSA enhances viral fusion induction. TSA modulates cellular component-related genes to tolerate the cell-cell fusion of FUVAC. These combination therapy would improve their therapeutic potential especially in FUVAC-resistant tumors.

第二世代遺伝子改変miRNA標的配列搭載コクサッキーウイルスB群3型の非臨床毒性試験

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近年、ウイルスの腫瘍内での特異的増殖と腫瘍溶解性を誘導し、抗腫瘍活性を期待する「腫瘍溶解性ウイルス療法」（以下ウイルス療法と略）が開発され、国内外で承認が認められてきている。ウイルス療法の臨床的安全性と腫瘍特異性は確認されてきているが治療効果は限定的であり、さらなる開発が望まれる。我々はこれまでにコクサッキーウイルスB群3型（CVB3）が複数の肺癌細胞を特異的に溶解することを発見し、本ウイルスの副作用を減弱させた第一世代遺伝子改変CVB3-HPを開発した。現在では、高用量を投与してもCVB3の副作用を抑え込んだ第二世代遺伝子改変CVB3-BHPの作製に成功している。CVB3-BHPはウイルスゲノムの非翻訳領域にmiR-34aとmiR-217の標的配列を搭載した遺伝子改変ウイルスであり、本ウイルスは第一世代型であるCVB3-HPで認められた高用量投与時の副作用も消失しており、安全性が飛躍的に向上している。

次に、我々は臨床試験に向けて第二世代遺伝子改変CVB3-BHPの閉鎖系製造法の開発を試み、ディスポーザブル培養バッグを用いた細胞大量培養及びウイルス培養法を確立後、2段階のクロマトグラフィーを用いたウイルス精製法の開発に成功した。

上記製造した試験物を用いて薬効試験、生体内分布試験および毒性試験を実施した結果、どの試験においても被験物質投与による一般状態の異常は認められなかった。薬物動態予備試験において、投与から14日後に涙腺及び顎下腺にのみウイルスのゲノムが検出された。毒性試験において、高用量群で一過性の肝機能障害を認めた。薬効試験では、被験物質濃度依存的な腫瘍増殖抑制を認めた。これらの結果から、第二世代遺伝子改変CVB3-BHPを臨床応用できる可能性が示唆され、今後はさらなる安全性向上を試みつつ、PMDAとの薬事戦略相談に進む予定である。

SaCas9によるゲノム編集の安全性評価のために考慮すべきオフターゲット変異候補配列に関する検討

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[背景]
ゲノム編集を利用した遺伝子治療は、従来の遺伝子治療では不可能な遺伝子破壊や遺伝子置換による治療が可能である。一方で、目的外のDNA配列を編集してしまうオフターゲット変異がゲノム編集製品に特有の安全性上の懸念点であり、ゲノム編集を用いた遺伝子治療用製品の安全性評価のためには、その予測・評価が必要である。
[目的]
AAVベクターを利用したin vivoゲノム編集で頻用される*Staphylococcus aureus*由来Cas9 (SaCas9)により、どのような配列がオフターゲット変異を起こしうるかについての情報を得ることを目的として研究を行った。
[方法]
ヒトゲノム上に多数の相補結合配列を持つような特異性の低いガイドRNAを意図的に設計した。設計したガイドRNAとSaCas9の複合体によって、細胞から抽出したゲノムDNAを切断した。ハイスループットシークエンス解析によりその切断点を検出し、どのような配列が切断されているか評価した。
[結果]
オンターゲット部位を一か所持ち、1塩基以上の不適合箇所を有するオフターゲット部位を多数持つ特異性の低いガイドRNAが設計できた。設計したガイドRNA/SaCas9複合体による、本来の標的部位とは異なる配列の切断が多数検出された。そのほとんどが標的配列から数塩基のミスマッチを有する配列であったが、SaCas9の認識する典型的なPAM配列(NNGRR)とは異なる配列も多数切断されていた。
[結論]
SaCas9によるオフターゲット変異の予測・評価を行う際には、非典型的なPAM配列を有する配列も含めて、ヒトゲノム上に存在する標的配列との類似配列を評価する必要があると考えられる。

ファブリー病へのEx vivo Gene & Cell Therapy, Macroencapsulated Spheroid with Scaffold (MESS) Transplantation

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ファブリー病はライソゾームのalpha-galactosidase (GLA)酵素活性が低下し、細胞内にglobotriaosylceramide (Gb3)やそのリゾ体(lyso-Gb3)が蓄積する疾患である。主な治療はGLA酵素製剤による酵素補充療法（ERT）であるが、体内に入った酵素製剤の多くは体外に排出され、残りは臓器・組織内で短期間に活性を失うため、定期的な点滴加療が必要となる。以上よりGLAを分泌する細胞を生体に移植し、ERTの補助を果たせる細胞移植療法の開発を検討した。そこで移植細胞が長期間生存し、酵素を分泌し続ける環境を生体内で構築するために足場材μ-pieceに着目し、マウス胎児性線維芽細胞（MEF）とμ-pieceを混合した細胞塊CellSaicを作製した。このCellSaicをファブリー病モデルマウス腎被膜下に移植したところ、28日後も良好に生存しており、移植部位に赤血球が観察された（Kami. *Cell Transplant.* 2020）。次にGLAを過剰発現させたMEF（GLA-MEF）を樹立し、足場材とともに免疫隔離膜で作製したパック内に封入するMESS法の有効性について検討した（Kami. *Cell Transplant.* 2021）。このパックをモデルマウス背部皮下へ移植し、28日後に解析したところ、GLA-MEFの生存とGLA産生、さらにパック外へのGLA分泌が観察された。また肝臓中のGLA活性値も増加したが、lyso-Gb3量は変化がなかった。これはGLA-MEFでも分泌されるGLA量が少なかったと予想されたため、ライソゾーム酵素の分泌を増加させる必要がある。今後はこの増加方法を検討し、新たなMESS法による生体内のGLA活性増加とGb3/lyso-Gb3の除去を目指し、ヒトへの応用を視野にいれたい。

閉鎖系自動細胞調製システムを用いたRetroNectin®による遺伝子導入細胞の新製法

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タカラバイオでは、患者末梢血の単核球（PBMC）を用いた遺伝子導入CAR（キメラ抗原受容体）-T細胞遺伝子治療のための細胞製造法開発に取り組んでいる。細胞製造現場の課題として、作業工数の削減や細胞品質の安定化がある。閉鎖系自動細胞調製システムCliniMACS Prodigy®を使用した自動培養法を確立することで、作業者の工数を削減し、かつ作業者の手技に頼らない安定した細胞数・細胞品質を達成できる製造メニューを確立することが可能である。作業工数、細胞回収量及び細胞特性などの観点から新規製造法と従来のマニュアル製法を比較し、低コストで高品質な細胞製造法であることの実証を目指す。CliniMACS Prodigy®のメニューであるCustomized Application (CAP) ServiceとT Cell Transduction Process（TCT）を組み合わせRetroNectin®を用いたCAR-T製造法を構築した。PBMCから分離した凍結Apheresateから作成されたメニューを用いてT cell（CD3+ CD4+ cell, CD3+ CD8+ cell）の分離と培養、RetroNectin®とRetroviral Vectorによる遺伝子導入を実施した。作業全体は工数管理され各工程での工数を算出し、また製造された細胞について免疫表現型解析によってCD3+ CD4+ cell /CD3+ CD8+ cellの増殖率・回収細胞数をまとめた。作業工数は自動培養法によって現行法より大きく削減された。また、細胞回収量についても安定した細胞回収が期待される。

キメラ抗原受容体を樹状細胞(dendritic cell: DC)に組み込んだ新規細胞療法CAR-DC

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CAR-T細胞はT細胞に腫瘍表面抗原を認識する一本鎖抗体とT細胞活性化に必要な細胞内シグナル伝達ドメインを融合させたキメラ抗原受容体 (chimeric antigen receptor : CAR)を導入した細胞である。CD19を標的とするCAR-T細胞は血液悪性腫瘍の患者に顕著な有効性を示しているが、固形腫瘍に対するCAR-T細胞療法に対する効果は十分ではない。従って、固形腫瘍に対して効果を発揮するように改良された新規細胞療法の確立は急務といえる。CAR-T細胞に加え、CAR-gdT細胞、CAR-NK細胞ならびにCAR-マクロファージ療法が開発されている。

近年、樹状細胞 (dendritic cell : DC)は腫瘍内で活性化し、産生するサイトカインにより腫瘍内環境が変化し、腫瘍内にT細胞が集積することが明らかになった。また、DCは活性化後、様々な外来抗原を捕獲・抗原提示することが可能であるから、heterogenousな固形腫瘍の複数の癌抗原を認識し、T細胞に提示し抗腫瘍効果を発揮することが期待される。そこで、我々は腫瘍抗原を特異的に認識するCARを介しDCを活性化させるCAR発現DC (CAR-DC)を作製した。多くのCAR-細胞療法は細胞内にシグナルを伝えるためにCD3z鎖を用いているが、我々のCAR-DCはTLRを用いた。CAR-DCは抗原特異的に活性化し、in vivoにおいて抗原特異的に固形腫瘍の増殖を有意差を持って抑制した。また、抗PD-1抗体と併用することにより、抗腫瘍効果は増強された。抗PD-1抗体によりCAR-DCの抗腫瘍効果が増強されたことから、CAR-DCの抗腫瘍効果にT細胞が関与していることが予想され、CAR-DC投与後に腫瘍にT細胞が多く浸潤していることを確認した。以上より、CAR-DCは固形腫瘍に対して有用な新たな細胞療法として期待される。

非小細胞性肺癌脳転移に対するHSV-TK発現脱落乳歯歯髄幹細胞(SHED)を用いた自殺遺伝子療法の有効性

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＜はじめに＞
肺癌は最も頻度の高い癌であり、予後が改善している一方で頭蓋内転移は増加している。近年、入手のしやすさからヒト脱落乳歯歯髄幹細胞(SHED)が再生医療分野で注目されている。我々はグリオーマに対して間葉系幹細胞を自殺遺伝子のvehicleとして用いた自殺遺伝子治療の開発を行なっており、応用を試みた。今回、非小細胞性肺癌(NSCLC)の脳転移モデルに対してSHEDをvehicleとしたThymidine kinase(TK)/gancyclovir(GCV) systemを用いた自殺遺伝子療法の有効性が確認できたので報告する。
＜方法＞
アミノ酸を一部改変したHSV-TK(modified TK)を作成しSHEDに導入して治療細胞(SHED-TK)を樹立した。wild-typeのTKとmodified TKとで導入後のviabilityを比較した。NSCLC細胞株（H1299、A549、H460）とSHED-TKをGCV存在下で共培養しbystander効果による抗腫瘍効果を検討した。SHED-TKのNSCLCに対する遊走能およびgap junctionを通したdyeの移行を評価した。H1299をヌードマウスの脳に移植して作成したモデルにSHED-TKを移植しGCVの投与を行い、抗腫瘍効果を評価した。
＜結果＞
wild-typeと比較してmodified TKはSHEDに対して細胞毒性が少なく、GCVへの感受性においても以前の報告と比べて同程度であった。in vitroではNSCLC細胞株に対してbystander効果による抗腫瘍効果を示した。特に抗腫瘍効果が高かったH1299でSHEDとの間のgapjunctionによるdyeの移行が見られた。SHED-TKは腫瘍のconditioned mediumに対する遊走を認めた。モデルマウスでは、コントロール群と比較してSHED-TKの移植およびGCVを投与した群では腫瘍による生物学的発光の抑制が見られ、マウスの生存期間の延長も認められた。
＜結語＞
HSV TKのアミノ酸改変により安全に治療細胞SHED-TKを作製することに成功した。SHED-TKはNSCLCに対して培養細胞系及びマウスモデルでbystander効果による著明な抗腫瘍効果を示した。

陰イオン交換-UHPLCによるアデノ随伴ウイルスベクターの中空粒子評価法に関する研究

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In vivo遺伝子治療において、長期の遺伝子発現が期待され非病原性で安全性の高いアデノ随伴ウイルスベクター（adeno-associated virus (AAV) vector）が現在の臨床開発の主流となっており、世界中で多くの臨床試験が実施されている。AAVベクターの品質特性の一つとして、ベクターゲノムを含まない空のウイルス粒子（中空粒子）がその目的物質由来不純物として混入することが挙げられる。現在、中空粒子が生じない製造法は確立されておらず、また製造法によって中空粒子の混入量が変化する場合もある。これらのことから、AAVベクターの有効性および安全性を評価する目的で、完全粒子に対する中空粒子の存在比率が測定されている。これまでに、中空粒子の存在比率を測定するための様々な分析手法が開発されている。本研究では、陰イオン交換-超高速液体クロマトグラフィー（UHPLC）を用いた中空粒子の存在比率の測定法確立を試みるとともに、その分析性能特性に関して研究を行った。

陰イオン交換-UHPLCの分離分析条件を検討した結果、高純度のAAVベクター試料に含まれる完全粒子と中空粒子を良好に分離・検出することができ、得られたクロマトグラムから算出された中空粒子の存在比率は、他の分析手法から算出される中空粒子の存在比率とほぼ同等であった。また、さらなる検討から、本陰イオン交換-UHPLCシステムを用いて、少量のAAVベクター試料から、比較的短時間で、僅かな中空粒子の混入までを再現性良く測定することが可能であることが示された。これらのことから、本陰イオン交換-UHPLCシステムはAAVベクター製品に含まれる完全粒子と中空粒子の存在比率を高感度かつ高精度で測定可能であり、品質評価手法の一つとして有用であると考えられた。

細胞内結晶化抑制型NEU1とCTSAの二重搭載AAV5ベクターを用いたミオクローヌスモデルマウスの遺伝子治療

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【研究目的】シアリドーシスとガラクトシアリドーシス（GS）は、それぞれリソソーム酵素ノイラミニダーゼ１（NEU1）とその活性化因子カテプシンA（CTSA）の欠損により、NEU1活性が低下し、末端シアル酸含有糖鎖の過剰蓄積及び中枢神経症状等を伴う常染色体潜性遺伝病であり、根本治療法は確立されていない。演者らは、アデノ随伴ウイルス（AAV）ベクターの内、脳内投与時に遺伝子導入効率が高く、アストロサイトへの導入も可能なAAV5に着目した。本研究では、細胞内結晶化を低減させた改変型（mod）NEU1とCTSA遺伝子を同時搭載したAAV5ベクターを作製し、GSモデルマウスの脳室内への投与による治療効果の検証を目的とした。
【方法】7週齢GSマウスにAAV5-*modNEU1/CTSA*を1.5×10^{13}vg/kg body weight で脳室内単回投与し、隔週でミオクロニー発作測定とオープンフィールド試験を行った。投与3週後に解剖・大脳抽出液を調製し、4-MU-NANA分解活性を指標にNeu 活性を、Z-Phe-Leu分解活性を指標にCtsa活性を測定した。末端シアル酸含有糖鎖量は、レゾルシノールアッセイにより評価した。また、anti-CD68抗体を用いた脳凍結切片免疫染色を実施した。
【結果・考察】ベクター投与マウス大脳では、Ctsa活性は野生型の12%まで回復した。Neu1基質である末端シアル酸含有糖鎖量は非投与群の72%まで低下した。GSマウスの凍結脳切片で観察される、活性化ミクログリアマーカーのCD68陽性細胞数は、ベクター投与群で顕著に減少していた。さらにGSマウスで起こるミオクロニー発作や活動性低下が抑制された。以上から、AAV5ベクターの脳室内単回投与により、欠損酵素活性の回復と蓄積基質の分解が起こり、脳内炎症反応の抑制と共に、中枢神経症状の改善が検証された。

細胞内非結晶性NEU1及びCTSA遺伝子同時搭載AAVPHP.eBを用いたガラクトシアリドーシスモデルマウスに対する遺伝子治療

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【目的】リソソーム病であるシアリドーシス(SD)とガラクトシアリドーシス(GS)は、リソソーム酵素ノイラミニダーゼ１(NEU1)とその活性化因子カテプシンA(CTSA)の欠損により、NEU1活性が低下し、その基質であるシアリル糖質の過剰蓄積及び中枢神経障害や肝脾腫等の全身症状を伴う常染色体潜性遺伝病である。これらの疾患に対する根本治療法は確立されていない。演者は、アデノ随伴ウイルス(AAV)ベクターの内、静脈内投与時に中枢神経系への遺伝子導入効率が高い、AAVPHP.eBに着目した。本研究では、細胞内結晶化を阻止した改変型(mod)NEU1とCTSA遺伝子を同時搭載したAAVPHP.eB-*modNEU1/CTSA*ベクターを作製し、GSモデルマウスの脳室内投与による治療効果を検証した。
【方法】7週齢GSマウスにAAVPHP.eB-*modNEU1/CTSA*を1.5×10^{13}vg/kg及び3.0×10^{13}vg/kgで脳室内投与し、隔週でミオクロニー発作測定とオープンフィールド試験を行った。投与3週後に解剖・大脳抽出液を調製後、4-MU-NANA分解活性を指標にNeu 活性を、Z-Phe-Leu分解活性を指標にCtsa活性を測定した。シアリル糖質の蓄積量をレゾルシノール法により測定した。脳凍結切片を作製し、anti-CD68抗体を用いた免疫染色を行った。
【結果・考察】AAVPHP.eB-*modNEU1/CTSA*を脳室内投与したGSマウスの大脳において、Ctsa活性は1.5×10^{13}vg/kgの投与量で野生型の22%、3.0×10^{13}vg/kgの投与量で野生型の62%まで回復した。またNeu1基質であるシアリル糖質量は野生型レベルまで低下した。GSマウスの脳内で観察される、活性化ミクログリアマーカーのCD68陽性細胞は減少していた。さらに、GSマウスで起こるミオクロニー発作や、オープンフィールド試験での活動性(行動距離・時間)の低下が抑制された。以上から、AAVPHP.eB-*modNEU1/CTSA*の脳室内投与により、欠損酵素活性の回復と蓄積Neu1基質の分解が起こり、脳内炎症反応の抑制とともに、中枢神経症状が改善したと考えらえる。

組換えウイルスベクターを用いたアルツハイマー病の能動免疫療法

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アルツハイマー病（AD）はアミロイドβ（Aβ）とタウの凝集体が脳に蓄積して生じる認知症である。1999年Dale Schenk らはADのモデルマウスを凝集Aβで免疫し、脳に蓄積したアミロイドが除去できることを初めて見出した。直ちにヒトで治験が行われたが、自己免疫性と思われる脳炎が多発した為治験は中止された。しかし、ヒトでも免疫によりアミロイドが除去できることが分かった。脳炎を回避するためにAβに対するモノクロ―ナル抗体の治験が行われ、抗体によってもアミロイドの除去に成功したが、認知機能に対する効果は見られなかった。アミロイドの蓄積は発病の20年以上前から始まっていることが分かり、発病後の介入ではもはや遅いと考えられるようになった。そこで診断・治療のパラダイムシフトが行われ、認知症発症前のADをバイオマーカーで診断し介入できるようになった。このようにして条件付きながら米国FDAの承認を得たのがAducanumabである。現在Lecanemab, Donanemabの第III相試験が行われており、うまく行けばAβ抗体療法がADに対する最初の疾患修飾薬になると思われる。

抗体薬は脳炎は回避できるが脳浮腫や脳出血が起こることがある。繰り返し静脈注射が必要で、非常に高価であり、安全であれば能動免疫療法のほうがよい。我々はAβ遺伝子を組換えたAAV、SeVを経口ないし経鼻投与する方法を開発し、その有効性、安全性をマウスと老齢サルで示してきた。本法は遺伝子治療に当たるとしてハードルが高かったが、ゾルゲンスマの承認、新型コロナに対するウイルスベクターワクチンの使用により、ハードルはかなり低くなった。さらに本法は当初ワクチンと考えられたが、診断・治療のパラダイムシフトにより超早期ADに対する能動免疫療法となることが可能になった。ここではこれまでの知見をまとめて発表する。

Early distribution of 18F-labeled AAV9 vectors in the cerebrospinal fluid after intraventricular or intracisternal infusion in non-human primates

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The delivery of adeno-associated virus (AAV) vectors via cerebrospinal fluid (CSF) has emerged as a useful method for widespread transduction in the central nervous system. While infusion into the cerebral ventricles is a common protocol in preclinical studies of small animals, cisterna magna has been recognized as an alternative target for clinical studies that can be reached in a less invasive manner using an intrathecal catheter via the subarachnoid space from a lumbar puncture. Unlike the classical circulation theory that the CSF moves as a unidirectional bulk flow from the choroidal plexus to subarachnoid villi, recent in vivo magnetic resonance imaging revealed the dynamic motion of the CSF influenced by various factors including cardiac pulsations, pulmonary respiration, and postures. We herein evaluated the early distribution of fluorine-18-labeled AAV9 vectors ([^{18}F]FB-AAV) infused into the lateral ventricle or cisterna magna of non-human primates by positron emission tomography (PET). Positron emitter-labeled tyrosine- mutant AAV9 expressing green fluorescent protein (GFP) was prepared by amino group-modification of capsid protein using *N*-succinimidyl 4-[fluorine-18] fluorobenzoate. The [^{18}F]FB-AAV were infused via the cisterna magna in two male macaques and via lateral ventricle in the other two male macaques. Accumulation of [^{18}F]FB-AAV was analyzed at 30-min intervals over 4 hours by PET. On images of average uptakes for 4 hours, [^{18}F]FB-AAV were distributed into subarachnoid space around the spinal cord as well as basal cisterns in all four monkeys. In intracerebroventricular infusion, [^{18}F]FB-AAV was distributed into the cisterna magna through the 4th ventricle within the initial 30 min. Subsequently, the accumulation of the [^{18}F]FB-AAV spread to the basal cisterns and subarachnoid space of the spinal cord and surface of the brain cortex. On histological examination of GFP expression, neurons in the cervical spinal cord were efficiently transduced by both infusion routes. For gene therapy that primarily targets the spinal cord and brainstem such as amyotrophic lateral sclerosis, intracisternal magna infusion is expected to be as effective as an intraventricular infusion.

Modulation of neurodegeneration by peripheral immune system in Niemann-Pick disease type C

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Niemann-Pick disease type C (NPC) is an autosomal-recessively inherited lysosomal storage disorder affecting an estimated 1 in 120,000 live births worldwide. Mutations in NPC1 or NPC2 gene represent approximately 95% or 5% of total patients with NPC, respectively. Their gene products, NPC1 and NPC2 proteins, function cooperatively in late endosomes and lysosomes to transport unesterifed cholesterol to the plasma membranes. Typical clinical feature of the disease is neurovisceral accumulation of unesterified cholesterol and several forms of glycosphingolipids. NPC can present with a broad range of clinical manifestation from a neonatal acute fatality to an adult-onset chronic disease associated with neurodegeneration. The development of neurological symptoms, including cerebellar ataxia, laughter-induced cataplexy, dystonia, and progressive dementia, affects quality of life of the patients drastically. Hence, it is essential to explore the pathogenic events that trigger and/or promote the neurodegenerative process for future clinical interventions. In this study, we addressed an involvement of immune system in neuropathogenic process using a murine model of NPC, npc1 mutant mice. Breaching of blood-brain barrier and infiltration of monocyte-derived macrophages correlated spatially and temporally with the loss of cerebellar Purkinje cells, which is a hallmark of neurodegeneration in NPC. Reduction of circulating monocytes using Ccr2-knockout mice ameliorated Purkinje cell degeneration. Moreover, involvement of acquired immune system in the Purkinje cell loss was investigated by crossing npc1 mutant mice with Rag1 knockout mice. The npc1 mutant mice that lack Rag1 exhibited enhanced cerebellar ataxic phenotype. These results imply that delivery of lymphocytes may be effective for the treatment of neurodegeneration in NPC. Importantly, we confirmed that peripheral delivery of CD4 and CD25-double-positive regulatory T lymphocytes ameliorated cerebellar ataxia and neurodegeneration of Purkinje cells in npc1 mutant mice. Depletion of regulatory T lymphocytes enhanced cerebellar ataxic phenotype. Moreover, neonatal bone marrow transplantation ameliorated cerebellar ataxia and neuronal degeneration. Our results disclose a previously unrecognized neuropathogenicity of immune system in NPC and would benefit future remedies for devastating neurological diseases.

筋萎縮性側索硬化症における蛋白質翻訳機能障害と治療への応用

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筋萎縮性側索硬化症（ALS）は運動神経の変性により全身の筋力低下を生じる神経難病の1つである。我々はALSの病態として、原因蛋白質であるRNA結合蛋白質TDP-43による神経軸索内への何らかのmRNA輸送が障害されている可能性を考え、TDP-43による輸送標的mRNAを検索しリボソーム蛋白質mRNAを同定した。培養神経細胞においてTDP-43の発現低下により軸索でのリボソーム蛋白質mRNA量は減少し、それに伴いリボソームの機能である蛋白質翻訳やリボソームRNAプロセシングが軸索で障害された。ALS患者神経組織の運動神経軸索走行部位においてもリボソーム蛋白質mRNA量の減少が確認された。さらにTDP-43発現低下培養神経細胞およびTDP-43過剰発現ショウジョウバエにおいて、リボソーム蛋白質の発現増加によりそれぞれでみられる軸索伸長障害、複眼変性が軽減された。

以上の知見は神経細胞でリボソーム機能を上昇させることがALSの治療につながる可能性を示唆しており、現在アデノ随伴ウイルスを用いた関連遺伝子の発現増加による遺伝子治療の開発を進めている。

プラズマ遺伝子導入法のゲノムリアレンジメントフリー性

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[背景]
本研究室では、プラズマ遺伝子導入法という放電プラズマを用いた遺伝子導入を確立した。プラズマ遺伝子導入法では、電気的および化学的刺激が複合的に作用して、生物学的応答であるエンドサイトーシスが惹起され細胞内に遺伝子などの外部巨大分子が導入されることを明らかにしている。本法の利点は株化細胞だけでなく初代培養細胞に対しても高効率な遺伝子導入が可能であること、エンドサイトーシスによって遺伝子が導入されるため、細胞に対して低侵襲であること、また高度な技術、高価な装置や試薬が必要なく非常に低コストであることにある。
[目的]
これらの利点から、プラズマ遺伝子導入法は細胞医療への可能性があると考え、細胞医療実現を目指すための第一歩として本法の安全性の高さを検証したのでその結果を報告する。
[方法]
細胞医療は重篤な疾患にのみ限定されており、汎用的な治療法として用いるためには安全面の課題を解決する必要がある。そこで既存の遺伝子導入法であるエレクトロポレーション法を比較対照とし本法の安全性を評価した。エレクトロポレーション法ではゲノムへのランダムインテグレーションが頻繁に生じるのに対してプラズマ法ではその発生確率が十分に低いことが確認された。また、細胞の生存率もプラズマ法はエレクトロポレーション法に比べて遙かに高いことが確認された。
[結果]
これらの結果から、プラズマ遺伝子導入法は細胞に対して安全に遺伝子を導入できることが明らかになった。今後、染色体変異の頻度および副作用の発現の可能性について詳細に検討すると共に、I型糖尿病モデルマウスを用いて本法の治療効果を検証していく。
[結論]
プラズマ遺伝子導入法は他の手法と比べてゲノムへの障害性が著しく低いため安全性の高い遺伝子導入法であることが確認でき、医療応用の可能性が示唆された。
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Mdmx plays a crucial role in neuronal damage after ischemic stroke

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[Background] Mdmx and Mdm2 are two major suppressor factors for the tumor suppressor gene p53. Mdmx suppresses the transcriptional activity of p53 and enhances the binding of Mdm2 to p53 for degradation. In the central nervous system, Mdmx suppresses the transcriptional activity of p53 and enhances the binding of Mdm2 to p53 for degradation. Accumulated research has shown that Mdmx-p53 interaction plays a central role in neuronal cell death after cerebral infarction, and its suppression reduces ischemic cell damage, but Mdmx dynamics in cerebral infarction remained obscure.
[Objective] This study aims to elucidate the functional role of Mdmx in ischemic stroke and to develop a treatment for stroke through elevated Mdmx.
[Material and Method(s)] 8-week-old C57BL/6 male mice were subjected to 60 min transient middle cerebral artery occlusion. Primary neurons were isolated from the cortex of embryonic day 16 (E16) mouse embryos and subjected to oxygen glucose deprivation following up to 24 h reperfusion. Recombinant AAV9 vector that overexpressed Mdmx were applied to the primary neuronal culture and incubated for 3 days. Western blot and immunofluorescence staining were used to detect Mdmx expression in neurons. Cell viability assay was conducted to evaluate neuron survival after hypoxia/recovery.
[Results] Hypoxia/recovery induced decreased Mdmx and elevated p-Mdmx expression both in vitro and in vivo. Overexpression of Mdmx via AAV-Mdmx transduction significantly increased Mdmx expression and reduced ischemic vulnerability hypoxia/recovery in neurons.
[Conclusions] These findings indicate Mdmx exerts a crucial role in neurons and further studies are needed to elucidate Mdmx dynamics in vivo.

Targeted Gene Delivery to the Brain with Smartly-Coated AAV9 Assisted by High Intensity Focused Ultrasound Enhances Safety and Efficacy

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Adeno-associated viral (AAV) vector is a highly efficient gene-delivery platform for treating intractable diseases due to its low invasiveness and long-term gene expression capability. However, high systemic doses of AAV vector induce serious side effects, such as hepatotoxicity and nephrotoxicity. Therefore, a novel AAV delivery system, which suppresses non-specific accumulation in healthy tissues/organs, such as the liver and kidneys, is urgently needed. We have recently developed a smart delivery system for biomolecules comprising tannic acid (TA) and phenylboronic acid-conjugated polymers [*Biomacromolecules 2020 Sep 14;21(9):3826-3835*, *PCT/JP2020/021301*]. TA is a polyphenol that forms a complex with biomolecules, such as proteins *via* hydrophobic interaction and hydrogen bond in aqueous solution. Moreover, TA forms boronate esters with phenylboronic acid-conjugated polymers resulting in the construction of nanoparticles capable of encapsulating biomolecules in their core. This supramolecular chemistry technique has potential as a novel approach for designing smart coatings for AAVs toward enhanced safety and delivery efficiency. We have examined the delivery ability of AAV coated with TA and block copolymers. The coated AAV was constructed by mixing of AAV9 (Serotype 9) -luciferase(luc), TA and phenylboronic acid-conjugated polymers in aqueous solution through **sequential 'self-assembly'.** The sizes of AAV9 and coated AAV9 were found to be 29 nm and 46 nm in diameter, respectively. The spherical shape of the coated AAV9 was confirmed using transmission electron microscopy. In healthy mice, coated AAV9-luc achieved high luciferase activity in the brain, suppressing liver and kidney accumulation.

In the current study, we studied whether the brain targeting of the smartly-coated AAVs can be enhanced by combining bubble liposomes (BLs) and high-intensity focused ultrasound (HIFU) to promote the permeability of the blood brain barrier (BBB). Thus, we injected echo-contrast gas (C3F8) entrapping liposomes with HIFU exposure for transient BBB opening. Smartly-coated AAV9-luc was delivered to the brain after pre-treatment with BLs and HIFU, which resulted in 10-fold increased gene expression in the brain at the focused-US exposure site. Furthermore, this BLs-HIFU-assisted delivery method significantly reduced liver and kidney accumulation (around 1/9). These results suggest that combining BLs and HIFU together serves as a powerful approach for altering the permeability of BBB to enabling the smartly-coated AAV9-luc to be delivered at the focused brain site. Our data demonstrate successful prevention of the AAV vector from non-specific accumulation in healthy tissues/organs, increasing the safety and efficiency of therapy.

Protocol optimization for generation of retroviral vector-producing human mesenchymal stem cells (VP-hMSCs)

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Background: Human mesenchymal stem cells (hMSCs)-based cell therapy is a promising strategy for cancer treatment because of the inherent tumor-homing ability of hMSCs. We have previously reported that retroviral vector-producing hMSCs (VP-hMSCs) administered to tumor-bearing mice systemically were able to achieve efficient transduction of therapeutic genes to cancer cells through their accumulation at tumor site and *in situ* production of infectious progeny viral particles. Human amniotic MSCs (hAMSCs), which can be easily obtained from fetal appendage and expanded *in vitro*, are good candidate as novel cellular vehicle for this approach. In order to generate VP-hMSCs efficiently, we recently established optimal electroporation protocols for hAMSCs. In this study, we further evaluated the productivity and functionality of retroviral vectors (RVs) from VP-hAMSCs generated by optimized protocols.
Methods: For generation of VP-hMSCs, hAMSCs and bone marrow derived hMSCs (hBMSCs) were transfected by electroporation of retrovirus vector harboring reporter gene and retrovirus packaging plasmids under different electroporation protocols. Four days after electroporation, the resultant VP-hMSCs and their supernatant were collected. Cell viability of VP-hMSCs was determined using flow cytometry. The physical (genomic) and biological (infectious) titer of RVs produced from VP-hMSCs was quantitated by qRT-PCR for physical titer and colony formation assay for biological titer, respectively.
Results: Among optimal electroporation protocols, cell viability of VP-hMSCs after electroporation was negatively correlated with the voltage of electrical pulse applied to the hMSCs, whereas positively correlated with the biological-to-physical titer ratio of RVs, suggesting that infectious RVs can be produced efficiently from VP-hMSCs with high viability. The co-culture experiments of a human pancreatic cancer cell line PANC1 with VP-hMSCs generated by optimized protocols revealed the high transduction of PANC1 cells with RVs.
Conclusion: Our findings suggested that cell viability of hMSCs post-electroporation is a crucial factor for generation of functional VP-hMSCs. Experiments to demonstrate the effect of cell division on the production of the highly infectious RVs are ongoing.

大規模製造に向けたゾーナル超遠心による短時間高精度組換えアデノ随伴ウイルスベクター精製法の開発

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背景：組換えアデノ随伴ウイルスベクター（rAAV）は臨床応用が進んでいるが、大量投与に伴う有害事象が報告された。製剤には、治療遺伝子であるベクターゲノム全長をもつ完全体、一部を持つ中間体、ゲノムを持たない中空粒子の他、宿主細胞由来タンパク質やプラスミドが含まれる。また、精製開始材料が細胞から培養上清に移行しており、大規模で高精度な精製法が必要とされている。塩化セシウム（CsCl）密度勾配超遠心を用いた精製法は、完全体とその他を分ける方として有効であるが、大規模製造には課題がある。また2日間の遠心によりCsClによるrAAV感染力低下が著しい。そこで、大容量のサンプルを処理できるゾーナルローターを用いて、独自のプロトコルにてrAAV精製を試みた。
方法：複数の密度のCsCl溶液とAAV9-ZsGreen1を含む培養上清（最大1000mL）を用いて4-10時間の超遠心を行い分画を回収した。各分画において、定量PCR、ウェスタンブロッティング、ZsGreen1陽性細胞率のフローサイトメトリーにより、rAAVゲノムコピー、カプシド量、感染力価を評価した。精度は、分析用超遠心機（AUC）と透過型電子顕微鏡（TEM）で解析し、パッケージングされたゲノム領域の解析をベクタープラスミドの全領域を標的としたプライマーとプローブを用いたdroplet digital PCR (ddPCR)により行った。
結果：ゲノムコピー、カプシド量、感染力のある完全体分画とカプシド量のみの中空粒子分画が確認でき、AUCとTEMによりこれら完全体と中空粒子の分画が高精度に分離できた。興味深いことに、ddPCRより完全体分画ではベクター領域上の22箇所が同レベルで検出されたが、中空粒子分画ではITR領域が他の領域より高く検出され、中空粒子に多コピーのITR断片がパッケージングされていた。
結論：ゾーナルローターを用いた新規CsCl密度勾配超遠心法は高精度のrAAVを短時間で精製でき、安全で効率の高い治療ベクターの製造に活用できる。

日本人血友病患者の抗AAV中和抗体保有率

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背景：血友病を始めとした種々の難治性疾患に対して、アデノ随伴ウイルス（AAV）ベクターを用いた遺伝子治療の開発が進んでいる。AAVベクターの全身投与による遺伝子治療は、既感染に伴う抗AAV中和抗体（NAb）を保有すると治療効果が減弱する。本研究では日本人の健常者と血友病患者におけるNAbの保有率を検討した。

方法：健常人血清は、自治医科大学にて男性、女性共に20、30、40、50、60代、各10名ずつの合計100検体が健常ボランティアから供与され、国内の血友病診療施設より血友病患者216名の血清を得た(血友病Aが175名（81%）、血友病Bが41名（19%）)。NAbは被験血清とウシ胎仔血清を1:1とした混合血清がヒト肝臓細胞株Huh-7へのAAVの遺伝子導入を50%以上阻害した場合をNAb陽性と判断した。

結果：種々のAAV血清型に対するNAb陽性率は、AAV1が22.7%、AAV2が25%、AAV3Bが29.2%、AAV5が22.7%、AAV6が23.1%、AAV7が24.1%、AAV8が20.4%、AAV9が21.3%、AAVrh10が23.6%であった。健常人と血友病患者の陽性率に明らかな差を認めなかった。陽性者の44.8%が全てのAAV血清型に対して陽性を示し、単独のAAV血清型のみに陽性を示す症例よりも多かった。年代別に解析すると、10-40代の各AAV血清型の陽性率は4.5-20%であるのに対し、50代は24-34%、60代以上は56-74%であった。過去の我々の報告に比べ40-50代の陽性率は明らかに低下していた。

結論：日本人血友病患者では、約75%が抗AAV中和抗体陰性であり、特に若年代では比較的多くの患者が遺伝子治療の対象となりうる。過去の我々の調査と比較すると、陽性率のピークは60代にシフトし、年長者では過去の既感染がNAb陽性の要因であると考えられる。

M1ミクログリア標的化アポトーシスペプチドによる新生児低酸素性虚血性脳症への新規治療法開発

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【背景】脳性麻痺の原因となる新生児低酸素性虚血性脳症(Hypoxic Ischemic Encephalopathy; HIE)は脳性麻痺の原因の半数を占めるが、低体温療法以外の有効な治療法はなく、新たな治療法の確立が期待されている。
【目的】HIEにおける急性期の炎症には、脳内の免疫担当細胞であるミクログリアが関与しており、中でも、活性型ミクログリア(M1ミクログリア)が、炎症性サイトカインを産生し、神経細胞障害を引き起こすことで障害の発症に深く関わっていることが報告されている。そこで、我々はこのM1ミクログリアの選択的アポトーシス誘導が、HIEの新規治療法となるのではないかと考え検討することとした。
【方法】以前に同定したM1ミクログリアに選択的に結合するペプチド配列で標識したアポトーシスペプチド(MG1-KLA)を作成。新生仔マウスの左総頸動脈の血流を遮断した上で低酸素状態に暴露することでHIEモデルマウスを作成し、24時間後に患側である左脳室内へMG1-KLAペプチドの投与を行った。HIE手術から72時間、2週間後にミクログリアを中心とした組織学的評価を、またHIE手術から2週間後に行動機能解析を行い、治療効果を評価した。
【結果】コントロール群(PBS投与)では、患側脳にミクログリアの集積を認め、虚血による神経細胞の脱落を伴う脳萎縮がみられたが、MG1-KLA群では、ミクログリアの集積および脳萎縮が著明に抑制された。また、コントロール群で認められた行動機能障害についても、MG1-KLA群ではその障害は有意に抑制された。
【結論】MG1-KLAペプチド投与群では、HIEによるミクログリアの集積、神経細胞障害、行動機能障害が有意に抑制され、治療効果を示した。M1ミクログリア標的化アポトーシスペプチドは、HIEへの新規分子治療法としての臨床応用が期待されると考えられた。

Establishment of a Pancreatic Cancer Rat Model using the Pancreas-Targeted Hydrodynamic Gene Delivery

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Background: A development of the pancreatic cancer animal model is an unmet need to develop the novel therapy and biomarkers for the disease which is a leading cause of cancer-related death worldwide. To establish the model, we have examined the applicability of the pancreas-targeted hydrodynamic gene delivery (HGD) method to establish a pancreatic cancer rat model in efficient manner.
Material and Methods: The pancreas-targeted HGD was utilized to deliver human pancreatic cancer related genes to the pancreas of wild rats to examine the development of cancer animal models. The genes include the plasmid expressing *KRAS* wild-type (WT), $KRAS^{G12D}$ variant, *MYC*, and *YAP* genes. The combinatorial delivery and repeat HGD were examined for more effective and malignant tumor development.
Results: $KRAS^{G12D}$-induced pancreatic intraepithelial neoplasia lesions showed malignant transformation in the main pancreatic duct at four weeks and developed acinar-to-ductal metaplasia (ADM), which led to PDAC within five weeks and the gene combination of $KRAS^{G12D}$ and *YAP* enhanced these effects. The repeat HGD of $KRAS^{G12D}$ + *YAP* combination at four weeks showed ADM in all rats and PDAC in 80% of rats one week later. Metastatic tumors in the liver, lymph nodes, and subcutaneous lesions and nervous invasion were confirmed. $KRAS^{G12D}$ and *YAP* combined transfer contributes to the E- to N-cadherin switch in PDAC cells and to tumor metastases. In addition, activation of the various oncogenic pathways including Akt, Erk, etc. were confirmed.
Conclusions: This research developed an easy-to-use, reproducible pancreatic cancer animal model utilizing pancreas-targeted HGD to deliver human pancreatic cancer-related genes to the pancreas of wild rats. This pancreatic cancer model will speed up pancreatic cancer research for novel treatments and biomarkers.

Reovirus-mediated anti-fibrotic effects: functional restoration of hepatic stellate cells following reovirus treatment

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Liver fibrosis is induced by various types of hepatic damages, including virus infection and alcoholic hepatitis. Hepatic damages lead to the production of transforming growth factor-β (TGF-β). TGF-β activates hepatic stellate cells, which play a crucial rule in liver fibrosis by differentiating into myofibroblasts and producing large amounts of extracellular matrices (ECM). Excessive production of ECM results in liver fibrosis. Advanced liver fibrosis and cirrhosis are major risk factors for hepatocellular carcinoma. Although there are many patients suffering from liver fibrosis, there are few effective anti-fibrotic drugs. Reovirus, which is a non-enveloped virus containing a 10 segmented dsRNA genome, has attracted much attention as an oncolytic virus due to the promising properties, including efficient antitumor effects and lack of pathogenicity. We previously demonstrated that reovirus showed efficient antifibrotic effects on the CCl4-induced liver fibrosis mice following intravenous administration (The 27th JSGCT annual meeting 2021), however, it remained to be elucidated whether functions of liver stellate cells were restored by reovirus treatment. In this study, we examined the function of hepatic stellate cells after reovirus treatment.

First, we examined the intrahepatic distribution of reovirus following intravenous administration. Immunohistochemical and western blotting analysis of virus capsid protein demonstrated that reovirus was taken up by not only hepatocytes but also hepatic stellate cells in the liver. These data suggested that reovirus directly mediated anti-fibrotic effects on activated liver stellate cells after cellular uptake of reovirus. Next, we investigated whether lipid accumulation was restored by reovirus treatment in LX-2 cells, which is a human liver stellate cell line. In normal state, hepatic stellate cells store vitamin A in lipid droplets. Pre-incubation of LX-2 cells with TGF-β resulted in the significant reduction in the numbers of lipid droplets, while the numbers of lipid droplets were increased by reovirus treatment, and were similar to those in the naive cells, which were not treated with TGF-β or reovirus. Furthermore, we examined the cell migration of LX-2 cells by scratch assay. TGF-β treatment significantly promoted the migration of LX-2 cells. Reovirus treatment significantly suppressed the cell migration. The migration levels of reovirus-treated LX-2 cells were similar to those of the naive cells. These results indicated that reovirus treatment not only reduced the fibrotic marker expression but also improved the cellular functions of hepatic stellate cells. Finally, we performed mRNA-seq transcriptome analysis to characterize the reovirus-treated LX-2 cells. Reovirus-treated cells and naive cells showed similar expression profiles of extracellular matrix organization-related genes, cell migration-related genes and cell adhesion-related genes, while expression levels of these genes were up-regulated in TGF-β-treated cells. These data suggested that properties of the activated hepatic stellate cells were altered to quiescent state by reovirus treatment.

Comparability assessment after transfer of the manufacturing process for a next generation CAR-T (CD19-JAK/STAT CAR-T, TBI-2001)

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Background: TBI-2001 is a CAR-T cell product that expresses a next-generation CAR construct containing anti-CD19 scFv and the improved signal transduction domains CD28-ΔIL2RB-CD3ζ (YXXQ) (JAK/STAT), which leads to the activation of cytokine-related JAK/STAT signaling pathways. This CAR construct has shown enhanced antitumor activity in preclinical studies using CAR-transduced T lymphocytes by increasing the proliferation and persistence of T lymphocytes when compared to cells transduced with a CAR lacking the JAK/STAT signaling domain. Takara Bio Inc. (Takara) has developed the manufacturing process for generating TBI-2001 for clinical use. As we plan to manufacture TBI-2001 at the Princess Margaret Cancer Centre (PM-CC) in Canada for the first-in-human trial in patients with CD19+ B-cell lymphoma, chronic lymphocytic leukemia or small lymphocytic lymphoma, the manufacturing process was transferred and a comparability assessment of TBI-2001 test batches manufactured at Takara and the PM-CC was performed to confirm that the controlled process used for the manufacture of TBI-2001 minimizes product variability.
Methods: Both Takara and the PM-CC separately manufactured and tested two full-scale test batches. One of the PM-CC's batches was prepared from a fresh Leukopak and a fresh Peripheral Blood Plasma pack. The other batch was manufactured using aliquots from the same cryopreserved PBMC and plasma that were used for Takara's test batches, therefore providing a side-by-side comparison of test batches prepared from the same apheresis product. All of the process steps for the manufacture of the test batches including the processing and cryopreservation of PBMC and plasma were identical at both sites and also to the process steps that will be used to manufacture TBI-2001 batches for the clinical trial. The comparability of PM-CC's batches was assessed against Takara's batches based on the data from final product specification testing, additional characterization tests, and in-process control testing.
Results/Conclusion: The results from final product specification testing and additional characterization tests from the PM-CC's batches were all within the pre-determined target ranges. The in-process control test results also supported comparability of the batches. It was concluded that the PM-CC's batches were comparable to Takara's batches. The manufacturing process for generating TBI-2001 was successfully transferred for the upcoming clinical trial at the PM-CC.

A Microfluidic 3D Endothelium-on-a-Chip Model to Study Transendothelial Migration of T Cells in diseases

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Recruitment of T cells is a crucial component in the inflammatory cascade of the body which involves the trafficking of T cells through the vascular system and their stable arrest to vessel walls at the site of inflammation, followed by extravasation and subsequent infiltration into tissue. Using patients' own immune system proved to be a promising avenue for combatting cancer although therapeutic efficacy varies significantly depending on the tumor type. To improve this treatment and expand its use to other tumor types, new models that better recapitulate the *in vivo* processes should be developed.Previously, we described an assay to study 3D T cell dynamics under flow in real time using a high throughput, artificial membrane-free microfluidic platform that allows unimpeded extravasation of T cells towards a chemotactic trigger or melanoma cells. The endothelial vessel and tumor/trigger compartment were separated by empty collagen gel, which made it easy to track migration of T cells, but more difficult to look at tumor cell killing. In this research we decreased the distance between endothelial vessel and the other compartment in two different ways. We assessed whether we would still have a proper barrier of the endothelium by measuring TEER over time, look at the effect on the number of T cells that showed transendothelial migration and at tumor cell killing. This improved model presents new possibilities to expand our knowledge regarding the process of transendothelial migration and killing of immune cells during diseases. Summary: T cell recruitment is a crucial component in the inflammatory cascade of the body and involves extravasation of T cells from blood vessels. We describe a novel assay using a high-throughput device to study this process in healthy and diseased settings.

Targeted genome repair in selectively expanded mouse long-term hematopoietic stem cells

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First-line treatment for the most of genetic blood disorders is allogenic hematopoietic stem cell (HSC) transplantation, which only available for ~30% of patients. Gene therapy and targeted genetic correction in HSCs could possibly cure the diseases as alternates, and the preliminary results of genome-editing in HSCs are considered promising with the emergence of CRISPR/Cas9. We recently demonstrated that cDNA insertion via non-homologous end-joining (NHEJ) to the double-strand break (DSB) site created by CRISPR/Cas9 could cure X-linked severe combined immunodeficiency (X-SCID) mice, although the genome edited HSCs repopulated in only 25% of mice to cure. The results suggested us that we need more number of HSCs needs to be genome-edited. We also observed NHEJ-mediated cDNA integration disrupted cDNA or the integration site in some cases, suggesting that more accurate integration method may be needed. Taken together, here we aimed to (1) expand long-term repopulating HSCs (LT-HSCs) to achieve more genome-editing with higher repopulation ability and (2) develop a method for homologous-direct repair (HDR) in HSCs.

First, we enriched mouse LT-HSCs by sorting Lineage marker negative (Lin-), cKit+, Sca1+, CD150+, and CD34- cells from bone marrow, and expanded using PVA-containing media. LT-HSCs were expanded to XXX times in YYY days. Second, we optimized HDR-based genome-editing in expanded LT-HSCs. For the optimization, we chose Ptprc (CD45) as the target. Only a single amino acid polymorphism causes CD45.1 and CD45.2, which are detectable with selective antibodies. After the optimization of the delivery method of Cas9 ribonucleoprotein (RNP) and single stranded DNA repair template to LT-HSCs, we achieved ~10% of conversion from CD45.2 to CD45.1. We then transplanted the genome edited HSCs. After the engraftment, we observed only ~1% of donor HSC-derived peripheral blood cells was CD45.1.

The current study demonstrated that precise genome editing of expanded LT-HSCs is feasible, however, further improvement is necessary for the realization of this method to cure genetic blood disorders.

CRISPR-Cas3を利用した次世代CAR-T細胞療法の開発

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CRISRP-Cas9を用いたゲノム編集による遺伝子改変は、その簡便性から、遺伝子の機能解明など基礎研究の発展に貢献しているだけでなく、遺伝子治療、再生医療や創薬などの医療分野などで活用されている。一方で、本ゲノム編集技術は、オフターゲット変異などが懸念されており、これらの現象がしばしば医療応用への障害になっている。最近、我々は、ヒト細胞においてCRISPR-Cas3によるゲノム編集が可能であることを発見し、本ゲノム編集技術が、オフターゲット変異を引き起こす可能性が低いことを示した。キメラ抗原受容体（CAR）-T細胞療法は、がん治療、特にB細胞性腫瘍に高い治療効果が発揮され、免疫チェックポイント阻害療法続く次世代のがん免疫療法として注目を集めている。CAR-T細胞療法は、自家細胞治療であるため、製造コストが高額になる。また、悪性腫瘍の大部分を占める固形がんに対しては有効性が乏しいことなどの課題がある。最近、玉田らはケモカインやサイトカインを発現ことにより、固形癌でも高い抗腫瘍効果を示すPrime CAR-T技術を開発した。本研究の目的は、ゲノム編集技術CRISPR-Cas3とPrime CAR-T技術の二つの技術を融合して、固形癌でも効果を示し、より汎用性に優れた他家CAR-T細胞療法を開発することである。我々は、CRISPR-Cas3を用いてT細胞の患者正常組織の傷害と免疫拒絶を抑えた他家T細胞の開発を目的として、ヒト白血病T細胞株（Jurkat細胞）を用いてTCR α 鎖定常領域（TRAC）遺伝子および β 2-microglobulin（B2M）遺伝子のノックアウトを試みた。その結果、TRAC遺伝子では、ノックアウト効率が79%であった。B2M遺伝子に関しては71%のノックアウト効率であった。今回の成果より、ヒト免疫細胞において、CRISPR-Cas3が目的遺伝子に対して高効率に欠失変異を加えるが可能なことから、本ゲノム編集技術が養子免疫細胞療法の開発に利用できると考えられる。

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