The 1st Meeting of Hirosaki Medical Science Forum

# 第1回 弘前メディカルサイエンスフォーラム

## 知の結集、融合、そして創造的変革へ
## Together! Toward Innovation

## プログラム・抄録集
Program and Abstracts

**会　期**

**2019年11月30日(土)**
**9:00～17:00（参加受付 8:00～ ※予定）**

**会　場**

**弘前大学大学院医学研究科**
**健康未来イノベーションセンター**
**〒036-8562　弘前市在府町5**

**会　長**

**若林　孝一**（弘前大学大学院医学研究科長）

**学会ＨＰ**

**http://www.med.hirosaki-u.ac.jp/~inter2/hmsf/**

弘前メディカルサイエンスフォーラム　第１回学術集会事務局
実行委員長　富田　泰史（弘前大学大学院医学研究科　循環器腎臓内科学講座　教授）
〒036-8562　青森県弘前市在府町５
TEL：0172-39-5057　FAX：0172-35-9190　E-mail：hmsf-1st@hirosaki-u.ac.jp

共催：弘前大学大学院医学研究科
後援：弘前市

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**参加費：1,000円**

**参加費と引き換えに参加証をお渡ししますので、「所属」「氏名」をご記入のうえ、会場では必ずご着用ください。原則として参加証の再発行はできません。**

弘前メディカルサイエンスフォーラム　第１回学術集会事務局
弘前大学大学院医学研究科　循環器腎臓内科学講座
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# ご挨拶

会長
**若林孝一**
弘前大学大学院
医学研究科長

実行委員長
**富田泰史**
循環器腎臓内科学講座
教授

医学の進歩は目覚ましく、免疫チェックポイント阻害薬などの分子標的薬、遺伝子変異に基づくがんゲノム医療、AIを活用した医療システムの開発、ロボット支援下手術（ダ・ヴィンチ手術）など枚挙にいとまがありません。その土台となるのは医学研究です。医学研究なくして医学の進歩はありえません。昨今、医学・生命科学をはじめとする基礎科学研究全体の衰退が叫ばれています。複数の要因が絡み合った結果と推察されますが、弘前大学大学院医学研究科も例外ではありません。そこで、医学研究科における基礎医学研究をさらに活性化すべく、弘前メディカルサイエンスフォーラムを立ち上げました。このフォーラムの目的は、医学の発展のため、医学研究科一丸となって基礎医学研究を活性化することです。第1回の学術集会を2019年11月30日（土）に開催致します。

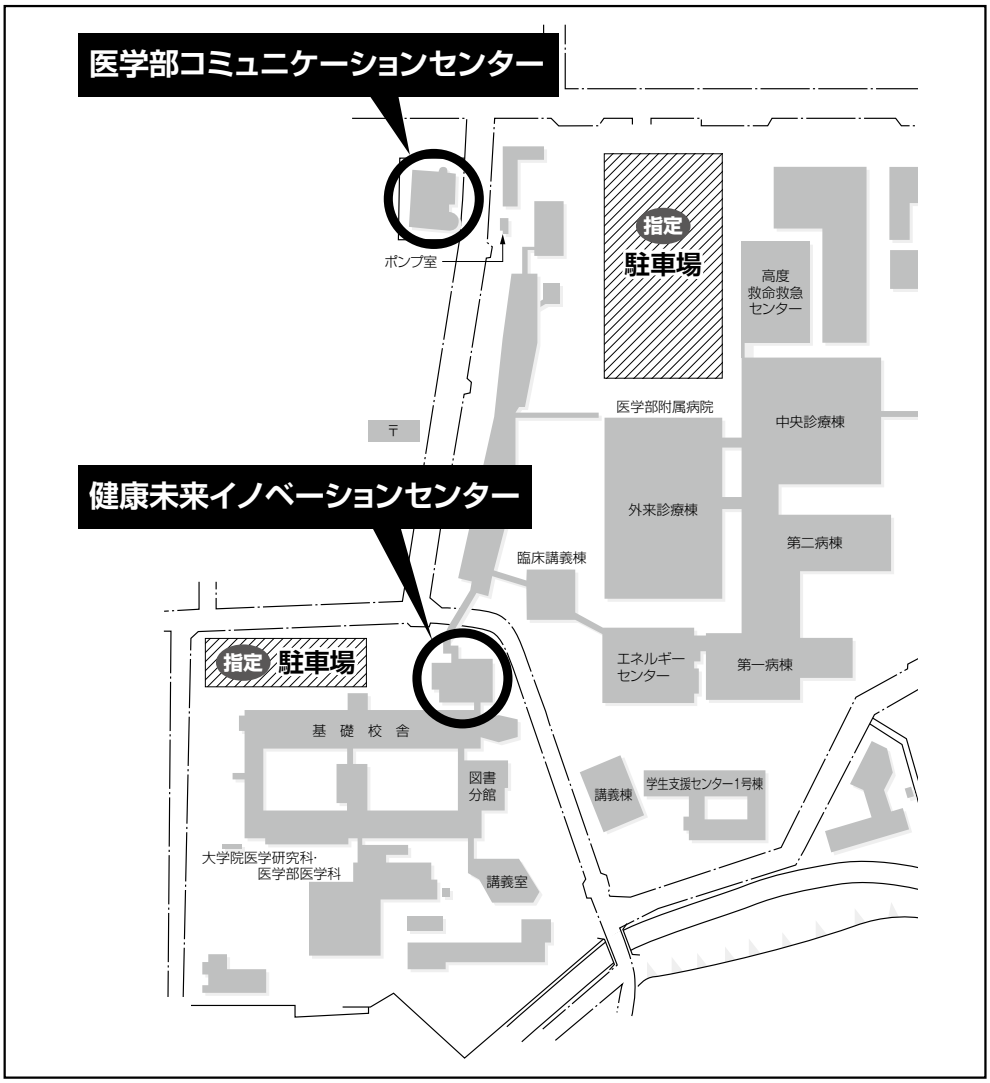
今回のテーマは「知の結集、融合、そして創造的革新へ（Together! Toward Innovation）」です。魅力ある創造的な研究は、様々な分野あるいは知の融合から芽生え、そして発展していきます。さらにそのヒントは毎日の実験における注意深い観察、あるいは日常診療から生まれるClinical Questionsの中に隠されています。常に探究心を持ち続け、見出されたQuestionsを大切にし、講座や学部の垣根を越えて議論しながら、皆で一丸となってその課題に取り組むことがこれまで以上に求められています。様々な研究者たちとの議論の中から創造的な研究が生まれると確信しています。

今回の学術集会の特別講演では国内外から著名な医学研究者をお招きし、最新の研究成果だけでなく、発見に至る過程や秘話など、研究をさらにEncourageするような内容でのご講演をお願いしています。シンポジウムでは「知の結集と融合から生まれる創造的研究」をテーマとし、弘前大学内あるいは国内外との共同研究を積極的に展開している先生方に、知の結集と融合から生まれる創造的な研究の一端をご紹介いただきます。さらに応募演題の中から高得点演題を選出し、プレナリーセッションで発表していただきます。プレナリーセッションならびにポスターセッションではAward（副賞があります）を設けておりますので、積極的にご応募ください。弘前大学医学研究科における基礎研究ならびにトランスレーショナル研究の活性化が、今回の弘前メディカルサイエンスフォーラムの大きなミッションです。皆様の積極的な参加をお待ちしています。皆で弘前大学における医学研究を盛り上げていきましょう！

# 会場案内＆アクセス

## 健康未来イノベーションセンター

〒036-8562　青森県弘前市在府町５（医学部基礎棟）




### 駐車場

フォーラム参加の方は、指定駐車場に限り無料です。病院駐車場か医学部基礎校舎前をご利用ください。
病院駐車場をご利用の方は、参加受付より無料駐車券を受け取って下さい。
駐車場は台数に限りがございますので、満車の際は近くの有料駐車場をご利用ください。

### アクセス方法

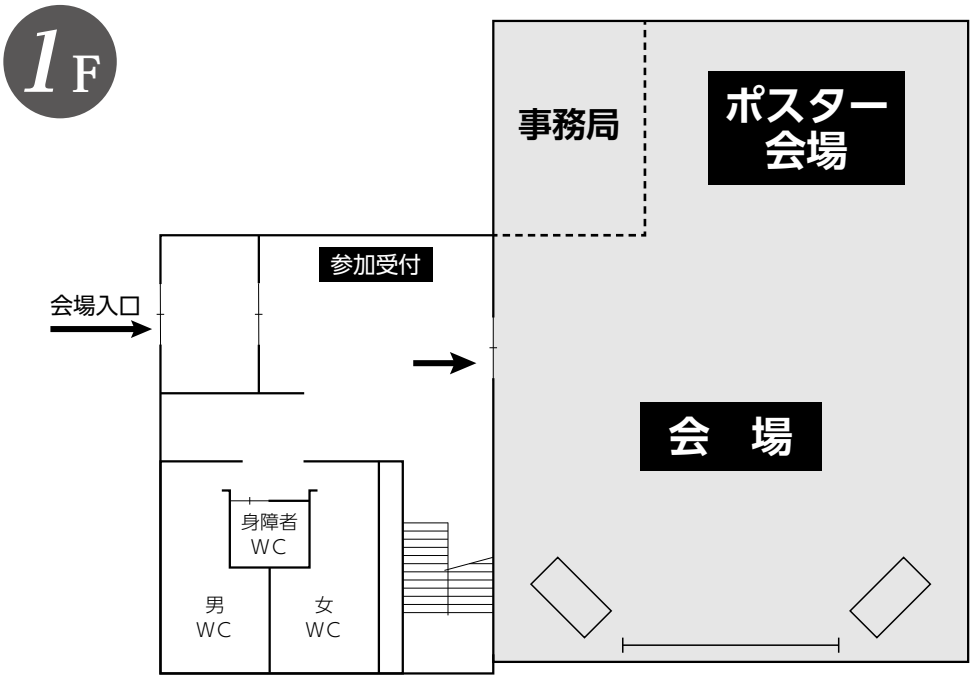
弘前駅から

◎徒歩→約35分　　◎タクシー利用→約10分

◎バス利用→約20分

- ＪＲ弘前駅前（中央口）６番のりば
  「駒越線」乗車、「大学病院前」で下車
- ＪＲ弘前駅前（中央口）８番のりば
  「金属団地・桜丘線」乗車、「本町」で下車
  ※土手町循環100円バスのご利用が便利です。

# 会 場 案 内 図



# 参加者へのご案内

## ■ 参加受付

※参加費は下記の通りです。

| | |
|---|---|
| 参加費 | 1,000円 |
| | 学生／初期研修医：無料<br>※ただし、証明できるものを提示してください。 |

- 参加費と引き換えに参加証をお渡しいたしますので、「所属」「氏名」をご記入の上、会場内では必ずご着用ください。再発行はいたしません。

## ■ 参加受付

［受付時間］ 11月30日（土） 8：00 ～ ※予定
［受付場所］ 健康未来イノベーションセンター 入り口付近ホール

## ■ 授賞式・懇親会のご案内

会終了後、授賞式・懇親会を開催いたしますので、奮ってご参加ください。
［日時］ 11月30日（土） 17：30～19：00 ※予定
［会場］ 弘前大学医学部コミュニケーションセンター（MCC）1階
［懇親会参加費 ］ 無料

## ■ 駐車場

フォーラム参加の方は、指定駐車場に限り無料です。病院駐車場か医学部基礎校舎前をご利用ください。
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## ■ その他

- 会場の健康未来イノベーションセンターは全館禁煙および敷地内禁煙です。
- 会場内で呼び出し音のある携帯電話等の利用はご遠慮願います。
- 会場内での録音、写真撮影およびビデオ収録は固くお断りいたします。
- 会場内では、指定の場所以外でのご飲食はご遠慮ください。
- 会場にクロークはございません。

# 発表に関するご案内

## ■ 座長の方へ

- 担当セッション開始10分前までに必ず次座長席にお着きください。
  座長受付はございません。
- 進行は座長に一任いたします。時間厳守にてお願いいたします。
- Keynote lectureは15分、プレナリーセッションは1演題12分（発表時間10分＋質疑応答2分）です。

## ■ プレナリーセッション発表者の方へ

- PC受付は、フォーラム当日8：00~8：45とし、USBメモリのみで受付いたします。
  指定時間内に、必ずPC受付で動作確認をしてください。
- 発表データはWindowsのみといたします。その他の場合は、事前に必ず事務局にご相談ください。
- ファイル名は「演題番号（半角）発表者名（漢字)」としてください。(例：PL-01 弘前太郎)
- 進行は座長の指示に従い、時間厳守でお願いします。
- 発表言語は英語もしくは日本語になりますが、発表スライドは全て英語で作成してください。

**【発表データの作成】**

- OSはWindows、アプリケーションはMicrosoft　PowerPoint 2010/2013/2016、文字はOS標準フォントをご使用ください。

  例：[日本語] MS ゴシック・MSP ゴシック・MS 明朝・MSP 明朝・メイリオ

  [英　語] Times New Roman・Arial・**Arial Black**・Arial Narrow・Century・Century Gothic・Courier New・Georgia

- 画面サイズは「標準（4：3)」に統一します。

**【発表時のご注意】**

- 次演者は、次演者席にお着きください。
- 発表時は演台上のマウスとキーパッドをご自身で操作してください。
- 発表時間の終了1分前と終了時に合図にて時間をお知らせいたします。
  くれぐれも時間厳守でお願いいたします。

## ■ 質疑応答

- 発言を希望される方は、あらかじめ会場内のマイク付近で待機してください。
- 発言者は座長の指示に従って所属と氏名を述べたのち、簡潔にご発言ください。

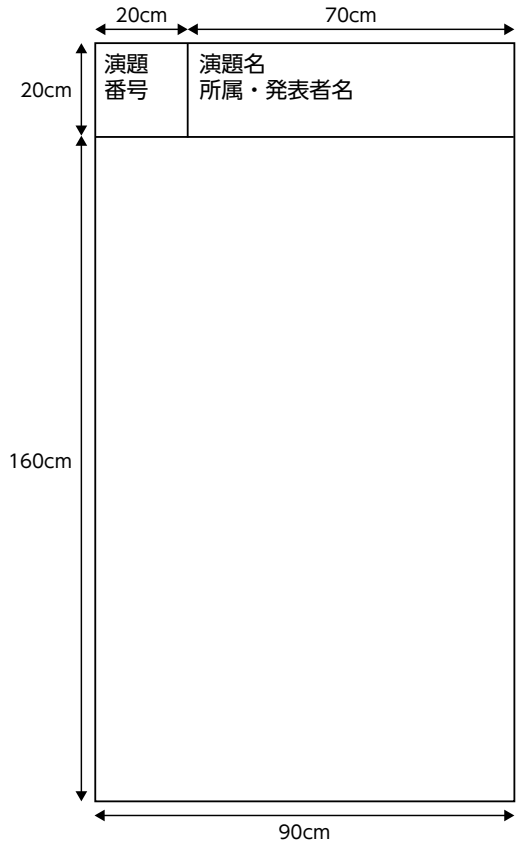
# ポスター発表に関するご案内

## ■ ポスター発表について

- 掲示場所　　会場後方のポスター掲示用パネル
- 掲示時間　　フォーラム当日**8：00～8：45**の間に掲示をしてください。
- 発表時間　　13：50～14：50　演題番号の奇数と偶数に分かれて対応をお願いいたします。
  下記の時間になりましたら、質疑応答のためご自身のポスター前に待機してください。
  13：50～14：20　演題番号が奇数の方（PO-01・PO-03…）
  14：20～14：50　演題番号が偶数の方（PO-02・PO-04…）
- 撤去時間　　フォーラム終了後から30分間とし、以降は事務局で撤去、処分します。

## ■ ポスター作成要領

- ポスターは全て英語で作成してください。
- ポスターパネルのサイズは縦180cm×横90cmです。演題番号は予め表示されています（下図参照）。
  演題名・所属・発表者名は演者ご自身でご用意ください。
- 文字や図表のサイズ、レイアウトを工夫し、離れたところからでもわかりやすい判読しやすいポスターを作成してください。なお、「Background」「Methods」「Results」「Conclusion」などを明確にし、Backgroundは左上部に、Conclusionは右下部になるようにレイアウトしてください。



# スケジュール

## 11月30日（土）AM

**8:00–8:00**
受付開始

**8:55–9:00**
開会の挨拶
会長 若林　孝一（弘前大学）

**9:00–9:50**
特別講演1
座長 富田　泰史（弘前大学）
「基礎研究はどこまで循環器病の謎を解いたか ―新しい循環器病学の時代へ」
演者 小室　一成（東京大学）

**9:50–10:20**
教育講演
座長 横山　良仁（弘前大学）
「リサーチマインドは臨床医の必須アミノ酸」
演者 大山　　力（弘前大学）

**10:20–11:50**
プレナリーセッション
座長 伊東　　健（弘前大学）　石橋　恭之（弘前大学）

Keynote lecture
「Relationships between OCT, ERG and morphology in animal models for retinitis pigmentosa」
演者 Mitsuru Nakazawa (Hirosaki University Graduate School of Medicine)

PL-01「Inhibition of protease activated receptor-1 suppresses progression of cardiac hypertrophy and fibrosis in renin-overexpressing hypertensive mice」
演者 Yoshikazu Yokono (Hirosaki University Graduate School of Medicine)

PL-02「Activated Macrophage via RAGE Signaling Induces Insulin Resistance and Deficit of Retrograde Axonal Transport in Diabetic Polyneuropathy」
演者 Sho Osonoi (Hirosaki University Graduate School of Medicine)

PL-03「Adult onset-multiple system atrophy model mice」
演者 Kunikazu Tanji (Hirosaki University Graduate School of Medicine)

PL-04「Anti-inflammatory effect of transplantation of multilineage differentiating Stress Enduring cell for spinal cord injury」
演者 Toshihide Nagaoki (Hirosaki University Graduate School of Medicine)

PL-05「Deficiency of p62 exacerbates doxorubicin-induced cardiac dysfunction」
演者 Michiko Tsushima (Hirosaki University Graduate School of Medicine)

PL-06「Identification of a novel autophagy receptor that regulates intracellular bacteria proliferation」
演者 Keiya Uriu (Faculty of Agriculture and Life Science, Hirosaki University)

**12:00–12:50**
ランチョンセミナー
座長 藤井　穂高（弘前大学）
「細胞外小胞形成の分子機構」
演者 森田　英嗣（弘前大学）

**13:00**

## 11月30日（土）PM

| | | |
|---|---|---|
| 13:00 | 13:00–13:50<br>特別講演2 | 座長 富田　泰史（弘前大学）<br>「From “sea-snake venoms” to “genetic risk of atherosclerosis”」<br>演者 Nobuyo Maeda (University of North Carolina at Chapel Hill) |
| 50 | | |
| 14:00 | 13:50–14:50<br>ポスター<br>セッション | 13:50-14:20　奇数の演題番号 |
| 20 | | 14:20-14:50　偶数の演題番号 |
| 50 | | |
| 15:00 | 14:50–15:40<br>特別講演3 | 座長 田坂　定智（弘前大学）<br>「マクロファージを用いた難治性呼吸器疾患に対する新規治療戦略」<br>演者 鈴木　拓児（自治医科大学） |
| 40 | | |
| 16:00 | 15:40–17:00<br>シンポジウム | 座長 水上　浩哉（弘前大学）　富田　泰史（弘前大学）<br>テーマ「知の結集と融合から生まれる創造的研究」<br>基調講演「研究のすすめ」<br>演者 若林　孝一（弘前大学）<br>S–1「臨床講座と基礎講座との融合」<br>演者 今泉　忠淳（弘前大学）<br>S–2「医学と理工学との融合：成果と課題」<br>演者 福田　幾夫（弘前大学）<br>S–3「血管・リンパ管網を形成する三次元ヒト生体組織エンジニアリング」<br>演者 下田　浩（弘前大学） |
| 17:00 | 17:00–17:05<br>閉会の挨拶 | 会長 若林　孝一（弘前大学） |
| 05 | | |

| | | |
|---|---|---|
| 17:30 | 17:30–19:00<br>授賞式・<br>懇親会 | 司会 伊東　健（弘前大学）<br>場所：医学部コミュニケーションセンター |
| 18:00 | | |
| 19:00 | | |

（敬称略）

特別講演 1

# 基礎研究はどこまで循環器病の謎を解いたか
## ー新しい循環器病学の時代へ

東京大学循環器内科 教授、日本循環器学会 理事長
小室　一成

我が国は超高齢社会となり、循環器病の患者数、死亡者数が激増している。我が国の死因のトップはがんであるが、高齢者ではがんと循環器病の死亡者数はほぼ同じであり、患者数は循環器病ががんよりもはるかに多い。平均寿命と健康寿命の約10年間の解離の原因としても循環器病ががんよりも圧倒的に重要である。今後健康長寿を目標としている我が国において循環器病はがんと並んで重要な疾患である。私は、研修医の時に将来何を専門にするか、がんにするか循環器病にするか迷ったものである。臨床としては循環器病が面白いが、研究となるとがんに魅力を感じていた。当時循環器の研究としては、血行力学といった生理学的研究が主流であった。その学問自体は高度に洗練されており感動したが、一方で生理学的な解析では疾患発症の分子機序を解明することはできないと感じていた。幸い恩師になる先生から循環器病学もこれからは生化学や分子生物学的な研究によって病態を解明していかなくてはならないと言っていただき、循環器病学を志そうと決心した。

私は循環器病の中でも特に心臓に特異的な収縮弛緩といった動的な機能の異常から発症する心不全に魅力を感じた。1990年代から遺伝子改変マウスが心不全研究にも使用されるようになり、初めて心機能といった動的な異常を分子レベルで解析することが可能となり、心不全研究は一気に加速した。最近ではそこにiPS細胞も加わり、従来不可能であったヒト心筋細胞を用いた研究も可能となった。

最近我が国の科学力の伸び悩みが方々で叫ばれているが、循環器の基礎研究はその最も極端な例である。例えば循環器基礎研究の専門誌への投稿論文数を見ても、諸外国は軒並み増えているのに対し、我が国からの投稿数は10年前と比較し伸び悩むどころか半減している。本来臨床研究にしろ、創薬やデバイス開発にしろ、独創的な基礎研究の成果に基づいて行われるべきであり、すべての基盤となる基礎研究なくしてその後の発展はない。がんは基礎研究により原因が解明され、原因に基づいた治療が行われるようになった結果、不治の病と思われていたものが治る時代になった。一方治療が進んでいたと思われていた循環器病は病態の解明が進まず対症療法に甘んじているために未だに治すことができていない。基礎研究から橋渡し研究を経て臨床研究を行い、さらにそこから得られた知見をもとに基礎研究を行うといった循環型の研究により初めて真実が見え、有効な治療法が確立されるのであろう。

**小室　一成**
（こむろ　いっせい）

**略　歴**

| | |
|---|---|
| 昭和57年 | 東京大学医学部医学科卒業 |
| 昭和59年 | 東京大学医学部附属病院第三内科医員 |
| 平成 元 年 | ハーバード 大学医学部博士研究員 |
| 平成 5 年 | 東京大学医学部第三内科助手 |
| 平成10年 | 東京大学医学部循環器内科講師 |
| 平成13年 | 千葉大学大学院医学研究院循環病態医科学教授 |
| 平成18–20年 | 千葉大学医学部附属病院副病院長 |
| 平成21年 | 大阪大学大学院医学系研究科循環器内科学教授 |
| 平成24年 | 東京大学大学院医学系研究科循環器内科学教授 |

**受賞歴**

ベルツ賞、米国心臓病学会賞、日本循環器学会賞、国際心臓研究学会賞
持田記念学術賞、高峰譲吉賞など

**研究テーマ**

心不全の病態解明、心臓血管の発生・再生・老化

**所属学会**

日本循環器学会（代表理事）、日本腫瘍循環器学会（代表理事）
日本医学会連合（理事）、日本内科学会（理事）、日本心臓病学会（理事）、日本心不全学会（理事）、日本脈管学会（理事）、日本心血管内分泌代謝学会（理事）、日本循環制御学会（理事）、国際心臓研究学会（理事）、アジア太平洋心臓学会（次期理事長）など

**編集委員**

Journal of Clinical Investigation, Circulation, Cardiovascular Research
Arteriosclerosis, Thrombosis, and Vascular Biology, Circulation Journal
Journal of Molecular Cellular Cardiology, Heart & Vessels
International Heart Journal（editor-in-chief）, Annals of Vascular Diseases
Genes to Cells

特別講演２

# From “sea-snake venoms” to “genetic risk of atherosclerosis”

Robert H Wagner Distinguished Professor,
Department of Pathology and Laboratory Medicine,
University of North Carolina at Chapel Hill
Nobuyo Maeda

When you begin to work in a laboratory for the first time, whether as a technician or as a beginning PhD student, a project assigned to you is somewhat accidental. However, if you find a joy in working on the bench, whether you use cutting edge techniques or mundane repetitive procedures, or ultimate questions are big or small, your experience stays with you throughout your research career. Not that you keep working on the topic forever or use the same techniques over and over, but a thread of scientific thoughts remains with you as you move into new fields. I began my research career in the Department of Chemistry at Tohoku University and my PhD work was on the structure of snake venom toxins. I became fascinated with the amount of information protein and genome sequences contain in their seemingly unimportant parts. This interest led me to a postdoctoral work on molecular evolutions at the University of Wisconsin, Madison. After 3 years, I moved to the laboratory of Oliver Smithies, where I had a most exciting and productive time. An Initial appointment of six month became seven years. Any genes I worked on revealed new information of how genomes evolve. My particular focus was on homologous recombination between repetitive genes because it accelerates genome evolution. At that time, Oliver successfully demonstrated that targeted modification of the mammalian genome using homologous recombination is feasible – the work which led to his receiving a Novel Prize in 2007. Dreaming that this gene targeting would allow me to develop a small animal model for a human complex disease, I picked atherosclerosis because the studies on genetic risk factors for this very common disease were limited at that time. Mice deficient of Apolipoprotein E were born in 1981 at the University of North Carolina where I moved as a Faculty Researcher. The current scientific community emphasizes hypothesis-driven, mechanistic works, but I never liked that aspect of research. Instead, I am still fascinated by the question of how gene variations arise and how the resulting small changes affect the disease of individuals. So I find personal enjoyments in devising novel approaches to model human problems in mice.

Nobuyo Maeda

1) EDUCATION:

Ph.D. (Bio-Organic Chemistry), Tohoku University, Japan, March 1977
M Sc. (Bio-Organic Chemistry), Tohoku University, Japan, March 1974
B.Sc. (Chemistry) Tohoku University, Japan, March 1972

PhD Thesis : Isolation and characterization of neurotoxins from the venoms of sea snakes, and the use of amino acid sequences in taxonomy.

2) POSITIONS HELD:

Robert H Wagner Distinguished Professor, Dept of Pathology and Laboratory Medicine, U of North Carolina at Chapel Hill. 2003-present
Director, Pre-doctoral Training Program for Integrative Vascular Biology. 2002-2015
Adjunct Professor, Dept of Nutrition, U of North Carolina, 2000 - present
Professor, Dept of Path and Lab Med, U of North Carolina, 1996 - present
Associate Professor, Dept of Path and Lab Med, U of North Carolina, 1988 - 1996
Associate Scientist, Laboratory of Genetics. U of Wisconsin, 1986 - 1988.
Assistant Scientist, Laboratory of Genetics. U of Wisconsin, 1983 - 1986.
Research Associate, Laboratory of Genetics. U of Wisconsin, 1981 - 1983.
Research Associate, Physiological Chemistry, U of Wisconsin, 1978 – 1981.
Postdoctoral Fellowship from the Japanese Society for Promotion of Science, Tohoku U., Dept of Chemistry , 1977 - 1978.

3) Representative Publications (Refereed Articles total >320)


- Kakoki M, Bahnson EM, Hagaman JR, Siletzky RM, Grant R, Kayashima Y, Li F, Lee EY, Sun MT, Taylor JM, Rice JC, Almeida MF, Bahr BA, Jennette JC, Smithies O, Maeda-Smithies N. Engulfment and cell motility protein 1 potentiates diabetic cardiomyopathy via Rac-dependent and Rac-independent ROS production. JCI Insight. 2019;4:e127660.
- Tomita H, Zhilicheva S, Kim S, Maeda N. Aortic arch curvature and atherosclerosis have overlapping quantitative trait loci in crosses between 129S6/SvEvTac and C57BL/6J apolipoprotein E-null mice. Circ Res. 2010;106:1052-60.
- Nakata Y, Maeda, N. Vulnerable atherosclerotic plaque morphology in apolipoprotein E-deficient mice unable to make ascorbic acid. Circulation 2002;105:1485-90.
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- Doetschman T, Gregg RG, Maeda N, Hooper ML, Melton DW, Thompson S, Smithies O. Targetted correction of a mutant HPRT gene in mouse embryonic stem cells. Nature 1987;330:576-8.
- Maeda N. Nucleotide sequence of the haptoglobin (Hp) and haptoglobin-related (Hpr) gene pair: Hpr contains a retrovirus-like element. J. Biol. Chem. 1985;260:6698-6709.
- Maeda N, Tamiya N. The primary structure of the toxin Laticauda semifasciata III, a weak and reversibly acting neurotoxin from the venom of a sea snake, Laticauda semifasciata. Biochem. J. 1974;141:389-400.

特別講演３

# マクロファージを用いた難治性呼吸器疾患に対する新規治療戦略

自治医科大学 呼吸器内科 准教授
鈴木　拓児

肺ではリン脂質を主体とした界面活性物質であるサーファクタントが、表面張力を低下させることで肺胞の虚脱を防ぐという生体の呼吸にとって必須の機能を担っている。肺サーファクタントはⅡ型肺胞上皮細胞により産生・分泌・再利用され、その一方で肺胞マクロファージによって取り込まれて処理されることにより、産生と分解のバランスが保たれて恒常性が維持されている。組織マクロファージは定常状態では末梢血由来の造血細胞に依存せずに維持されていることが近年明らかとなり、各臓器には特異的な機能を持つ組織マクロファージを維持する微小環境があることが示唆されている。

肺胞蛋白症は末梢気腔内にサーファクタント由来物質が過剰に貯留し呼吸不全に至る疾患群であり、その大部分はサーファクタントを処理できない肺胞マクロファージが病態の中心である。1958年にRosenらによって肺末梢気腔内に好酸性物質が貯留する疾患として報告され、1960年代には既に全肺洗浄という現在行われている治療法が有効であることが報告されている。しかしながらその病態の解明には40年近い歳月とGranulocyte/macrophage colony stimulating factor（GM-CSF）ノックアウトマウスの解析という基礎研究結果のセレンディピティが必要であった。実際の肺胞蛋白症患者においてはGM-CSF欠乏症ではなく、GM-CSFシグナル伝達の障害は、抗GM-CSF抗体の発見（1999年）やGM-CSF受容体遺伝子変異の発見（2008年）など、いずれも日本人による研究成果によって後に明らかとなった。本講演では、肺サーファクタント恒常性の維持における肺胞マクロファージの役割と疾患、そしてマクロファージを用いた新規細胞治療法についての研究を紹介する。

**鈴木　拓児**
（すずき　たくじ）

**【略歴】**

平成６年 、東北大学医学部卒業後、聖路加国際病院にて研修医、内科医、内科チーフレジデントとして勤務。
平成９年、東北大学大学院医学系研究科入学・東北大学加齢医学研究所呼吸器腫瘍研究分（貫和敏博教授）入局。 東北大学加齢医学研究所附属病院勤務、大学院在学中に東京大学医学部分子予防医学（松島綱治教授）にて免疫学・ケモカインおよび包括的遺伝子発現解析の研究の指導を受け 、平成13年、東北大学大学院医学系研究科卒業（医学博士）。
平成13年より東北大学加齢医学研究所博士研究員、東北大学付属病院遺伝子呼吸器内科医員、助手として勤務。肺癌の遺伝子治療、がん免疫、化学発癌、EGFR遺伝子変異解析などの研究に従事した 。
平成17年よりシンシナティ小児病院医療センター肺生物学分野（Bruce Trapnell 教授）Visiting Research Scientist、Research Fellow、Instructor
平成26年よりシンシナティ大学・ シンシナティ小児病院医療センター肺生物学分野Assistant Professorとして勤務。びまん性肺疾患、肺胞蛋白症など肺稀少疾患および肺胞マクロファージを中心に呼吸器における自然免疫の研究に従事。 現在も兼任。
平成25年より新潟大学医学部非常勤講師（現在も兼任）。
平成29年４月より自治医科大学呼吸器内科（萩原弘一教授）にて准教授として勤務。
平成31年４月より山口大学医学部非常勤講師（兼任）。

**【学会】**

日本内科学会（認定内科医 、総合内科専門医 、指導医）、日本呼吸器学会（呼吸器専門医、指導医、代議員、英文誌編集委員会 委員、細胞・分子生物学学術部会 部会長、学術講演会プログラム委員会 委員）、日本免疫学会、日本癌学会、日本肺癌学会、日本アレルギー学会、日本感染症学会、日本結核病学会、アメリカ胸部疾患学会（ATS）

**【賞罰】**

平成14年　AACR Scholar-in-Training Award（93rd Annual Meeting American Association for Cancer Research,）
平成15年　日本肺癌学会ポスター賞（第44回日本肺癌学会）
平成16年　APSR Best Poster Award（9th Congress of the Asian Pacific Society of Respirology,）
平成17年　RMCB研究奨励賞（第25回Respiratory Molecular Cell Biology）
平成17年　持田記念医学薬学振興財団留学補助金
平成23年　ATS Travel Award（American Thoracic Society 106th International Conference）
平成24年　The annual ATS Assembly on Pediatrics Scientific Abstract Award（American Thoracic Society 107th International Conference）
平成26年　Cincinnati Children’s Hospital Medical Center Trustee Award
平成28年　ATS Neonatal and Developing Lung Interest Group（NDLIG）Award（American Thoracic Society 111th International Conference）
平成30年　アステラス病態代謝研究会優秀発表賞・最優秀理事長賞
平成31年　日本呼吸器学会・熊谷賞

ランチョンセミナー

# 細胞外小胞形成の分子機構

弘前大学農学生命科学部 分子生命科学科 細胞分子生物学分野 准教授
森田　英嗣

細胞は、外界からの刺激に応じて様々な物質を細胞外に放出しており、高等生物ではこの機構は細胞同士のコミュニケーションにおいて重要な役割を持つ。このような分泌の多くは、分泌物を蓄えた輸送小胞が細胞膜と融合し、内容物が外界に放出されることによって行われているが、近年、小胞そのものを放出するという新たな形態の分泌が注目を集めている。このようなエクソソームに代表される『細胞外小胞』には、タンパク質のみならずmicroRNAなど様々な物質が含まれており、新たな細胞間コミュニケーションの手段として脚光を浴びている。

エクソソームに代表される細胞外小胞の多くは、内腔側に多数の小胞を含んだ多胞体（multivesicular body：MVB）と呼ばれる後期エンドソームの一種が、細胞膜と融合することにより生じるとされている。2001年にSundquistらのグループによって、多くのRNAエンベロープウイルスがエクソソーム形成過程を乗っ取ることで、キャプシドを皮膜させ細胞から出芽することが明らかにされて以来、多くの研究者がこの小胞形成過程を解析するようになり、小胞形成の分子機構の理解が飛躍的に進展した。この過程には、酵母のクラスE-VPS遺伝子産物として知られるESCRT（endosomal sorting complex required for transport）複合体が関与しており、機能的に5つのサブ複合体（ESCRT-0～III, VPS4）に分類される因子群によって制御されている。本セミナーではESCRT因子群が関与する細胞外小胞形成の分子機構について最新の情報を交えて解説する。

（Morita, E. *Annu Rev Cell Dev Biol*. 2004, Morita, E. *Cell Host Microbe* 2007, Morita, E. *EMBO J* 2007, Morita, E. *Proc Natl Acad Sci U S A*. 2009, Morita, E. *Cell Host Microbe* 2011, Tabata, K. *Cell reports* 2016）

**森田　英嗣**
（もりた　えいじ）

**略歴：**

1996年　弘前大学 理学部 生物学科卒業

2002年　東北大学大学院 医学系研究科 博士課程（医科学専攻）修了

2000年　日本学術振興会・特別研究員DC2

2002年　東北大学大学院医学系研究科免疫学分野・助手 菅村和夫 研究室

2002年　ユタ大学生化学講座・博士研究員 Wesley I. Sundquist Lab

2009年　大阪大学微生物病研究所細胞制御分野・特任助教 吉森保 研究室

2011年　大阪大学微生物病研究所分子ウイルス分野・助教 松浦善治 研究室

2011年　大阪大学微生物病研究所感染症国際研究センターウイルス研究グループ・特任准教授

2014年　弘前大学農学生命科学部分子生命科学科・准教授

現在に至る

教育講演

# リサーチマインドは臨床医の必須アミノ酸

弘前大学大学院医学研究科 泌尿器科学講座 教授
大山　力

日本専門医機構は専門医制度整備指針の理念として「専門研修の中で、医師としての人格の涵養、患者中心の診療、リサーチマインドの修得などの多面的な学習の視点を保持し、信頼される医療を目的に、初期臨床研修、専門研修、生涯学習へとシームレスな学習課題を設定することが望まれる。」としている。この理念の中で、私が最も重要視したいのは「リサーチマインド」である。初期研修医の多くが大学病院以外での研修を選択する時代になり、若い医師たちが基礎研究に接する機会は激減している。私はこの「リサーチマインド」とは「なぜだろう？どうしてだろう？と疑問に思う」心、そして「もっと良くするにはどうしたらよいのか？」と改善策を探る前向きな心から湧いてくる心だと思う。好奇心・探求心・向上心と言い換えてもよかろう。医師の生涯学習は文字通り医師である限り一生継続するものだが、その精神的バックグラウンドになるものが「リサーチマインド」であろう。

わが身を振り返って幸運だったと思うのは、医師になって３－４年目で基礎研究に接する機会を得たことである。糖脂質研究の世界的権威、シアトルの箱守仙一郎先生のもとで研究に従事した福士泰夫先生が帰国され、米国での研究生活の楽しさを話して下さった。私の基礎研究は糖脂質から始まったが、糖鎖研究の対象分子は、糖タンパク、プロテオグリカンへと推移し徐々に分子量が大きくなっていった。主に癌の転移や免疫と糖鎖との関連に関する研究を行ったが、その経験は私の癌診療の考え方に大きな影響を与えている。

ある日、日常診療で実施している経直腸前立腺生検に疑問を感じた。「患者さんは痛い思いをしている。我々も合併症でつらい思いをしている。その割に検出率が低すぎないか？生検患者を絞り込めないか？」1996年、米国留学中に着想したPSA糖鎖の癌性変異を標的にした新規前立腺癌診断法は、20年以上を経てようやく形を成してきた。

そして、手術支援ロボットに出会えたのも幸運であった。手術支援ロボットは骨に囲まれた狭い空間での手術のつらさを解消してくれた。ロボット支援前立腺全摘除術の次は、当然、膀胱全摘除術である。ロボットを使用した膀胱全摘除術も大変快適であった。そして、自然な流れで回腸新膀胱をロボットで造りたくなった。幸いなことに弘前には初代　舟生富寿先生時代から培ったU字回腸新膀胱があった。多少試行錯誤はあったが、完全腹腔内操作の課題であった総手術時間も５時間30分台になってきた。何より、患者さんの術後の元気の良さと笑顔が心の支えとなった。

基礎研究の経験は自分の診療形態に大きな影響を与え、実臨床でのclinical questionは基礎研究のテーマになっている。基礎研究であれ、臨床研究であれ、リサーチマインドは良医になるための必須アミノ酸だと思う。

プレナリーセッション：Keynote lecture

# Relationships between OCT, ERG and morphology in animal models for retinitis pigmentosa

弘前大学大学院医学研究科 眼科学講座 教授
中澤　満

**Background:** Among the various conditions associated with inherited retinal degeneration, retinitis pigmentosa (RP) is the most common clinical entity and is the second most common cause of the legal blindness in Japan. RP comprises genetically and clinically heterogeneous disease complex. Recent developments in the spectral-domain optical coherence tomography (SD-OCT) technology have provided valuable information regarding structural changes in many retinal diseases. However, the precise pathological background behind certain SD-OCT findings is still unknown because it is not possible to perform retinal biopsy in patients with RP. The aim of this study was to understand the relationship between the findings of SD-OCT of previously reported animal models of RP associated with known genetic mutations and their background structural and functional changes. The result of this study may give us some implications regarding pathological and pathophysiological background for SD-OCT findings of patients with RP.

**Methods:** We reviewed our previous publications reporting the SD-OCT findings of animal models of RP and summarized the characteristic findings in SD-OCT of four different kinds of animal models (Royal College of Surgeons [*RCS*$^{-/-}$] rats, the rhodopsin [*RHO*] P23H transgenic rats, *RHO* S334ter transgenic rats and the retinal pigment epithelium 65 knockout [*Rpe65*$^{-/-}$] mice) of human RP. The SD-OCT and full-field scotopic electroretinography (ERG) were longitudinally recorded to both qualitatively and quantitatively characterize the retinal degeneration of each animal model. The histological (hematoxylin and eosin staining) and electron microscopic appearances were prepared at certain time points of animal models.

**Results:** Despite the various abnormal structural changes found in these different animal models, common SD-OCT findings were summarized as progressive thinning of the outer nuclear layer (ONL) and hyperreflective change in the photoreceptor inner and outer segment (IS-OS) layer. The electron microscopic findings varied depending on genetic abnormalities from mild disarrangement of photoreceptor outer segment discs to severe destruction of the IS-OS. The ERG findings revealed various degrees of disease severity in these animal models.

**Conclusion:** SD-OCT is sensitive enough to detect even mild changes in the photoreceptor OS. Conversely, SD-OCT cannot qualitatively differentiate the pathological and functional differences occurred in the photoreceptors associated with different genetic abnormalities. These findings can be applied in the clinical practice for RP.

シンポジウム：基調講演

# 研究のすすめ

弘前大学大学院医学研究科長
若林　孝一

私自身、研究らしいことを始めて30年以上が経過しました。本講演ではこれまでの研究生活を振り返り、「研究のすすめ」について述べてみたいと思います。

## 1．研究を支えるのは「人」と「時間」

学校（school）や学者（scholar）の語源である「スコレー（scholē）」という古代ギリシャ語は「ヒマ」を意味します。充分に時間をかけて知的好奇心を満たす。そのことが将来何の役に立つかは後の時代や人が決めてくれることです。私の米国留学中の恩師は「その人のペースでやらせること」を重視しました。周りからは「ほうっておく」ように見えるかもしれませんが、発想には効率というものが存在しません。そのためにも、大学院に進んで自分の学問、研究のための時間を作ることです。大学院生や若手研究者を大きく育てるためにも「もっと時間を」と心から思います。

## 2．鉄は熱いうちに打て

科学技術政策研究所の調査によれば、Top10%論文の投稿時年齢で最も割合が高いのが35～39歳です。つまり、若い時期、特に30代中盤までが研究者にとって最も重要な時期であると言えます。私自身は25歳で基礎の大学院に入り、助手になったのが32歳でした。振り返ると最も研究に専念できたのが30代であったと思います。

## 3．Unexpected natureが重要

日本人は周囲と異なることに引け目を感じ、共通したものに安心を覚えます。しかし、研究では意識して他とは異なるもの（オンリーワン）を目指すべきではないかと思います。

## 4．英文論文を書くために

英語で論文を書くために必要なのは、「論理的思考」と「論文としての英語表現」でしょう。そのためにも、研究に没頭できる環境が重要だと思います。

## 5．自らを豊かに

研究によって磨かれた論理的思考は臨床医としてのものの見方を伸ばすことにも通じます。また、新知見を見つけた時の感動は何物にも代えがたいものです。さらに、国際学会や留学の機会を得ることができ、共同研究を通して仲間の輪が広がります。今は少し時間があれば、すぐに海外旅行ができる時代ですが、旅行者と実際に現地に住んでみるのとは大きな違いがあります。留学では学問の背景にある歴史や文化も学ぶことができます。

シンポジウム１

# 臨床講座と基礎講座との融合

弘前大学大学院医学研究科 脳血管病態学講座 教授
今泉　忠淳

医学はサイエンスの一分野であり、医療もサイエンスに基づかなくてはならないことは明らかです。佐藤敬学長は、「すべての医師は科学者でなくてはなりません」と述べています。近年、医療は高度化し、医学研究はボーダーレスになってきており、一人の医師が臨床と研究の両立していくことはなかなか大変です。そのような状況の中で、弘前大学大学院医学研究科における医学研究を推進するには、共同研究の重要性が増してきていると考えます。

医学研究というものは、本来、臨床医学の現場で疑問に思ったことから始まるものと考えます。その疑問を研究・解明していくには、研究のノウハウや技術が必要となります。その際には、基礎医学系講座にコンサルトすることが助けになります。臨床医学の現場で観察されることを、分子や生理の言葉で語ることができれば、その知見は、臨床の現場にフィードバックすることができるかもしれません。

一方、基礎医学系講座で、生命現象に関する新しい仮説を提唱したり、新しい技術を開発したり、新しい遺伝子の機能を見つけたりした場合には、それを臨床医学の研究に応用することにより、その研究の輝きが増します。そこで、臨床医学系講座との共同研究を提案できれば、医学研究科らしい研究を展開できる可能性があります。

弘前大学大学院医学研究科の研究を活発にするには、講座間の敷居を低くし、臨床医学系講座、基礎医学系講座のネットワークを形成し共同研究を促進することが重要と考えます。臨床医学系講座に所属する若い人たちには是非、大学院に進学していただきたいし、大学院生は、必要に応じて、基礎医学系の講座を大いに利用していただきたいと思います。

以上のような観点から、これまで行ってきた、脳血管病態学講座と臨床系講座との共同研究の一部を紹介したいと思います。

## シンポジウム２

# 医学と理工学との融合：成果と課題

弘前大学大学院医学研究科 胸部心臓血管外科学講座 教授
福田　幾夫

心臓血管外科領域では高齢化に伴う疾病構造と形態の変化に対して動物実験モデルの作成は困難である。大動脈疾患では、大動脈内の血流・圧・剪断応力が病態の解明や手術術式の有用性検証に重要である。これらを計測し可視化するためには、モデル実験を欠かすことはできない。動物愛護の観点からも動物実験への制限が加わり、解決法として数理理論モデルによる実験が有用である。実験モデルとしてはCADを用いてこれに理論数式を代入することによるcomputer simulation model、CTや解剖学的観察から作成した実体モデルでの計測実験ある。理論モデルでは実体との乖離が問題となり、この整合性の確認のためには実体モデルでの計測実験やMRIを用いた生体内の血流観察に実験結果をフィードバックすることが重要である。大動脈内の血流の可視化を実体モデルおよび理論モデルで解明した研究を紹介し、今後の課題について検討したい。

シンポジウム３

# 血管・リンパ管網を形成する三次元ヒト生体組織エンジニアリング

弘前大学大学院医学研究科 生体構造医科学講座／神経解剖・細胞組織学講座 教授
下田　浩

iPS細胞の樹立により加速化する再生医療の技術開発において、ヒト細胞を用いた立体臓器・組織の創出と応用が近年つよく求められている。三次元化された生体組織は再生医療への応用のみならず、米国NIHとFDAが進める「Organ on a chip」プロジェクト（創薬・医薬品評価のためのヒト細胞による実験動物代替モデルの開発）に代表されるように新しい医学研究モデルとしても注目されている。ヒト立体組織の開発と応用には高い解剖学的正確性が求められると同時に、その形態形成と機能発現を再現するために組織内に「血管・リンパ管網」を導入することが最も重要な課題とされている。

最近我々の研究グループは、細胞外マトリクスの多層ナノコーティング技術を用いて種々のヒト細胞を三次元組織化する世界に類を見ない細胞集積法による三次元組織構築技術を開発し、様々なヒト組織モデルの創製を行うとともに、新たな創薬・医学研究モデルおよび再生医療材料としての実用性の評価に取り組んでいる。本技術により結合組織内に形成される血管およびリンパ管は、これまでの単層培養された血管・リンパ管内皮細胞の索状構造とは異なり、明瞭な管腔形成を示し、その構造は生体内の血管やリンパ管とほぼ同等の解剖学的特性を有している。さらに、生体内の微小循環系に匹敵する血管・リンパ管のネットワークを構造体内で自発的に構築することも大きな特徴である。本構造体は様々な生理活性因子を産生する微小環境を有し、これを工夫することにより血管・リンパ管網を備えた様々なハイブリッド型ヒト三次元組織構造体の構築が可能である。未だ稚拙で未熟な成績であるが、我々のグループが創出した三次元生体組織とその特徴、ならびに基礎・臨床医学研究および再生医療・創薬応用に向けた研究成果の一部を報告させて頂ければ幸いである。

PL-01

# Inhibition of protease activated receptor-1 suppresses progression of cardiac hypertrophy and fibrosis in renin-overexpressing hypertensive mice

○ Yoshikazu Yokono [1], Masato Narita [1], Yosuke Kawamura [1], Kenji Hanada [1], Michiko Shimada [1], Tomohiro Osanai [2], Hirofumi Tomita [1]

[1] Hirosaki University Graduate School of Medicine Cardiology and Nephrology
[2] Hirosaki University Graduate School of Health Sciences Department of Health Promotion

**Backgrounds:**

Enhancement of the renin-angiotensin system (RAS) causes hypertension and cardiovascular remodeling, which finally leads to heart failure. Recent evidences have demonstrated that coagulation pathway is involved in cardiovascular remodeling induced by RAS. Protease activated receptor-1 (PAR-1) is widely expressed in the vasculature and the heart, and plays important roles in pro-inflammatory process in the cardiovascular system. However, the relationship between RAS activation and PAR-1 signaling remains unknown. Therefore, we tested the hypothesis that inhibition of PAR-1 signaling may have a protective effect on the progression of cardiac remodeling induced by chronic RAS activation in renin-overexpressing hypertensive mice (Ren-Tg).

**Methods & Results:**

We treated 12-16 weeks-old male wild type mice (WT) and Ren-Tg with continuous subcutaneous infusion of PAR-1 antagonist SCH79797 (25mg/kg/day) or vehicle for 4 weeks. Prothrombin fragment 1+2 (F1+2) and factor Xa (FXa) in plasma were greater in Ren-Tg than in WT (F1+2:10.3 ± 3.1 vs 4.7 ± 0.6 ng/ml, FXa: 18.0 ± 3.6 vs 9.9 ± 4.8ng/ml, WT n=4, Ren-Tg n=8, both $p<0.05$). After treatment period, left ventricular wall thickness calculated as interventricular septum plus posterior wall thickness measured by echocardiography was greater in Ren-Tg than in WT (0.25 ± 0.003 vs 0.18 ± 0.002 mm, WT n=4, Ren-Tg n=6, $p<0.05$), and SCH79797 attenuated the increase to 0.22 ± 0.01 mm in Ren-Tg (n=6, $p<0.05$ vs Ren-Tg without SCH79797). The ratio of heart weight to body weight was greater in Ren-Tg than in WT (6.1 ± 0.4 vs 4.6 ± 0.7 mg/g, WT n=4, Ren-Tg n=6, $p<0.05$), and SCH79797 attenuated the increase to 5.2 ± 0.1 mg/g (n=6, $p<0.05$). The area of cardiac fibrosis evaluated by Masson-trichrome staining was greater in Ren-Tg than in WT (2.6 ± 0.2 vs 1.4 ± 0.3%, n=3, $p<0.05$), and SCH79797 attenuated it to 1.6 ± 0.3% in Ren-Tg (n=3, both $p<0.05$). Cardiac mRNA expressions of TNF-$\alpha$, TGF-$\beta$1, COL3A1 and $\beta$-MHC were all greater in Ren-Tg than in WT, and SCH79797 attenuated their increases in Ren-Tg (all $p<0.05$). In isolated cardiac fibroblast, thrombin and FXa enhanced ERK1/2 phosphorylation and SCH79797 attenuated it (both $p< 0.05$).

**Conclusions:**

Inhibition of PAR-1 signaling attenuates cardiac hypertrophy and fibrosis in Ren-Tg via inhibition of inflammatory cytokine production. These results support the involvement of PAR signaling in the development of heart failure induced by RAS, and may provide novel therapeutic target for the treatment of hypertensive heart failure.

PL-02

# Activated Macrophage via RAGE Signaling Induces Insulin Resistance and Deficit of Retrograde Axonal Transport in Diabetic Polyneuropathy

○ Sho Osonoi [1)], Hiroki Mizukami [1)], Rikuo Shindo [1)], Takanori Sasaki [1)], Kazuhisa Takahashi [1)], Saori Ogasawara [1)], Kazunori Sango [2)], Yasuhiko Yamamoto [3)], Hiroshi Yamamoto [3)], Soroku Yagihashi [1)]

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[3)] Department of Biochemistry and Molecular Vascular Biology, Kanazawa University Graduate School of Medical Science

**Background:** Diabetic polyneuropathy (DPN) is one of the most prevalent complications of diabetes. While inflammation is one of its multifactorial pathophysiology, the detailed mechanism is not fully understood. Advanced glycation end products (AGEs) signaling via its receptor RAGE activates macrophage (Mφ) and evokes local inflammation in diabetic complications. Infiltration of proinflammatory Mφ (M1) activates TNFα-JNK pathway and causes insulin resistance (IR) in adipose tissue. These suggest that activation of RAGE in Mφ may similarly increase IR in DPN through the defect of retrograde axonal transport (RAT), which is partially regulated by IR. The aim of this study is to clarify the role of RAGE and Mφ in the nerve inflammation of DPN and their relationships to IR and RAT.

**Methods:** C57BL/6 mice (W) and RAGE null mice (R) were rendered diabetic with streprozotocin (STZ) (D). After 8 weeks, DPN was confirmed by delay of nerve conduction velocities (NCVs). Infiltration of M1 in sciatic nerve (SN) was determined by immunofluorescence (IF). Western blotting for phosphorylated (p)-AKT and p-JNK was performed to evaluate IR of SN with insulin stimulation. For RAT, dorsal root ganglia (DRG) was quantitatively evaluated by IF for Fluoro-Gold (FG) 7 days after subcutaneous injection of FG into the hindlimb footpad. Cell size of DRG neurons was morphometrically measured. To evaluate the dynamic RAT, primary cultures of mice DRG neurons were used. Neurons were exposed to LysoTracker to label intra-axonal acidic organelles with fluorescence and treated with TNFαor PBS(control). Transported movements of organelles were captured by time-lapse microscopy. IF for p-JNK was performed on neurons.

**Results:** NCVs were significantly delayed in WD ($p<0.01$, vs W), while maintained in RD. The number of infiltrating M1 was significantly more in SN of WD ($p<0.05$, vs W and RD). decreased p-AKT and elevated p-JNK expressions were seen in SN of WD, but not in RD. The frequency of transported FG positive neurons in DRG was significantly decreased in WD ($p<0.05$, vs W), while maintained in RD. Cell size of the DRG neuron was significantly decreased in WD ($p<0.05$, vs W), while maintained in RD. In real time observation in vitro, we observed dynamic transportations of intra-axonal organelles, which were divided into four categories (static, anterograde, retrograde and bidirectional). The movements were mainly retrograde in control but they were static or attenuated in axons of TNFα treated neurons. Axons of TNFα treated neurons showed an intensified signal for p-JNK in IF.

**Conclusion:** RAGE evoked inflammation in SN with M1 infiltration, which associated with IR and RAT deficit. These changes may contribute to DRG neuronal atrophy and progression of DPN.

PL-03

# Adult onset-multiple system atrophy model mice

○ Kunikazu Tanji [1)], Yasuo Miki [1)], Fumiaki Mori [1)], Yoshikazu Nikaido [2)], Hidemi Narita [1,3)], Koichi Wakabayashi [1)]

[1)] Department of Neuropathology, Institute of Brain Science, Hirosaki University Graduate School of Medicine.
[2)] Department of Anesthesiology, Hirosaki University Graduate School of Medicine.
[3)] Department of Rehabilitation Sciences, School of Health Sciences, Hirosaki University of Health and Welfare.

**Background:**

Multiple system atrophy (MSA) is an adult-onset neurodegenerative disorder characterized by various degrees of symptoms of parkinsonism, cerebellar ataxia, and autonomic failure. There seems to be approximately 12,000 patients in Japan. Pathological studies revealed that glial cytoplasmic inclusions (GCIs) were present in oligodendrocytes in the brain of the patients. Further neuropathological examination demonstrated that α-synuclein (Syn) is a major component of GCIs. Based on these findings, several MSA models, in particular genetically modified MSA mouse models, have generated, and have provided substantial contributions to what is currently known about MSA. Although relevant transgenic (Tg) model mice manifest MSA pathologies like GCIs, phosphorylated Syn deposits and abnormally aggregated Syn, Syn overexpression during the developmental stage could induce a latent developmental disturbance or compensatory effects within cells and tissues. More importantly, given that MSA is an adult-onset disease, it would be most ideal for Syn expression to increase in adulthood in model animals. To this end, we generated a new MSA model mouse by the Cre-loxP system technique.

**Methods:**

We first generated mice harboring a loxP-flanked (flox) controlled Syn gene. After these mice were successfully generated, they were mated with mice in which Cre/estrogen receptor (ER) was specifically expressed under the proteolipid protein (plp) promoter in oligodendrocytes (MSA model mice). After confirmation that abnormal Syn inclusions deposit, pathological, biochemical, and behavioral analyses were also performed in resultant MSA model mice.

**Results:**

When we injected tamoxifen into resulting MSA model mice, we confirmed that these mice exhibited numerous Syn inclusions in their oligodendrocytes in a manner resembling that of GCIs in human MSA patients. Additionally, the MSA model mice replicate some biochemical and clinical features of MSA patients. For instance, a filter-trap assay showed that lysates in the higher-density fractions of MSA model mice were labeled by an antibody for phosphorylated Syn, as shown in human MSA patients. Furthermore, rotarod tests revealed that significant deficits in motor coordination and balance in the MSA model mice at longer time points after Syn induction.

**Conclusion:**

Our MSA model offers a unique approach to advancing our understanding of the mechanisms underlying the initial disease progression and developing novel therapeutics for MSA.

PL-04

# Anti-inflammatory effect of transplantation of multilineage differentiating Stress Enduring cell for spinal cord injury

○ Toshihide Nagaoki, Gentaro Kumagai, Kenya Saruta, Toru Asari, Sunao Tanaka, Kanichiro Wada, Yasuyuki Ishibashi

Department of Orthopedic Surgery, Hirosaki University Graduate School of Medicine

**Background:** Transplantation of Mesenchymal stem cell (MSC) was initiated as a treatment for spinal cord injury (SCI). Multilineage differentiating Stress Enduring (Muse) cell is a rare pluripotent subpopulation within MSC and positive for SSEA-3 as known as a pluripotent marker. The effect of Muse cell transplantation for SCI is unclear. The purpose of this study is to investigate the anti-inflammatory effect of Muse cells for SCI.

**Methods:** MSC were cultured from mice inguinal adipose tissue. MSCs were separated into Muse cell (SSEA-3+) and Non-Muse cell (SSEA-3−) using flow cytometry. Neurotrophic factor (brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF)) was measured by analyzing the culture medium of Muse cell and Non-Muse cell with ELIZA (n=5). SCI was induced at the T10 level of female mice (C57BL/6, 8 weeks old). One week after SCI, Muse cells and Non-Muse cell were transplanted intralesionally. One week after transplantation, mice were sacrificed and the spinal cord was dissected. To evaluate Anti-inflammatory effect, M1 macrophages (inflammation marker, CD86) and M2 macrophages (anti-inflammation marker, CD206) were measured (n=6). Low M1 / M2 ratio means the anti-inflammatory effect is high.

**Results:** The amount of secretion in Muse cell were 269 ± 26.9 (BDNF pg/ml), 1479 ± 16.6 (VEGF) and 6590 ± 2106 (HGF). The amount of secretion in Non-Muse cell were 134 ± 8.6 (BDNF), 1160 ± 42.9 (VEGF), 2382 ± 935.2 (HGF). All neurotrophic factors were secreted significantly more in Muse cells than in Non-Muse cells ($p<0.05$). Muse cells had significantly lower the M1 / M2 ratio than non-Muse cells (Muse 0.31, Non-Muse 2.77, $p<0.05$).

**Discussion:** MSC secreted neurotrophic factors and attenuated inflammation in vitro and in vivo previously. In this study, Muse cells secreted more neurotrophic factors and attenuated inflammation in the injured spinal cord compared with Non-Muse cells.

**Conclusion:** Muse cells had higher anti-inflammatory effects than non-Muse cells both in vitro and in vivo. Muse cells may be useful as a transplantation source for SCI.

PL-05

# Deficiency of p62 exacerbates doxorubicin-induced cardiac dysfunction

○ Michiko Tsushima [1)], Junsei Mimura [1)], Liu Jun [1)], Hiromi Yamazaki [1)], Shuya Kasai [1)], Hirofumi Tomita [2)], Ken Ito [1)]

[1)] Department of Stress Response Science, Center for Advanced Medical Research, Hirosaki University Graduate School of Medicine
[2)] Department of Cardiology and Nephrology, Hirosaki University Graduate School of Medicine

**Background:** An anticancer drug, doxorubicin (Dox) is known to induce chronic cardiotoxicity and heart failure. p62/SQSTM1 is a ubiquitin-binding protein, which plays an important role in selective autophagy and mitophagy. Although Dox is known to induce p62, the impact of p62 on Dox-induced cardiotoxicity remains obscure.
**Methods:** For *in vitro* study, H9c2 rat myoblast cells were used, and p62 knockdown experiments were performed by using siRNA. H9c2 cells were treated with 1 μM Dox for 6 or 24 h depending on experiments. H9c2 cell viability was analyzed by using CCK-8 kit, respectively. Apoptosis was evaluated by immunoblotting of cleaved caspase-3. For *in vivo* study, wild-type (WT) or p62 knockout ($p62^{-/-}$) mice were given saline or Dox (5 mg/kg BW by weekly (4 times), i.p., 20 mg/kg cumulative dose). At 4 weeks after last injection, cardiac function was evaluated by echocardiography, and cardiac gene expression and histological analysis were performed.
**Results:** In H9c2 cells, Dox-induced cell death was significantly increased in p62 knockdown compared to control (54.1 ± 8.8 vs 39.9 ± 4.8 %, $p<0.01$). p62 knockdown also increased Dox-induced apoptosis in H9c2 cells. In Dox-treated $p62^{-/-}$ mice, exacerbated left ventricular fractional shortening was observed compared with WT mice (46.5 ± 2.2 vs 49.8 ± 1.6%, $p<0.05$). Although, gene expression of fibrosis marker (Col1a1, TGF-$\beta$) and heart congestion marker (Nppa, Nppb) in hearts was not significantly different between $p62^{-/-}$ and WT mice, down regulation of Nrf2-mediated antioxidant system in $p62^{-/-}$ hearts was observed.
**Conclusion:** Our results indicate that p62 has a protective effect against Dox-induced cardiotoxicity by preventing apoptosis. Deficiency of p62 in mice exacerbates Dox-induced LV contractile dysfunction suggesting that p62 is a promising therapeutic target for Dox-induced cardiotoxicity.

PL-06

# Identification of a novel autophagy receptor that regulates intracellular bacteria proliferation

○ Keiya Uriu [1)], Masashi Arakawa [1)], Mai Hirose [1)], Krisana Asano [2)], Akio Nakane [2)], Eiji Morita [1)]

[1)] Department of Biochemistry and Molecular Biology, Faculty of Agriculture and Life Science, Hirosaki University.

[2)] Department of Microbiology and Immunology, Hirosaki University Graduate School of Medicine

**Background:** Most of the cytoplasm-invasive bacteria, such as *Salmonella* and *Group A streptococcus (GAS)* can be recognized by cellular autophagic machinery and degraded after delivering to the lysosome. This selective autophagic machinery, called Xenophagy, is known as one of the cellular innate immune systems that has a role in the self-defense against for the bacterial infection. For the target recognition in selective autophagy, receptor model has been proposed, based on the idea that LIR (LC3 interacting region) containing receptors, such as p62, NDP52, and OPTN, recognize and bridge both targets and LC3 on the autophagic isolation membrane. However, it has been known that none of the known receptors has critical roles in the elimination of the invaded bacteria, suggesting the existence of other unknown receptors. In this study, we performed quantitative IP-mass spectrometry analysis using LC3 mutants that modified its binding affinity to LIR, and identified novel LIR containing LC3 binding protein, named LIRP8, and we clarified its role in Xenophagy.

**Methods:** We prepared a series of truncated mutants of LIRP8 and identified LIR that have roles in the direct binding to the LC3. Subcellular localization of LIRP8, LC3, and Galectin-3, a marker of the ruptured membrane, was tested in bacteria-infected cells or membrane rupture inducing reagent, LLOMe, treated cells. Time-lapse imaging was performed to measure the duration period required for the removal of damaged membranes using LIRP8 knockout cells. Gentamycin protection assay was performed to measure the growth rate of the intracellular *Salmonella* in these cells.

**Results & Conclusion:** LIRP8 was recruited to the LC3 positive compartment seen around membrane rupture site that induced by LLOMe or bacteria infection. One of four putative LIR was essential for the binding to LC3 and co-localization with LC3. LIRP8 recruitment was seen in a series of ATG knockout cells, suggesting that that LIRP8 function in upstream of the autophagosome formation. Intracellular bacterial growth was up-regulated, and duration period required for the removal of the damaged membrane was extended in LIRP8 KO cells, suggesting that LIRP8 function as a novel autophagy receptor that recognizes membrane rupture site and has a critical role in the removal of invaded bacteria.

# ポスターセッション

## 13:50〜14:20　奇数の演題番号

**PO-01** Involvement of Neurodegenerative and Neuroinflammatory Processes in the Pathogenesis of Postoperative Delirium in Elderly Mice
Yoshikazu Nikaido

**PO-03** Phosphorylated TDP-43 aggregates in skeletal and cardiac muscles are not specific to amyotrophic lateral sclerosis
Fumiaki Mori

**PO-05** Simultaneous detection of two single-nucleotide mutations corresponding to the T790M and L858R mutations in the EGFR gene by oligoribonucleotide interference-PCR.
Keisuke Baba

**PO-07** Diabetic state enhance MHC Class I expression on breast cancer.
Kazuhiro Kudoh

**PO-09** Development of human parvovirus B19 virus-like particle (VLP) vaccine
Sakika Kimura

**PO-11** Histamine release from intestinal mast cells induced by staphylococcal enterotoxin A (SEA) evokes vomiting reflex in common marmoset
Hisaya K Ono

**PO-13** An Optical Coherence Tomographic Analysis of RDH5-/- Mice Retina
Yuting Xie

**PO-15** Prenatal Heavy Metals Exposure and Newborn Leucocyte TL in Relationship with the Modifying Effects of Selenium
Kyi Mar Wai

**PO-17** Phospholipase C-related inactive protein type-1 deficiency affects anesthetic electroencephalogram activity induced by propofol and etomidate in mice
Tomonori Furukawa

**PO-19** Effect of glycosaminoglycans on retinoic acid-induced neural differentiation of P19 embryonal carcinoma cells
Ikuko Kakizaki

**PO-21** Morphological analysis of peritoneal dissemination of ovarian cancer based on levels of carbonyl reductase 1 expression
Fumie Oyama

**PO-23** The elucidation of transcriptional regulation of *Tspo* gene
Shuji Shimoyama

**PO-25** Body weight loss in postnatal GCN1L1 knockout mice
Liu Jun

**PO-27** Vitamin A deficiency impairs host resistance to *Listeria monocytogenes* infection through excessive apoptosis of macrophages.
Nahoko Tanaka

**PO-29** Goblet cells are involved in translocation of staphylococcal enterotoxin A in the intestinal tissue of house musk shrew (Suncus murinus)
Shouhei Hirose

## 14:20〜14:50　偶数の演題番号

**PO-02** Stimulation of CD11c positive dendritic cells by TSLP in animal model of eosinophilic otitis media.
Ryutaro Hara

**PO-04** ER-associated degradation (ERAD) machinery controls viral-protein homeostasis that is essential for the flavivirus propagation
Kotaro Ishida

**PO-06** Development of neural network system for case-finding of cancer patients using medical accounting ICD-10 code
Masashi Matsuzaka

**PO-08** Development of new therapeutic agents of preterm birth by glycosaminoglycan chain remodeling of urinary trypsin inhibitor
Seigo Tanaka

**PO-10** Transrational study between pancreatic cancer stroma and clinical image
Shintaro Goto

**PO-12** Aortic valve calcification via each inflammatory and non-inflammatory signaling
Zaiqiang Yu

**PO-14** Ability of uterine NK cell cytokine production of unexplained RPL women by the stimulation using semen
Ayako Taima

**PO-16** Excess glutamate may cause dilation of retinal blood vessels in Glutamate/Aspartate transporter-deficient Mice.
Takayuki Gonome

**PO-18** Food intake behavior for individuals with specific IgE (sIgE) positive
Mina Misawa

**PO-20** Melanoma differentiation-associated gene 5 positively modulates TNF-$\alpha$-induced CXCL10 expression in cultured HuH-7 and HLE cells
Shogo Kawaguchi

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Shinichiro Suto

PO-01

# Involvement of Neurodegenerative and Neuroinflammatory Processes in the Pathogenesis of Postoperative Delirium in Elderly Mice

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**Background:** Postoperative delirium (POD) after major surgery produces acute cognitive decline and various abnormal behaviors in elderly patients. Although several neurophysiological, cerebrovascular and inflammatory mechanisms of development of POD have been proposed, the pathogenesis of POD remains unknown. In this study, we examined the effects of abdominal surgery on postoperative diurnal activity and cognitive behavior of aged mice. We also performed a proteomic analysis in order to reveal what kinds of protein were enriched by the onset of POD-like behavioral changes.
**Methods:** Aged C57BL/6J mice were subjected to laparotomy under isoflurane anesthesia. Sham-operated aged mice were only anesthetized by isoflurane. After emergence from the anesthesia, each animal was kept in a single-housed homecage to assess diurnal activity. Cognitive behavior in open-field tests and y-maze tasks were examined at 12, 24, 36 and 48 hr after the surgery. Following the last behavioral tests, mouse hippocampal samples were collected to perform proteomic analysis with sequential window acquisition of all theoretical fragment ion spectra mass spectrometry (SWATH-MS).
**Results:** Laparotomized aged mice showed less moving in the homecage than sham-operated aged mice during 24-36 hr after the surgery. In the y-maze tasks, operated mice exhibited a deficit of spontaneous alternation at 36 hr after the surgery. There were no behavioral differences between animals in the open field tests. SWATH-MS and following proteomic analysis inferred that enriched hippocampal proteins of laparotomized aged mice were related to Huntington's and Parkinson's diseases and inflammation pathways.
**Conclusion:** Aged mice showed POD-like acute declines in spatial cognition and diurnal activity after abdominal surgery. These behavioral changes might be partly attributed by abnormal hippocampal protein profiles involved in neurodegeneration and neuroinflammation.

PO-02

# Stimulation of CD11c positive dendritic cells by TSLP in animal model of eosinophilic otitis media.

○ Ryutaro Hara, Naomi Kudoh, Atsushi Matsubara

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**Background:** Th2-type allergic inflammation, such as bronchial asthma (BA), has been considered consequence of acquired immune response by inhaled specific antigen. However, more and more attention has recently been paid to the aspect of innate immunity and the role of Group 2 innate lymphoid cells (ILC2s). Furthermore, it has been revealed that epithelial derived cytokines such as thymic stromal lymphopoietin (TSLP), IL-25, IL-33 are initiator of Th-2 allergic reaction in BA. Eosinophilic otitis media (EOM) is an intractable otitis media which is known as Th2-type eosinophilic inflammatory disease similar to BA. Our group conducted model animal of EOM and has been continued pathophysiological studies of EOM. We have reported that TSLP immunopositive cells were found in submucosal area of middle ear epithelium in animal model of EOM. CD11c positive dendrtitic cells (DCs) are activated by TSLP and activate ILC2s in turn. that recognize foreign antigens and induce an immune response. In the present study, we explored distribution and co-localization of DCs and TSLP receptor (TSLPR) immunopositive cells using animal model of EOM.

**Methods:** After three times weekly intraperitoneal injection of OVA to Hartley guinea pigs as general sensitization, daily application of intratympanic OVA stimulation to the right ear was performed for 7 days and for 14 days. Saline was injected to the left ear and used as control. Immunostaining and double immunofluorescent staining for CD11c and TSLPR were performed. The sections were observed to detect the immunopositive cells and their distribution.

**Results:** We found CD11c immunopositive cells in the submucosal area of the middle ear epithelium, particularly in the submucosal area around eustachian tube. The number of CD11c positive cells was significantly larger than control side. Immunopositive cells for TSLPR showed similar distribution with CD11c positive DCs.

**Conclusion:** We suggest that TSLP stimulates CD11c positive DCs in the submucosal area of the middle ear epithelium in OVA stimulated side, and DCs stimulated by TSLP induced Th2-type immunoreaction in animal model of EOM.

PO-03

# Phosphorylated TDP-43 aggregates in skeletal and cardiac muscles are not specific to amyotrophic lateral sclerosis

○ Fumiaki Mori [1)], Mari Tada [2)], Tomoya Kon [3)], Yasuo Miki [1)], Kunikazu Tanji [1)], Hidekachi Kurotaki [4)], Masahiko Tomiyama [3)], Akiyoshi Kakita [2)], Koichi Wakabayashi [1)]

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**Background:** Amyotrophic lateral sclerosis (ALS) is characterized pathologically by the occurrence of phosphorylated TDP-43 (pTDP-43)-immunoreactive neuronal and glial inclusions in the central nervous system. Recent studies have shown that pTDP-43 aggregates also occur in the skeletal muscles in a certain proportion of ALS patients. The aim of this study was to clarify the distribution and incidence of pTDP-43 aggregates in the skeletal and cardiac muscles of patients with ALS, and also those of patients with muscular and non-muscular diseases.

**Material and methods:** Five regions of muscle (tongue, cervical muscle, diaphragm, iliopsoas muscle and heart) were examined histologically and immunohistochemically in patients with ALS (n = 30), muscular diseases (n = 10) and non-muscular diseases (n = 10).

**Results:** Two types of pTDP-43 aggregates were distinguishable morphologically: dense filamentous and short linear inclusions. These inclusions were found in at least one of the five muscle regions in all 30 cases of ALS; skeletal muscles in 28 cases and myocardium in 12. pTDP-43 aggregates were also found in 7 of 10 patients with muscular diseases, including myositis, muscular dystrophy and mitochondrial myopathy, as well as in 5 of 10 patients with non-muscular diseases. In ALS, pTDP-43 aggregates were most frequent in the diaphragm (19 cases). In contiguous sections stained with hematoxylin and eosin and anti-pTDP-43, muscle fibers with dense filamentous inclusions demonstrated single-fiber atrophy with vacuolar degeneration.

**Conclusion:** The present findings indicate that pTDP-43 aggregates in skeletal and cardiac muscles are not specific to ALS and that the phenomenon occurs in various conditions irrespective of whether muscular disease is present. pTDP-43 aggregates are associated with myogenic degeneration of skeletal and cardiac muscles.

PO-04

# ER-associated degradation (ERAD) machinery controls viral-protein homeostasis that is essential for the flavivirus propagation

○ Kotaro Ishida [1], Masashi Arakawa [1], Keisuke Tabata [2], Ryosuke Suzuki [3], Takehiro Sugimoto [4], Tetsuya Okada [4], Kazutoshi Mori [4], Eiji Morita [1]

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**Background:** The infection of pathogenic flavivirus, such as dengue virus (DENV), Zika virus (ZIKV), and Japanese encephalitis virus (JEV), induces reconstitution of ER membrane to create a specific compartment, known as the viral-replication organelle, which is considered as a place for efficient viral genome replication and virion assembly. In this research, we performed extensive proteome analysis and identified host factors involved in the replication organelle biogenesis. Furthermore, we focus on the function of the identified protein: valosin-containing protein (VCP), and clarified the role of VCP mediated ER-associated degradation (ERAD) machinery in the viral life cycle.
**Methods:** We performed several different mass spectrometry analyses for biochemically purified replication organelle. To investigate the role of VCP and ERAD pathway, we prepared, Derlin1, Derlin2, or SEL1L, knockout cells by genome editing, or VCP knockdown cells by siRNA treatment, and tested the abilities of virus propagation in these cells. To test the possibility that the viral proteins can be the targets for the ERAD pathway, we performed cycloheximide (CHX) chase assays, and measured stabilities of each viral protein. We also investigated the localization of these host factors in flavivirus infected cells.
**Results&Conclusion:** JEV replication was significantly impaired in VCP, Derlin2, SEL1L deficient cells, but not in Derlin1 deficient cells, suggesting that Derlin2/Sel1L mediated specific ERAD pathway is involved in flavivirus life-cycle. CHX chase assay revealed that the stabilities of each viral protein were varied. The treatment of proteasome inhibitor blocks the degradation of unstable viral non-structural (NS) proteins, suggesting that this process is driven by ERAD pathway. Unstable NS proteins, as well as ERAD factors, localize at the convoluted membrane (CM) of replication organelle, suggesting that CM is the place for the NS-proteins degradation. Flaviviruses genome encodes single-polypeptide, and all viral proteins are matured from it. For these viruses, probably controlling the degradation, but not the production, is the only way to maintain the viral protein homeostasis that is essential for the viral propagation.

PO-05

# Simultaneous detection of two single-nucleotide mutations corresponding to the T790M and L858R mutations in the EGFR gene by oligoribonucleotide interference-PCR.

○ Keisuke Baba [1,2], Toshitsugu Fujita [1], Sadatomo Tasaka [2], Hodaka Fujii [1]

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**Background:**

Various gene mutations causing carcinogenesis have been known, and treatments specific to such mutations have been developed. For example, a *de novo* single-nucleotide mutation in the *EGFR* gene can cause the development of lung cancer. EGFR tyrosine kinase inhibitors (EGFR-TKIs) are used for clinical treatment of such lung cancers, but acquired resistance often mitigates their efficacy. Accordingly, monitoring of *de novo* and acquired nucleotide mutations is essential for clinical treatment of lung cancers with EGFR-TKIs. Previously, we reported that oligoribonucleotide interference-PCR (ORNi-PCR) can accurately and cost-effectively detect single-nucleotide mutations. ORN is a short ribonucleic acid and enables selective amplification of mutated genes by blocking amplification of wild-type alleles matching ORN sequences.

**Methods:**

Here, we applied ORNi-PCR to simultaneous detection of two single-nucleotide mutations corresponding to the *de novo* L858R mutation and the T790M mutation as acquired in the *EGFR* gene in lung cancer cells. Genomic DNA (gDNA) and complementary DNA (cDNA) were extracted from lung cancer cell lines (e.g. NCI-H1975), diluted, and used for ORNi-PCR.

**Results:**

First, we established optimal experimental conditions for ORNi-PCR to simultaneously detect the two mutations from gDNA extracted from lung cancer cells. The established conditions were also applied to ORNi-PCR using cDNA reverse-transcribed from extracted RNA. In addition, we found that ORNi-PCR can detect lung cancer cells possessing the two mutations mixed with a large number of cells harboring wild-type sequences even when the cancer cells constitute only 0.2% of the total cell number.

**Conclusion:**

ORNi-PCR was proved to be a useful method for simultaneous detection of L858R and T790M mutations in clinical examination. The sensitivity of ORNi-PCR is comparable to that of the Peptide Nucleic Acid (PNA) - Locked Nucleic Acid (LNA) clamp PCR method. In addition, cost of ORNi-PCR is less than one tenth of that of PNA-LNA clamp PCR. It is also important to show that the established method can be applied to patients' clinical samples, and such studies are undergoing.

PO-06

# Development of neural network system for case-finding of cancer patients using medical accounting ICD-10 code

○ Masashi Matsuzaka [1,2], Rina Tanaka [3], Yoshihiro Sasaki [1,3], Hirotake Sakuraba [4], Shinsaku Fukuda [4]

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**Background:** Hospital-based cancer registry is the project to find cancer patients from all patients visiting the hospital without omission and to register medical information about cancers with a database. Case-finding of cancer patients is time-consuming because it relies on man power without established algorithmic procedure. Although artificial intelligence, e.g. machine learning and deep learning, has been applied in a variety of fields, remarkable results have not been achieved yet in the field of medical services. The aim of this study was to construct a neural network (NN) system, which is a subset of machine learning, for the case-finding by using medical accounting data or 10th edition of international classification of disease (ICD-10) code, which is assigned to all patients visiting the hospital. The NN system would save man power in case-finding without omission.

**Methods:** Hospital ID and disease name coded into ICD-10 assigned to patients visiting Hirosaki University Hospital from 2016 to 2018 were extracted from AppLink medical accounting database. Hospital ID of cancer patients in the same periods were obtained from the hospital cancer registry database. The cancer patients from AppLink database were labelled as 1 and the other as 0 with reference to the cancer registry data. Four layer NN system with input layer dimension 7609 and output layer 2 (0 or 1) was trained using the labelled data set of 2016 and 2017, and tested by 2018 data set as validation.

**Results:** Characteristics of the cancer patients were well balanced in 2016, 2017 and 2018. Digestive organs were the most common site of cancers, and respiratory system were the second. Origins of the cancer were also well balanced in the observed period. In AppLink data, neoplasms were the most common category of diseases, and diseases of digestive system were the second. The trained NN system gives a probability of cancers from 0 to 1 in each patient characterised by assigned ICD-10 codes. Area under the ROC curve recognising the probability as a predictive indicator was 0.986 (sensitivity 0.98, specificity 0.95). The optimal cut-off point of the probability derived from the ROC curve was 0.073. Given that the optimal cut-off point was accepted, the sensitivity was 0.74 and the specificity was 0.92 in 2018 data.

**Conclusion:** The accuracy of the trained system was very high in the training data. A predictive ability of the algorithm was also performing well in the validation data. Decrease in the sensitivity observed when the cut-off point optimised in the training data was applied to validation data would be caused by fluctuation of disease name assignment. For more accurate NN system for case-finding, other data, e.g. medical lab results, may well be added to the input layer.

PO-07

# Diabetic state enhance MHC Class I expression on breast cancer.

○ Kazuhiro Kudoh, Takanori Sasaki, Syo Osonoi, Hiroki Mizukami

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**Background**: Immune check point inhibitor is a new type drug for anti-cancer therapy. Anti-programmed cell death-1(PD-1) antibody can stimulate cytotoxic T-cell mediated immune response to cancer cells expressing surface MHC Class I. To avoid this attack, cancer cells downregulate MHC Class I resulting in escaping from the immune response of T-cells.

Incidence of diabetes mellitus (DM) is increasing worldwide. It is well-known that DM can suppress the host immune defence. Therefore DM may change the host immune response to cancer cells, while there are few reports of relationship between DM and cancer immunity. The aim of this research is to clarify whether DM modulates the expression of MHC class I of BC cells.

**Methods:** 110 cases of invasive carcinoma of no special type (NST) were examined. We devided these cases into two groups based on HbA1c (cut off value 6.5%).

Immunohistochemistry was performed with MHC Class I (EMR8-5, Abcam), and pNF-$\kappa$B (sc-101749, Santa Cruz) antibody. We assessed intensity and percentage of positive tumor cells. We multiplied intensity and percentage of cells stained at each intensity. We summed each values according to H-score ($\Sigma$ [intensity * percentage]) of previous report. We defined total sum as HLA score or pNF-kB score.

We also evaluated stromal tumor infiltrating lymphocytes (sTILs) of breast cancer by H&E section.

**Results:** HLA score was significantly higher in HbA1c-High group (average 175) than HbA1c-Low group (average 90) ($p<0.01$). Weak correlation between fed blood sugar (mg/dL) and HLA score ($p<0.01$, $r=0.338$), fed BS and pNF-kB score ($p<0.01$, $r=0.292$), HLA score and pNF-kB score ($p=0.02$, $r=0.238$), and HLA score and sTIL($p=0.04$, $r=0.221$).

**Conclusion:** Diabetic state sugar enhance the expression of MHC Class I in BC cells. The mechanism of the enhancement can be mediated by the upregulation of the phosphorylated NF-kB. These changes may increase therapeutic effects of immune check point inhibitors in BC cells with DM.

PO-08

# Development of new therapeutic agents of preterm birth by glycosaminoglycan chain remodeling of urinary trypsin inhibitor

○ Seigo Tanaka [1)], Ikuko Kakizaki [2)], Kanji Tanaka [1)], Tomoe Kodama [1)], Asami Ito-Fukuyama [1)], Shinichiro Suto [2)], Ryoki Takahashi [3)], Yoshihito Yokoyama [1)]

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**Background:** Preterm birth is estimated to annually affect 12,900,000 births or 9.7% of all births of the world. The prognosis of preterm infants has been improved by advances in neonatal medical care, however, preterm birth is still one of main causes of perinatal death. The establishment of adequate care for preterm birth is a problem that should be resolved immediately in perinatal medical care.

Urinary trypsin inhibitor (UTI) suppresses proteases, such as elastase, and IL-8 release. It is known to control cervix inflammation and cervical ripening. Although UTI is used as a vaginal preterm birth therapeutic drug in many clinical settings, the preterm birth protective efficacy of UTI has not been yet proved. UTI is a protective biological substance found in fetal urine and in large quantities in amniotic fluid. It is a small proteoglycan with a low-sulfated chondroitin 4-sulfate chain that binds to the tenth serine residue of a core protein as a glycosaminoglycan (GAG) moiety via the linkage region. The function of UTI is based on the protease inhibitor activity of its core protein structure. However, the structure of GAG moiety has not been elucidated.

In this study, we aimed to clarify the role of GAG chain in UTI function and to apply the study results for the development of more effective therapeutic drugs for the management of preterm birth.

**Methods:** We prepared GAG chain-remodeled UTIs by hydrolysis and/or transglycosylation reaction of testicular hyaluronidase. These UTIs were added to uterine cervical fibroblasts (UCFs) culture system obtained from gynecology operation, and the effects of UTIs on the release of IL-8, IL-6, MMP-8, and MMP-9 were examined.

**Results:** UTIs that were not hydrolyzed tended to reduce IL-8 release more strongly than GAG chain hydrolyzed UTIs. IL-6 did not show the constant tendency by a GAG chain hydrolysis or not. GAG chain hydrolyzed UTIs tended to reduce MMP-8 and MMP-9 release more strongly than UTIs that were not hydrolyzed.

**Conclusion:** During the process of cervical ripening in preterm birth, MMP-8 and MMP-9 are involved in the degradation of collagen, while IL-8 and IL-6 are involved in increase in HA production. Our study indicated that GAG chains of UTIs might reduce HA in the process of cervical ripening by reducing IL-8 release and have opposite effects on reducing MMP-8 and MMP-9 release related to the degradation of collagen. Our present study shows the possibility of improving the function of UTI by remodeling of GAG chain. Although further researches were needed to confirm these findings, the research suggested the possibility of becoming a new treatment for preterm birth.

PO-09

# Development of human parvovirus B19 virus-like particle (VLP) vaccine

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***Background:*** Human parvovirus B19 is a most serious cause of the infection of the fetus from the mother, since this virus infection to the pregnant woman induces the miscarriage with hydrops fetalis and anemia. However, the preventive vaccine for this virus has not been developed yet. At present, virus-like particles (VLPs) have been considered to be a candidate for the B19 vaccine, because of the absence of experimental system to amplify this virus in the cultured cells. In this study, we developed a B19-VLP production system and characterized biochemical properties of B19-VLPs. Furthermore, we established a novel method to detect B19 virus infection using erythroid progenitor cell-line: UT7/Epo-S1, and tested the ability to induce neutralizing antibodies. We also considered the possibility that the natural self-assembling protein nano-particles can be the carrier of the B19 antigens for the efficient induction of neutralizing antibodies.

***Materials and Methods:*** We optimized codon usage of the B19 capsid proteins, VP1 and VP2, and tested the improvement of their expression levels. VP1 and VP2 were expressed in 293T cells individually or simultaneously, and the relevant ratio of VP1 and VP2 amount for VLP formation was clarified by performing sucrose density gradient analysis, gel-filtration analysis, and electron microscopic observation. We also prepared B19 epitope fused to the self-assembling protein nano-particles and confirmed the presence of B19 epitope on the particle surface by testing the binding abilities of these particles to the UT7/Epo-S1. Purified B19-VLPs were immunized to the mice and investigated the ability to neutralize-antibody inductions.

***Result and Conclusion:*** Codon optimization significantly improved the expression level of B19-VPs. Efficient VLP assembly was observed in the nuclear extract of VPs-expressed 293T cells in case that VP1 and VP2 were expressed with a 1:1 ratio. These VLPs were formed spherical particle, 20nm in diameter, and bound to the UT7/Epo-S1. Immunization of B19-VLP to the mouse could induce neutralizing antibodies, suggesting that VLPs have the same structural characteristics with real viral particles. Natural particles that have B19 epitopes were also expressed in 293T cells, and these particles bound to the UT7/Epo-S1 efficiently. We are currently purifying these particles and testing the ability to neutralize-antibody induction.

PO-10

# Transrational study between pancreatic cancer stroma and clinical image

○ Shintaro Goto [1)], Tadashi Yoshizawa [1,2)], Kana Saito [1,3)], Takuya Shimanaka [1,3)], Hiroko Seino [1)], Takanobu Akaishi [1)], Toshihiro Haga [1)], Satoko Morohashi [1)], Hiroshi Kijima [1)]

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**Background:** Pancreatic cancer is one of the most aggressive cancers. The most common histopathological type is invasive ductal adenocarcinoma, which is characterized by infiltrative growth with abundant fibrous cancer stroma. The fibrous cancer stroma contains a lot of active fibroblasts called cancer-associated fibroblasts (CAFs), and is thought to promote the aggressive growth/invasion of pancreatic cancer. Histologically, the cancer cells/stroma ratio is various depending on the cases. This histological diversity of pancreatic cancer is one of our pathophysiological issues. On the other hand, contrast-enhanced computed tomography (CECT) is useful clinical modality because it enables to observe the internal properties of tumors without invasive procedures. In this study, we examined whether the preoperative clinical image correlates with the histological diversity of pancreatic cancer.
**Method:** We reviewed 40 cases of pancreatic cancers without preoperative chemotherapy which were diagnosed as invasive ductal adenocarcinoma, histopathologically. All cases were performed with dynamic CECT in our hospital. We calculated the density of cancer cells and the positive rate of immunoreactive α-smooth muscle actin (αSMA), a marker of the cancer stroma. Next, we analyzed dynamic CECT images, and draw out the time intensity curve of pancreatic cancer. Dynamic CECT images consist of non-contrast phase, arterial phase, portal phase and equilibrium phase. Time intensity curve represented the changes of CECT image value. We finally analyzed the correlations between the histopathological findings and the dynamic CECT images.
**Results:** There were significant correlations between the CT value at arterial phase and theαSMA positivity which indicating the cancer stroma. In contrast, there were no correlations between the cancer cells and CT values at any phases. We tried to clarify molecular/histological mechanisms why the αSMA positivity was significantly correlated with the time intensity curve of CECT.
**Conclusion:** The contrast medium of CECT infiltrates into the αSMA-positive cancer stroma. A lot of active CAFs regulate the pancreatic cancer microenvironment, and play an important role in the contrast medium infiltration.

PO-11

# Histamine release from intestinal mast cells induced by staphylococcal enterotoxin A (SEA) evokes vomiting reflex in common marmoset

○ Hisaya K Ono [1,2], Shouhei Hirose [2,3], Kouji Narita [2,4], Makoto Sugiyama [5], Krisana Asano [2,3], Dong-Liang Hu [1], Akio Nakane [2,3]

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**Background:** Staphylococcal enterotoxins (SEs) produced by *Staphylococcus aureus* are known as causative agents of emetic food poisoning. We previously demonstrated that SEA binds with submucosal mast cells and evokes mast cell degranulation in a small emetic house musk shrew model. Notably, primates have been recognized as the standard model for emetic assays and analysis of SE emetic activity. However, the mechanism involved in SEA-induced vomiting in primates has not yet been elucidated.

**Methods:** The emetic assay of SEs in common marmosets was estimated using Bergdoll's monkey feeding assay with some modifications. To clarify the mechanism of emesis induced by SEs, histological and pharmacological techniques were used for common marmosets and their gastrointestinal tracts.

**Results:** We established common marmosets as an emetic animal model. Common marmosets were administered classical SEs, including SEA, SEB and SEC, and exhibited multiple vomiting responses. However, a non-emetic staphylococcal superantigen, toxic shock syndrome toxin-1, did not induce emesis in these monkeys. These results indicated that the common marmoset is a useful animal model for assessing the emesis-inducing activity of SEs. Furthermore, histological analysis uncovered that SEA bound with submucosal mast cells and induced mast cell degranulation. Additionally, *ex vivo* and *in vivo* pharmacological results showed that SEA-induced histamine release plays a critical role in the vomiting response in common marmosets.

**Conclusion:** SEA induces histamine release from submucosal mast cells in the gastrointestinal tract and that histamine contributes to the SEA-induced vomiting reflex via the vagus nerve.

PO-12

# Aortic valve calcification via each inflammatory and non-inflammatory signaling

○ Zaiqiang Yu [1], Xu Liu [1], Wei Yang [1], Kazuyuki Daitoku [1], Tadaatu Imaizumi [2], Shigeru Motomura [3], Ikuo Fukuda [1], Ken-ichi Furukawa [3], Kazuhiko Seya [2]

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**Background:** Aortic valve stenosis (AVS) is the most frequent heart valve disease in the elderly, accompanied and accelerated by ectopic calcification of valves (AVC). The most effective treatment is surgical aortic valve replacement (SAVR) with massive invasion, and transcatheter aortic valve replacement (TAVR) for high risk patients. However, the mechanism of AVC remains unclear, and the effective medical therapy for inhibiting acceleration of AVC needs to be established in near future. We aimed to clarify the mechanism of AVC and establish the medical therapy to prevent acceleration of AVS.

**Methods:** Written informed consent was obtained from all patients. This study was approved by the institutional review boards of the Hospital of Hirosaki University. Human aortic valve interstitial cells (HAVICs) were obtained from AVS patients, and aortic regurgitation (AR) patients without AVC as a control group, which were isolated by collagenase digestion. The calcification was identified by Alizarin Red S stain. The expression of calcification related genes was measured by real time-PCR. Weston blot was used to measure protein expression.

**Results:** Firstly, we demonstrated that inflammatory cytokine tumor necrosis factor-a (TNF-a) induces calcification of HAVICs obtained from AVS patients. Gene expression of bone morphogenetic protein 2 (BMP2) and activity of alkaline phosphatase (ALP) were accelerated significantly in AVS group. We also found that gene and protein expression of matrix Gla protein (MGP), a known calcification inhibitor that antagonizes BMP2, was inhibited significantly in TNF-$\alpha$-induced calcification of HAVICs. In contrast, HAVICs overexpressing MGP had significantly decreased TNF-$\alpha$-induced calcification, and gene expression of BMP2 also was inhibited significantly. Secondly, the calcification of HAVICs also was accelerated in high inorganic phosphate (Pi) condition (3.2 mM) without inflammatory activated, and warfarin accelerated the calcification of HAVICs with presence of high Pi via pregame X receptor (PXR) pathway to inhibit gene expression of BMP2. At last, there are massive mesenchymal stem cell like cells (MSCLC) found in HAVICs showed with cellular maker CD45 negative and CD73/90/105 positive. These cells showed CD34 negative MSCLCs had more sensitive to high Pi than CD34 positive MSCLCs. We think that CD34 negative MSCLCs were the most closed to original cells of AVC.

**Conclusion:** These data showed that AVC was induced by each inflammatory via NF-$\kappa$B and non-inflammatory condition via PXR with high Pi. BMP2 and MGP was the most important key proteins to control progression of AVC. The role of CD34 negative MSCLCs in AVC also need to be further examined for innovation of medicine therapy to prevent from AVC.

PO-13

# An Optical Coherence Tomographic Analysis of RDH5-/- Mice Retina

○ Yuting Xie [1)], Takayuki Gonome [1)], Saeko Arai [1)], Kodai Yamauchi [1)], Natsuki Maeda-Monai [1)], Reiko Tanabu [1)], Sei-ichi Ishiguro [2)], Mitsuru Nakazawa [1)]

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**Background:** Fundus albipunctatus (FA), as a type of hereditary retinal dystrophy, is a rare eye disorder characterized by an impaired visual ability under dim-light conditions and the presence of numerous white dots that are especially abundant near the mid-periphery and perifovea of the retina. Although mutations of the retinaldehyde binding protein1 (RLBP1) and retinal pigment epithelium (RPE)-specific-65-kDa protein (RPE65) genes have been reported to be associated with FA, most cases of FA have been caused by mutations in the 11-cis retinol dehydrogenase 5 (RDH5) gene. The RDH5 gene encodes RDH5, which is predominantly expressed in the retinal pigment epithelium (RPE), where it converts the molecule 11-cis retinol to 11-cis retinal. 11-cis retinal is the recycling molecule in an integral operation of the visual cycle. It is the chromophore residing in rhodopsin and cone opsins and is needed for the conversion of light to electrical signals.

**Methods:** The mouse retina was segmented into four layers; the inner retinal (A), outer plexiform and outer nuclear (B), rod/cone (C), and retinal pigment epithelium (RPE)/choroid (D) layers. The thickness of each retinal layer of RDH5-/- mice was longitudinally and quantitatively measured at six time points from postnatal months (PM) 1 to PM6 using SD-OCT. Age-matched C57BL/6J mice were employed as wild-type controls. The data were statistically compared using Student's t-test. The fundus appearance was assessed, histologic and ultrastructural examinations, and electroretinography (ERG) analyses were performed in both groups.

**Results:** Layers A and B were significantly thinner in the RDH5-/- mice than in the wild-type C57BL/6J mice during the observation periods. Layers C and D became thinner in the RDH5-/- mice than in the wild-type mice after PM6. Although no abnormalities corresponding to whitish fundus dots were detected by SD-OCT or histologic examinations, the intracellular accumulation of low-density vacuoles was noted in the RPE of the RDH5-/- mice by electron microscopy. The photoreceptor nuclei appeared less dense in the RDH5-/- mice than in the wild-type mice. No differences were found in the amplitude of both a- and b-waves between two groups.

**Conclusion:** The results from the present study suggest that although it is difficult to detect qualitative abnormalities, SD-OCT can detect quantitative changes in photoreceptors even in the early stage of retinal degeneration induced by the RDH5 gene mutation in mice.

PO-14

# Ability of uterine NK cell cytokine production of unexplained RPL women by the stimulation using semen

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**Background:** Some women with recurrent pregnancy loss (RPL) have an abnormality of NK cell cytokines production. To evaluate cytokines production, we usually use drugs such as PMA and Ionomycin as a stimulator, but this method is not physiological. Semen looks an appropriate stimulator for uterine NK cell because semen is essential at the time of pregnancy. So we aimed to develop the physiological stimulation method using semen.
**Methods:** Endometrium(n=13), under approval by the ethics committee and patients' agreement, was evaluated for the NK cell (CD56+ cell) cytokine production by flow cytometry after stimulation by partner's semen or drugs. Additionaly, we have compared the cytokines production by NK cells with control, RPL women with immunological abnormality, and unexplained RPL women by cytokines production.
**Results:** The percentages of IFN-$\gamma$ and TNF-$\alpha$ producing NK cell were significantly lower in stimulation by semen than drugs ($p<0.01$). Moreover, NK1/NK2 ratio by the stimulation using semen is significantly lower than using drugs ($p<0.01$). And the percentages of IL-4 and IL-10 producing NK cell stimulated by semen were significantly lower in unexplained RPL compaired with control ($p<0.05$).
**Conclusion:** Semen stimuration show type2 shift better than drugs . Futhermore, in some of unexplained RPL women, NK1 shift may be occur and cause miscarriage after stimulation by semen.

PO-15

# Prenatal Heavy Metals Exposure and Newborn Leucocyte TL in Relationship with the Modifying Effects of Selenium

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Telomeres are repetitive DNA sequences (TTAGGG), located at the end of chromosomes. Telomere length (TL) shortening is considered as a biomarker of cellular aging and is associated with increased risks age-related health diseases including cardiovascular diseases, malignancy and overall mortality. The objectives of this study are 1) to evaluate the effect of prenatal exposure of arsenic (As), cadmium (Cd) and Lead (Pb) on TL; 2) to examine the mediation effect of selenium (Se) on heavy metals induced TL shortening. A birth-cohort study was conducted with 408 mother-infant pairs in Myanmar in 2016. First, pregnant women in their third trimesters were interviewed using a pretested questionnaire, and spot urine samples were collected after the interview. During the follow-up period (1 to 3 months afterwards), the umbilical cord blood samples were collected by the local health workers at birth. The metals concentrations were measured using inductively coupled plasma mass spectrometry under quality control. TL was measured by the quantitative real-time polymerase chain reaction. Relative TL was calculated as a ratio of telomere gene to single copy gene. To evaluate the mediation effect of selenium, the molar ratios of metals were calculated accordingly. Bivariate analysis was performed to examine the associations between each heavy metal and TL, individually and after Se mediation. Later, multivariable linear regression models were applied for adjusting the potential confounders of maternal age, education, smoking status, parity, birth weight and infant's sex. Relative TL was significantly shortened with higher As (β = -0.22; 95% CI: -0.32, -0.12), Cd (β = -0.19; 95% CI: -0.29, -0.10) or Pb (β = -0.10; 95% CI: -0.18, -0.01) concentrations. The effects of As (β = -0.16; 95% CI: -0.26, -0.06) and Cd (β = -0.17; 95% CI: -0.27, -0.06) on TL remained significant with Se mediation although the effect size became noticeably smaller. However, the effect of Pb exposure on TL was attenuated with higher Pb-Se molar ratio (β = -0.06; 95% CI: -0.14, 0.03). Prenatal As, Cd and Pb was significantly associated with newborn TL shortening. Although our study did not support the mediation effect of Se on TL shortening by As and Cd exposure, we found that the effect size was lower with Se mediation compared to individual metals exposure.

PO-16

# Excess glutamate may cause dilation of retinal blood vessels in Glutamate/Aspartate transporter-deficient Mice.

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**Background:**

Glutamate/aspartate transporter (GLAST) deficient ($GLAST^{-/-}$) mice are a recognized mouse model of normal tension glaucoma (NTG), as they demonstrate progressive retinal ganglion cell loss and optic nerve degeneration without elevated intraocular pressure and show a glaucomatous pathology, including glutamate neurotoxicity and oxidative stress in the retina.

Previous studies have shown that glutamate is a major excitatory neurotransmitter of the mammalian retina and its uptake is essential for neurotransmission at glutamatergic synapses. GLAST is expressed mainly in Müller cells, and it removes glutamate from the extracellular space to maintain glutamate homeostasis in the retina. Moreover, the glutamate uptake by GLAST into Müller cells provides a substrate for synthesizing glutathione, which is an important radical scavenger. Glutathione has a strong protective role against oxidative stress as an antioxidant in the retina. In addition, glutamate is the most common trigger for neurovascular coupling (NVC) in the brain. NVC is the mechanism whereby an increase in neuronal activity leads to local elevation in cerebral blood flow by significant vasodilation of neighboring microvessels to match the metabolic requirements of firing neurons.

In this study, we evaluated the changes in all retinal layers in $GLAST^{-/-}$ mice using spectral domain-optical coherence tomography (SD-OCT). In addition, we also evaluated whether or not the retinal blood vessels were dilated in $GLAST^{-/-}$ mice using SD-OCT. We suspected that dilation of the retinal blood vessels in $GLAST^{-/-}$ mice might indirectly indicate a high concentration of glutamate in the retina and that glutamate might also contribute to NVC in the retina.

**Methods:**

The fundus findings and SD-OCT images were longitudinally recorded at five time points from postnatal (P) 22 to P156 in $GLAST^{-/-}$ mice. As a control wild type, age-matched C57BL/6J mice were employed. The mouse retina was subdivided into five layers and the thickness of each layer was longitudinally measured by InSight® using SD-OCT pictures. The SD-OCT findings were compared with the histologic appearances. The diameter of the retinal blood vessels was measured by the ImageJ® software program using SD-OCT images. The data were statistically compared between both age-matched mouse groups.

**Results:**

The retinal blood vessels appeared more dilated in $GLAST^{-/-}$ mice than in wild-type mice. This tendency was statistically significant at all time points after P44 by analyses using SD-OCT images. The ganglion cell complex (GCC) and outer nuclear layer (ONL) were significantly thinner in $GLAST^{-/-}$ mice at all time points after P80 than in the wild-type mice. This tendency was more clearly indicated by SD-OCT than histologic sections.

**Conclusion:**

In the present study, we found for the first time the dilation of the retinal blood vessels and the thinning of the ONL in $GLAST^{-/-}$ mice, in addition to the thinning of the GCC.

**PO-17**

# Phospholipase C-related inactive protein type-1 deficiency affects anesthetic electroencephalogram activity induced by propofol and etomidate in mice

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**Background:** Intravenous anesthetics such as propofol and etomidate induce general anesthesia. Propofol and etomidate mainly exert their anesthetic actions via GABA A receptor ($GABA_A$-R). The $GABA_A$-R activity is influenced by phospholipase C-related inactive protein type-1 (PRIP-1), which is related to trafficking and subcellular localization of $GABA_A$-R. PRIP-1 deficiency attenuates the behavioral reactions to propofol but not etomidate. However, the effect of these anesthetics and of PRIP-1 deficiency on brain activity of CNS are still unclear. In this study, we examined the effects of propofol and etomidate on the electroencephalogram (EEG).

**Methods:** The cortical EEG activity (AP,+1.1 mm; L, 1.45 mm; mouse brain atlas) was recorded in wild-type (WT) and PRIP-1 knockout (PRIP-1 KO) mice using a biophysical amplifier and SleepWave data acquisition software. All recorded EEG data were offline analyzed, and the power spectral density and 95% spectral edge frequency of EEG signals were compared between genotypes before and after injections of anesthetics.

**Results:** PRIP-1 deficiency induced increases in EEG absolute powers, but did not markedly change the relative spectral powers during waking and sleep states in the absence of anesthesia. Propofol administration induced increases in low-frequency relative EEG activity and decreases in SEF95 values in WT but not in PRIP-1 KO mice. Following etomidate injection, low-frequency EEG power was increased in both genotype groups. At high frequency, the relative power in PRIP-1 KO mice was smaller than that in WT mice. Furthermore, immediately after anesthetic injections, the lower frequency shift of relative EEG power induced by propofol was greater than that induced by etomidate in WT mice.

**Conclusion:** The lack of PRIP-1 disrupted the EEG power distribution, but did not affect the depth of anesthesia after etomidate administration. Our analyses suggest that PRIP-1 is differentially involved in anesthetic EEG activity with the regulation of $GABA_A$-R activity.

PO-18

# Food intake behavior for individuals with specific IgE (sIgE) positive

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**Background:** The prevalence of allergic disease is increasing globally. In Japan, pollen-food allergy syndrome (PFAS) was added as a new clinical condition in a Treatment Guide for Food Allergy 2017 by AMED group. A person with PFAS might experience cross reaction with certain allergens in food. Therefore, the individual with specific IgE (sIgE) might unconsciously avoid eating certain foods from fear of an allergic reaction. We studied the associations of sIgE for major inhaled allergens with food intake behavior.

**Methods:** There were 1,073 participants in the Iwaki Health Promotion Project 2017 in Aomori. Participants had a checkup including blood sampling, and their dietary intake was estimated using a validated, self-administered diet history questionnaire. Subjects analyzed were aged 20 to 79 years who had completed the checkup and questionnaire, and their energy intake ranged from 500 to 5,000kcal/day. They were categorized into two groups for each pollen allergen according to immunoCAP class: negative (class 0 and 1) and positive (class 2 through 6). We examined the association between the sIgE for pollen allergens and intake of 5 foods that were known to have cross reaction with grass pollen allergen, 9 foods for cross reaction with weed pollen allergen, 1 food for house dust pollen allergen and 2 foods for Japanese cedar pollen (JCP) allergen.

**Results:** Subjects were grouped as 166 positive (78 males and 88 females) and 863 negative (345 males and 518 females) for grass pollen sIgE; 84 positive (40 males and 44 females) and 945 negative (383 males and 562 females) for weed pollen sIgE; 526 positive (244 males and 282 females) and 503 negative (179 males and 324 females) for JCP sIgE, and 227 positive (106 males and 121 females) and 802 negative (317 males and 485 females) for house dust sIgE.

Mann-Whitney U-tests found significant differences in intake of natto ($p=0.008$) between the grass pollen sIgE positive group in females; citrus ($p=0.032$) between the weed pollen sIgE positive group in males; and tomatoes ($p=0.026$), citrus ($p=0.035$), and rice ($p=0.020$) between the weed pollen sIgE positive group in females. After the Bonferroni Correction, differences in intake of natto between the grass pollen sIgE positive group in females remained significant showing intake of natto is smaller in the grass pollen sIgE positive group than the grass pollen sIgE negative group.

**Conclusion**: Individuals with grass pollen sIgE positive group tended to eat less natto. The influence of PFAS on natto requires further study.

**PO-19**

# Effect of glycosaminoglycans on retinoic acid-induced neural differentiation of P19 embryonal carcinoma cells

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**Background:** Although involvement of glycosaminoglycans (GAGs) in neural differentiation is suggested, the functions of the GAGs have not yet been elucidated in detail. We screened GAGs in order to investigate whether any of them have structures that affect neural differentiation.
**Methods:** *All-trans*-retinoic acid (RA) induced mouse P19 embryonic carcinoma cells were used as a model system for studying neural differentiation. Undifferenciated P19 cells were cultured for 4 days in the presence of 1 μM of RA as the aggregation, then they were trypsinized and further cultured for 3 days in the absence of RA as the monolayer. During culture various kinds of GAGs were added to the culturing media at 10 or 100 μg/ml. On day 7, cells were harvested and the expressions of neural or grail markers were analyzed by real time quantitative RT-PCR. Chondroitin sulfate E (ChSE) oligosaccharides were prepared by partial digestion with bovine testicular hyaluronidase and purified by gel filtration chromatography using a Bio-Gel P-4 column.
**Results:** The expression levels of neuron specific mRNAs, neurofilament light (NF-L, an intermediate filament) and β-tubulin 3 (a microtubular protein) were upregulated by addition of ChSE and heparin. The expression levels of glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) were upregulated by addition of ChSE. More upregulation was observed when ChSE was added during aggregation culture than either throughout culture or during monolayer culture both in mRNA and protein levels. ChSE oligosaccharides did not upregulate these expression levels at the concentration corresponding to the same molar concentration as ChSE polysaccharides.
**Conclusion:** Sulfation patterns of GAGs were suggested to be important to affect neural differentiation.

PO-20

# Melanoma differentiation-associated gene 5 positively modulates TNF-α-induced CXCL10 expression in cultured HuH-7 and HLE cells

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**Background:** The molecular mechanisms of innate immunity are closely associated with the development of non-alcoholic fatty liver disease (NAFLD). TNF-α and CXCL10 are key inflammatory molecules involved in the pathogenesis of metabolic inflammation like NAFLD. Melanoma differentiation-associated gene 5 (MDA5) is a member of the intracellular RNA helicase family proteins that plays a pivotal role in antiviral immune response. Previous studies have demonstrated that TNF-α induces the expression of MDA5 in some types of cells. However, the correlation between TNF-α and the expression of MDA5 in hepatocytes remains unknown.

**Methods:** We used two human hepatocellular carcinoma cell lines, HuH-7 and HLE, and the expression of MDA5 induced by TNF-α was analyzed by quantitative real time RT-PCR (qPCR) and western blotting. RNA interference (RNAi) against nuclear factor-kappa B (NF-κB) p65 or interferon regulatory factor 1 (IRF1) was performed and the expression of MDA5 was examined. Next, we performed the RNAi against MDA5 and analyzed the expression of TNF-α-induced CXCL10 by qPCR and ELISA. In addition, the phosphorylation of STAT1 was examined by western blotting.

**Results:** The expression of MDA5 had increased upon stimulation with TNF-α in a concentration-dependent manner. Gene silencing against p65 suppressed the expression of MDA5 in both cells. On the other hand, gene silencing against IRF1 significantly suppressed the expression of MDA5 in HLE, but not in HuH-7. Gene silencing against MDA5 suppressed the expression of TNF-α-induced CXCL10 in both cells. In HLE cells, gene silencing of MDA5 impaired STAT1 phosphorylation 24 h after stimulation with TNF-α. On the other hand, TNF-α-induced STAT1 phosphorylation was not detected in HuH-7 cells.

**Conclusion:** These results indicated that MDA5 positively modulated the TNF-α-induced CXCL10 expression in both STAT1-dependent and –independent manner. MDA5 may be critically involved in metabolic inflammation in the liver.

PO-21

# Morphological analysis of peritoneal dissemination of ovarian cancer based on levels of carbonyl reductase 1 expression

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**Background:** When carbonyl reductase 1 (CR1) is highly expressed in human ovarian cancer cells *in vivo*, tumor growth is reported to be inhibited. Conversely, when expression of CR1 decreases, tumor growth, invasion, and metastasis are reported to increase. Thus, the aim of the current study was to examine dynamic changes in ovarian cancer cells under different CR1 expression levels in artificial human peritoneal tissue (AHPT).

**Methods:** Serous ovarian cancer cells with different levels of CR1 expression were produced by transfection of HRA human ovarian carcinoma cells with CR1 DNA or CR1 siRNA. The transfected cells were seeded in AHPT and observed over time until peritoneal development of carcinomatosis. Apoptotic cells in the AHPT were compared using TUNEL staining and fluorescence-based flow cytometry.

**Results:** Cells transfected with CR1 DNA or CR1 siRNA did not differ from control cells in terms of their adherence to the mesothelium. After 24 h, when cells had invaded the tissue below the mesothelium, proliferation of CR1-overexpressing cells was inhibited while proliferation of CR1-suppressing cells increased. At 72 h, CR1-suppressing cells had invaded the stroma. CR1-overexpressing cells had a markedly higher rate of apoptosis than control or CR1-suppressing cells. Moreover, transmission electron microscopy revealed apoptotic bodies in cells overexpressing CR1. Differences in tumor growth depending on the extent of CR1 expression have been noted *in vivo*, and similar results were obtained in our *in vitro* model of AHPT. High and low levels of CR1 expression did not affect cell adherence to the mesothelium, but low levels did result in cells invading and proliferating below the mesothelium.

**Conclusion:** Our results have also demonstrated that tumor inhibition by CR1 involves an increase in apoptosis.

PO-22

# Sterilization of urethral indwelling catheters with high-power UVC LEDs

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**Background:** Urinary tract infections account for about 40% of nosocomial infections, of which about 80% are reported from urethral indwelling catheters. Once an infection occurs, patients are forced to extend their hospital stay, resulting in additional costs such as administration of extra antibiotics, which is an important issue in terms of patient disadvantage and medical costs. This study aims to develop a sterilization device for indwelling urethral catheters using high-power ultraviolet-C light-emitting diodes (UVC LEDs) in order to reduce the incidence of urinary tract infections.

**Methods:** In sterilization of the urethral indwelling catheter, it is important that not only the outer tube wall of the urethral indwelling catheter but also the inner tube wall can be sterilized. Therefore, the UVC transmittances of Foley catheters made of silicone rubber, which are widely used, were measured. In addition, since high-power UVC LEDs generate a great deal of heat, we created a cooling system and measured the temperature when driving the LEDs. Then, a sterilization unit using three high-power deep ultraviolet light-emitting diodes was created, and a sterilization experiment using E. coli bacterial solution was conducted.

**Results:** The UVC transmittance of the Foley catheter made of silicone rubber was 20-30%. This indicates that the inner wall of the indwelling catheter can be sterilized in a short time by using high-power UVC LEDs. From the temperature measurement when the LED was driven, the temperature rise of the LED when driven for 5 minutes was about 10-12 [°C] from room temperature. This confirmed that the sterilization unit cooling system we made was functioning properly and there was no risk of cold burns for the patient. In the sterilization experiments using E. coli solution, LRV 3.5 was achieved in about 14 seconds. Furthermore, the sterilization ability exceeding LRV 4.0 was shown in about 19 seconds.

**Conclusion:** To develop a urethral indwelling catheter sterilizer using high-power UVC LEDs, several basic characteristics related to high-power UVC LEDs were measured. As a result, it was found that the urethral indwelling catheter sterilizer using high-power UVC LEDs could become one of promising devices.

PO-23

# The elucidation of transcriptional regulation of *Tspo* gene

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**Background:** The 18-kDa translocator protein TSPO is used as an imaging target in positron emission tomography to detect neuroinflammation, and its expression is correlated with microglial activation. However, the mechanism underlying the transcriptional regulation of *Tspo* induced by inflammation is not clear. Accordingly, we focused on transactivation of *Tspo* gene expression in this study, and LPS was used as an inducer of neuroinflammation to elucidate the transcriptional regulation of *Tspo* in murine microglial cell line, BV-2.

**Methods:** To determine which transcription factor and *cis*-element regulates *Tspo* expression during LPS treatment, serial deletion constructs were designed for the *Tspo* reporter gene assay. After transfection of each vector, cells were treated with vehicle or 100 ng/ml LPS and a reporter gene assay was then performed. After identification of *cis*-element, the enrichment of transcriptional regulators were measured by Chromatin Immunoprecipitation assay.

**Results:** Reporter gene assay revealed that the deletion of AP-1 binding site significantly reduced the LPS-induced Tspo expression. Knockdown of c-Fos and c-Jun, the components of AP-1, reduced LPS-induced *Tspo* expression. Furthermore, the enrichment of Sp1 in the proximal promoter region of *Tspo* was increased in the presence of LPS. In addition, the binding of histone deacetylase 1 (HDAC1) to the enhancer region, which contains the AP-1 site, was decreased by LPS treatment, but there were no significant differences in HDAC1 binding to the proximal promoter region with or without LPS.

**Conclusion:** We showed that recruitment of both c-Fos and c-Jun, which are components of the AP-1 complex, to the enhancer region of the *Tspo* gene was increased upon LPS treatment in the microglial cell line BV-2. This evidence was supported by the observation that *Tspo* expression was reduced by knockdown of c-Fos and c-Jun. In addition, we found that the concentration of HDAC1 at the AP-1 binding site in the *Tspo* enhancer region was decreased by LPS treatment. These data suggest that LPS-induced *Tspo* gene expression in BV-2 was upregulated by AP-1 activation and that the release of HDAC1 from the AP-1 site was increased by LPS treatment.

PO-24

# Identification of chromosomal interactions by locus-specific ChIP

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**Background:**

Elucidation of molecular mechanisms underlying genome functions requires identification of molecules that interact with genomic regions of interest *in vivo*. To this end, we have developed locus-specific chromatin immunoprecipitation (locus-specific ChIP) consisting of insertional ChIP (iChIP) and engineered DNA-binding molecule-mediated ChIP (enChIP) to isolate genomic regions of interest. In those technologies, a locus of interest is tagged and then isolated by affinity-purification. Subsequently, molecules interacting with the locus (proteins, DNA, RNA) can be identified in combination with mass spectrometry or next generation sequencing (NGS).

**Methods:**

Here, we attempted to identify genomic regions that physically interact with target loci by locus-specific ChIP in combination with NGS. To this end, we isolated the promoter region of the *Pax5* gene from chicken DT40 cells or the 5'HS5 locus from human K562 cells by locus-specific ChIP. Subsequently, we identified genomic regions interacting with those regions by NGS analysis. In addition, we examined genome functions of identified genomic regions by locus deletion or ChIP assay.

**Results:**

We found that those target genomic regions interact with multiple genomic regions. In addition, deletion of a *Pax5* interacting genomic region in chromosome 11, which was marked by active enhancer histone modifications, resulted in moderate but significant down-regulation of *Pax5* transcription. Therefore, the *Pax5* interacting genomic region would function as an enhancer to activate *Pax5* transcription.

**Conclusion:**

These results showed that locus-specific ChIP combined with NGS are useful for non-biased identification of genomic regions that physically interact with a locus of interest and regulate genome functions. Previously, we also succeeded in identifying proteins and RNAs that interact with loci of interest by locus-specific ChIP. Thus, locus-specific ChIP may facilitate understanding of molecular mechanisms underlying genome functions, such as transcription.

PO-25

# Body weight loss in postnatal GCN1L1 knockout mice

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**Background:** In eukaryotes, GCN1 activates GCN2 kinase activity in response to amino acid starvation and attenuate general translation by phosphorylating eIF2α. GCN1 interacts with ribosome and GCN2 via N-terminal domain and C-terminal RWD-binding domain (RWDBD), respectively. GCN1-GCN2 interaction is necessary for the response and following selective translation of transcription factor ATF4 which induces expression of amino acid synthetase/transporter. To analyze functional roles of mammalian GCN1 homologue GCN1L1, we have previously analyzed two lines of GCN1L1 knockout mice, GCN1L1 exon 2 knockout mice (GCN1L1-/-) and GCN1L1 exons 46-53 knockout mice which lacks RWDBD (GCN1L1ΔRWDBD). Homozygous GCN1L1-/- and GCN1L1Δ RWDBD mice show embryonic and perinatal lethality, respectively, and these phenotypes are severer than that of GCN2 knockout mice.

**Methods:** To explore the function of GCN1L1 in tissue- and developmental stage-specific manner, we created conditional GCN1L1 knockout (CKO) mice by Cre/loxP system. GCN1L1 exon 2-floxed mice crossed with Rosa26-Cre-ERT2-expressing mice were intraperitoneally injected with 10 times of 75 mg/kg tamoxifen at 5 weeks old. Mice were sacrificed 10 days after the last injection and tissues were analyzed by genotyping PCR, RT-qPCR and immunoblot.

**Results:** Genotyping PCR detected deletion of GCN1L1 exon 2 in brain, lung, heart, liver, spleen, kidney, intestine, colon and skeletal muscle of CKO mice but not in control mouse tissues. GCN1L1 protein was efficiently diminished in the liver, intestine and colon, whereas GCN1L1 protein in brain was barely affected as reported previously. The body weight of CKO mice were strikingly reduced while and after the tamoxifen injection period, and one homozygous CKO mouse died before anatomy.

**Conclusion:** Tamoxifen-induced deletion of GCN1L1 exon 2 was detected at genomic DNA, mRNA and protein levels in most tissues analyzed except for brain. Although the data are still preliminary, the reduction in body weight of CKO mice implicates GCN1L1 is essential not only for embryogenesis but also for development of mouse at young age.

PO-26

# Histopathologic, enzymatic background for biosynthesis of aberrant glycosylated prostate-specific antigen and its clinical significance.

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**Background:** Although we previously reported that the prostate cancer (PC) diagnostic performance of serum aberrant Sia$\alpha$2,3Gal-glycosylated prostate-specific antigen (S2,3PSA) test or LacdiNAc-glycosylated PSA (LDN-PSA) test much superior to conventional PSA test (Ishikawa et al., *IJMS*, 2017; Yoneyama et al., *Cancer Sci.* 2019), the histopathologic and enzymatic background for biosynthesis of aberrant-glycosylated PSA in prostate tissue has still unknown. In this study, we investigate the origin of aberrant-glycosylated PSA, and also evaluated the clinical significance of aberrant-glycosylated PSA for PC detection.
**Methods:** Total RNA and protein were extracted from benign prostate gland and each Gleason pattern of PC tissue that was macro-dissected from FFPE prostate section in 68 patients who underwent radical prostatectomy in Hirosaki University. The expression level of sialylated- and LDN- synthesis-related glycosyltransferase genes were evaluated by droplet digital PCR, and also %S2,3PSA or LDN-PSA/total PSA levels were analyzed by automated immunoassay systems. We also retrospectively evaluated the avoidable prostate biopsy effect of base diagnostic model (age, DRE status, total PSA, and free/total PSA) combined with S2,3PSA or LDN-PSA test using decision curve analyses (DCA) in men who underwent a prostate biopsy in three academic urology clinics.
**Results:** The expression level of Sia$\alpha$2,6Gal- synthesis-related $\alpha$2,6sialyltransferase (*ST6GAL1*) was significantly decreased in Gleason 4 & 5 tissue compare to Gleason 3 and benign prostate gland. Sia$\alpha$2,3Gal- synthesis-related $\alpha$2,3sialyltransferases (*ST3GAL3,4,6*) was not vary from tumor malignancy. The %S2,3PSA level was also significantly increased in Gleason 4 & 5 tissues suggesting that upregulation of the Sia$\alpha$2,3Gal- ratio on PSA regulated by *ST6GAL1* expression. The expression level of LDN- synthesis-related β1,4-N-acetylgalactosyltransferase (*β4GALNT3&4*) and LDN-PSA/total PSA level were increased in Gleason 4 & 5 tissues compared to benign prostate gland suggesting that LDN- synthesis on PSA was increased in aggressive tumors. DCA showed that using a risk threshold of 30%, adding S2,3PSA or LDN-PSA test to the base diagnostic model permitted avoidance of even more biopsies without missing PC (8.0% vs. -0.3% (base model) or 5.6% vs. 1.8% (base model)).
**Conclusion:** Aberrant glycosylation (Sia$\alpha$2,3Gal- or LDN-) related glycosyltransferase expression significantly increased in PC tissue with higher Gleason pattern and aberrant glycosylated PSA mainly secreted from PC tissue. Addition of S2,3PSA and LDN-PSA test to conventional diagnostic model significantly improve avoidable biopsy effect in identifying patients with PC.

PO-27

# Vitamin A deficiency impairs host resistance to *Listeria monocytogenes* infection through excessive apoptosis of macrophages.

○ Nahoko Tanaka [1)], Hiroto Hiraga [1)], Hirotake Sakuraba [1)], Naoki Higuchi [1)], Chizuru Ariake [1)], Takato Maeda [1)], Yasuhisa Murai [1)], Rina Watanabe [1)], Shinji Ota [1)], Yui Akemoto [1)], Keisuke Hasui [1)], Shukuko Yoshida [2)], Krisana Asano [3)], Akio Nakane [3)], Shinsaku Fukuda [1)]

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**Background:** *Listeria monocytogenes* invades the cytoplasm of phagocytes and performs intracellur multiplication, and is often used as a tool for functional analysis of macrophages. Vitamin A regulates various immune system. Vitamin A deficiency (VAD) mice has malfunction of the barrier of the intestine. Thereupon, we aimed to evaluate the effects of retinoic acid on macrophages under VAD condition using *L. monocytogenes* infection model.

**Methods:** VAD mice and vitamin A sufficient (VAS) mice were infected *L. monocytogenes* and the spleens and livers were removed post infection. The spleens were sectioned and analyzed Terminal deoxynucleotidyl transferase-mediated UTP-biotin nick end labeling (TUNEL)-positive images were observed under microscope. The cells of the spleens were analyzed by fluorescence-activated cell sorting (FACS) and to be classify necrosis or apoptosis. Using Enzyme-Linked Immuno Sorbent Assay (ELIZA), the spleen and the liver were analyzed early cell death. RAW264.5, Macrophage cell line and peritoneal exudate cells (PECs) were infected *L. monocytogenes*, and the supernatants were measured and DNA fragmentation in the cells was determined to evaluate cell death. Unpaired-t test was used to determine the significance of the differences of numbers.

**Results:** In the uninfected mice, the number of TUNEL-positive cells did not change On the other side, the number of TUNEL-positive cells increased in VAD mice at 24 h after infection. Apoptosis of splenocyto in VAD mice was increased after *L. monocytogenes* infection. In addition, Apoptosis cells were analyzed the cells were mainly CD11b-posistive cells by FACS. In the case of RAW264.5 and PECs after infection, the VAD condition cells were increased apoptosis. Production of both IL-1β and TNF-α was significantly decreased in VAD condition. *In vivo*, the number of bacteria in the organs after L. monocytogenes infection increased significantly under VAD condition.

**Conclusion:** VAD impairs host resistance to *L. monocytogenes* infection through excessive apoptosis of macrophages. Vitamin A contributes to the maintenance of homeostasis by regulating macrophage functions, suggesting the importance of vitamin A supplementation for host defense.

PO-28

# Essential structure for the specific binding of hyaluronan to hyaluronan-binding protein (HABP) determined by a glycotechnological approach

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**Background:** Within the hyaladherin family, hyaluronan-binding protein (HABP) specifically binds to hyaluronan and is therefore often used in experiments for quantifying hyaluronan. Therefore, we prepared hyaluronan-chondroitin-hybrid oligosaccharides mainly composed of deca- and dodecasaccharides, to obtain information on the structure of hyaluronan oligosaccharides that interact with HABP. Using these oligosaccharides and modified hyaluronan-oligosaccharides, we examined the binding specificity of hyaluronan to HABP.
**Methods:** HA-Ch-hybrid oligosaccharides with defined structure were prepared by the transglycosylation reaction of bovine testicular hyaluronidase. Binding activities of oligosaccharides to HABP were estimated by a competitive ELISA-like method.
**Results:** A decasaccharide was known as the minimal chain length of hyaluronan required for binding to HABP. Decasaccharide, whose glucuronic acid was replaced by an unsaturated hexsaccharide at the non-reducing terminus or whose *N*-acetylglucosamine was replaced by *N*-acetylgalactosamine at the non-reducing terminus, lost the binding activities to HABP. This indicates an *N*-acetylglucosamine at the non-reducing termini is essential for the binding to HABP. It is possible to replace GlcUA $\beta$ -1-3GlcNAc from the second to fifth disaccharide units of the non-reducing termini with GlcUA $\beta$ -1-3GalNAc, therefore a series of five carboxyl groups in decasaccharide chain length is important for binding. It is likely that there is a specified part in the domain structure of the HABP, which strongly recognizes the structure of GlcNAc of the non-reducing termini. Also, next to that specific part (which recognizes GlcNAc), there is a basic part binding the series of five carboxyl groups (in the domain structure of the HABP). The parts of the hyaluronan-sugar chain that are longer than decasaccharide appear to be necessary for the stable binding of hyaluronan to HABP.
**Conclusion:** Essential structure of hyaluronan for binds to HABP was determined.

PO-29

# Goblet cells are involved in translocation of staphylococcal enterotoxin A in the intestinal tissue of house musk shrew (Suncus murinus)

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**Background:** Staphylococcal enterotoxin A (SEA) produced by Staphylococcus aureus is the most recognizable bacterial superantigen causing foodborne poisoning. Emesis is one of the primary symptoms of foodborne poisoning which is induced within 1 to 6 h after ingestion of toxin-contaminated food. To investigate the emetic mechanism of SEA, an animal model using house musk shrews was established. In this model, we have demonstrated that sensory stimulus of vomiting caused by SEA is transmitted from the abdominal viscera through the vagus and sympathetic nerve. However, the entry site of SEA from the gastrointestinal lumen remains to be clarified. In this study, we observed the localization of SEA in the gastrointestinal tissue of house musk shrew after peroral administration of SEA. In addition, we investigated an entry site of SEA during translocation across the gastrointestinal mucosal barrier in this animal.

**Methods and Results:** House musk shrews were per orally administered with recombinant SEA and localization of SEA in gastrointestinal tissues was investigated by immunohistochemistry and immunoelectron microscopy 30 min after administration. SEA was detected in a subset of intestinal epithelial cells and lamina propria in the villi of jejunum and ileum. This observation was also found in gastrointestinal loops. Morphological characteristics of the SEA-immunopositive cells indicated that goblet cells are an entry site of SEA. SEA entered mucus-expelling goblet cells and the induction of mucus secretion by alyll isothiocyanate resulted in an intensive SEA signal.

**Conclusion:** SEA translocates across intestinal epithelia via mucus-expelling goblet cells. In addition, mucus secretion by goblet cells is important for the translocation of SEA.

# 弘前メディカルサイエンスフォーラム規約

## 名　称

第１条：本会は弘前メディカルサイエンスフォーラム（以下「本フォーラム」という）と称する。

## 目的および事業

第２条：本フォーラムは国内・国外の研究者との交流を通じて医学研究の発展を図ることを目的とする。

第３条：本フォーラムは前条の目的を達成するために次の事業を行う。

１．総会および学術集会の開催。

２．その他、本フォーラムの目的達成のために必要な事業。

第４条：本フォーラムの事務局を弘前大学医学研究科内に置く。事務局は、本フォーラムの運営に関する庶務ならびに経理事務を行う。

## 会　員

第５条：本フォーラムの会員は原則として、弘前大学医学研究科ならびにその他の医療機関に在籍し、本フォーラムの目的および事業に賛同する医師ならびに研究者とする。ただし、非会員であっても本フォーラムの学術集会のみに出席する者は当日会員とする。

## 組　織

第６条：本フォーラムに次の役員を置く。

１．会長１名、幹事若干名、監事２名。

２．会長は弘前大学医学研究科長とする。

第７条：本フォーラムに次の組織を置く。

１．実行委員長１名。

２．実行委員長は実行委員会を組織する。

## 会　計

第８条：学術集会開催時に会員より会費を徴収する。年会費は原則として徴収しない。

第９条：本フォーラムの会計は事務局が管理する。

第10条：本フォーラムの会計年度は毎年４月１日に始まり翌年３月31日に終わる。

本フォーラムの決算については毎会計年度終了後、監事の監査を経た上で、役員会の承認を得なければならない。

## 付　則

１．本規約は令和元年６月１日から実施する。

２．本規約の変更は役員会の審議を経たのち、総会の承認を経て行われる。

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