# International Meeting on RECQ Helicases and Related Diseases 2018


- Home
- Greetings
- General Information
- Program
- Call for Abstracts
- Venue / Access
- Accommodation
- Registration
- Contact Us


伝説と情緒のまち
木更津市観光協会
Kisarazu city tourism association

## Greetings


**Koutaro Yokote, M.D., Ph.D.**
Department of Medicine
Chiba University Graduate School of Medicine


### Dear Colleagues,

It is our great pleasure and privilege to welcome you to the international meeting on RecQ Helicases and Related Diseases to be held on February 16-18, 2018 in Chiba, Japan.

Werner, Bloom, Rothmund-Thomson, Hutchinson-Gilford Progeria, Cockayne syndromes and xeroderma pigmentosum are rare genetic disorders that are referred to as progeroid syndromes. They are caused by mutations in genes that encode DNA helicases or are involved in DNA damage response and regulation of nuclear functions. The patients suffer from premature aging symptoms, and their precise mechanisms remains to be elucidated.

Currently, the world is experiencing the most advanced “population aging” in the human history. The United Nations predicts that 42.5% and 27.9 % of the citizens will be over 65 years old in 2050 in Japan and the USA, respectively. Therefore, most of developed countries are struggling to become prepared for the upcoming super-aged societies.

In accordance with this situation, the organizing committee of this symposium views aging science as an urgent need. This three-day conference will provide a unique opportunity for cutting-edge researchers, physician scientists and clinicians who are interested in RecQ helicases and related diseases as well as aging research to exchange the highest level of basic and clinical insights. In addition, patients and family sessions will cover topics aiming at improvement in health management and quality of life. The goal of this conference is to merge our knowledge and raise new translational science on progeroid syndromes and general aging.

We strongly hope, and are confident, that this conference will provide an exciting, stimulating and fruitful experience for all the participants. We look forward to seeing you and have discussion in Chiba soon.

This symposium is organized by the RECQ2018 steering committee with the Japan Intractable Diseases (Nanbyo) Research Foundation.

Sincerely,

# General Information

## • Dates

Friday, February 16 – Sunday, February 18, 2018

## • Venue

Kazusa Akademia Hall
Address : 2-3-9 Kazusakamatari, Kisarazu, Chiba 292-0818 Japan
Tel : +81-438-20-5555
URL : https ://www.okura-nikko.com/japan/kisarazu/okura-akademia-park-hotel/

## • Language

The official language of the congress is English. Sessions and posters will be presented in English. Simultaneous interpretation will not be provided.

## • Registration & Information

The Registration & Information Desk will be open during the following hours :

| Date | Open Hours |
|---|---|
| February 16 | 11 : 45 - 19 : 00 |
| February 17 | 8 : 00 - 18 : 00 |
| February 18 | 7 : 45 - 12 : 00 |

## • On-site Registration Fee

| Category | On-Site Registration Fee |
|---|---|
| General | JPY 25,000 |
| Student | JPY 10,000 |
| Accompanying Person | JPY 6,000 |
| 1Day | JPY 10,000 |
| Welcome Reception | JPY 4,000 |
| Banquet | JPY 3,000 |

## • Name Badges

All congress participants are kindly requested to wear their name badges at all times in order to attend the Scientific Sessions, Luncheon Seminar and Morning Seminar.

### • PC Center

Computers will be available for oral presentation speakers to preview their presentations.
Speakers are encouraged to report to the PC Center, located on the Foyer on the day prior or at least 1 hour prior to their scheduled presentation.
PC Center will be open during the following hours :

| Date | Open Hours |
|---|---|
| February 16 | 11 : 45 - 19 : 00 |
| February 17 | 8 : 00 - 18 : 00 |
| February 18 | 7 : 45 - 12 : 00 |

### • For Chairpersons

Chairpersons are requested to be in the session room, preferably 15 minutes in advance of the scheduled start time.

### • Information for Speakers

Speakers for oral sessions are requested to visit PC Center located on the Foyer to submit and check your presentation data.
The projection screen for your presentation has standard 4 :3 aspect ratio, which is XGA resolution (1024 × 768 pixels).

If you are bringing USB flash memory stick, please pass to the operator to download/copy it to the server. The secretariat will take responsibility to delete the data after the meeting.
Macintosh users are required to bring your own laptop.
If video data is included in your presentation data, we highly recommend you to bring your own laptop.
PowerPoint is the only application accepted.

## 【Precautions for Bringing Media】

1. All the equipment are compliant for Windows 10.
*Please note that it is not compatible with Macintosh.
2. Windows PowerPoint 2007/2010/2013/2016 are acceptable.
3. Please use standard fonts such as Arial, Century, Times New Roman, etc.
4. If you are using video data, we recommend you to bring your own laptop.
5. Video data for PowerPoint presentations should be able to run in codec with default state of Windows10 (OS) and Windows Media Payer12. To keep it link with PowerPoint, please save your data in the same folder.
6. In order to play the data properly at your presentation, please make sure to check the data on a different computer.
7. There should be only your presentation data saved in your media.
8. Please check your data from other PC beforehand to avoid copying the wrong data.
9. Please check your data with Virus Scanner.
10. Please be sure to bring your back-up data with you.

If you bring your own laptop, in order to avoid trouble arising from software incompatibility, speakers are requested to use their own laptop computer for data projection.

## 【Precautions for Bringing Your Own Laptop】

1. Please cancel the password, screensaver, and power-saving settings of your computer in advance.
2. The connection for the output connector “Mini D-sub 15 pin.” (See illustration on the bellow) is available. If you have a different output connector, please bring an appropriate conversion connector. Also, please remember to bring your computer’s AC adapter.

3. Please make sure to prepare a back-up data on media besides the one on your computer.
4. After reviewing your data at PC Center, please take your laptop with you to the Computer Operation Desk 20 minutes prior to your presentation. The Operation Desk is located at the front left side of your session room.

### • Poster Sessions

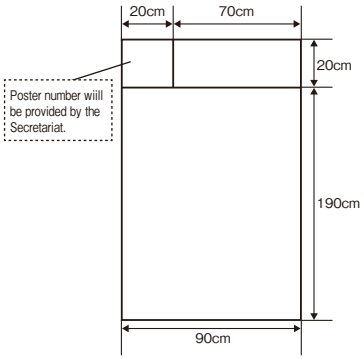
Speakers of the Poster Sessions are requested to stand in front of your poster during the Free Discussion period.
Please note that any posters left after the poster removing time will be removed and discarded without notice.
The congress organizer is not responsible for any loss or damage to each poster if it is not dismantled during the poster removing time.

### • Time for Mounting, Discussion, Removing

| Date | Poster Mounting Time | Free Discussion | Poster Removing |
|---|---|---|---|
| February 16 | 12 : 00 - 15 : 00 | 17 : 40 – 19 : 10 | |
| February 17 | | | |
| February 18 | | | 8 : 00 – 11 : 00 |

## • Size of Poster

Poster board measures
W90cm×H210cm (Vertical).
Poster numbers will be posted in the upper left of the board.
Please prepare a list of your abstract title, name and affiliation of presenter sized W70cm×H20cm to be put next to the poster number.




## • Social Programs

**Reception**

Time & Date : 19 : 30-21 : 30, Friday, February 16, 2018
Venue : Okura Akademia Park Hotel (5 minutes by walk. The hotel is adjacent to the venue.)
Fee : 4,000 JPY

**Banquet**

Time & Date : 19 : 30-21 : 30, Saturday, February 17, 2018
Venue : Takaraya (30 minutes by shuttle bus from the venue.)
※ Takaraya located in Kisarazu, Chiba was established in 1897.
Thereafter they have been serving the local taste of Kisarazu for more than 100 years with main menu of Edo-style seafood including popular a short-necked clam dish. They always provide the ingredients that are carefully selected in Kisarazu including the vegetables which they harvested near Boso peninsula.
Fee : 3,000 JPY

## *Meeting Scedule*

# *International Meeting on RECQ Helicases and Related Diseases 2018*

Friday 16 - Sunday 18 February 2018, Kazusa Akademia Hall, Chiba, JAPAN

---

| **Day1 Feb 16th (Fri)** | |
|---|---|
| 12:30-12:40 | **Opening Remarks**<br>Koutaro Yokote (Chiba University, Chiba, Japan) |
| 12:40-13:30 | **Plenary Lecture 1**<br>Chair: Koutaro Yokote (Chiba University, Chiba, Japan)<br>The Werner Syndrome: Past, Present & Future<br>George M. Martin (University of Washington, Seattle, USA) |
| | **Session 1 Clinical Features and Genetics of Werner Syndrome**<br>Chairs: Raymond J. Monnat (University of Washington, Seattle, USA)<br>Seijiro Mori (Tokyo Metropolitan Geriatric Hospital and Institute of Gerontology, Tokyo, Japan) |
| 13:30-14:00 | Lessons from Werner syndrome :A biased view from a rheumatologist<br>Makoto Goto (Nerima Hikarigaoka Hospital, Tokyo, Japan) |
| 14:00-14:30 | Genomic clues to Werner syndrome disease pathogenesis and cancer risk<br>Raymond J. Monnat (University of Washington, Seattle, USA) |
| 14:30-15:00 | International Registry of Werner Syndrome: Search for progeroid syndrome mutations and mechanisms<br>Junko Oshima (University of Washington, Seattle, USA) |
| | **Session 2 Bloom Syndrome and Stem Cell Aging in Skin**<br>Chairs: Nathan A. Ellis (University of Arizona, Tucson, USA)<br>Seiichiro Motegi (Gunma University, Maebashi, Japan) |
| 15:10-15:40 | NSMCE2 is required for the generation of sister chromatid exchanges at collapsed replication forks<br>Nathan A. Ellis (University of Arizona, Tucson, USA) |
| 15:40-16:10 | Altered Nucleolar Trafficking of the Blm Helicase in the Mouse Reduces Size, Increases Tumor Susceptibility and Genome Instability, and Facilitates Aging<br>Joanna L. Groden (The Ohio State University, Columbus, USA) |
| 16:10-16:40 | Stem cells orchestrate hair follicle aging program<br>Emi Nishimura (Tokyo Medical and Dental University, Tokyo, Japan) |
| | **Patients and Families Session**<br>Chairs: Joanna L. Groden (The Ohio State University, Columbus, USA)<br>Yoshiro Maezawa (Chiba University, Chiba, Japan) |
| 16:40-17:00 | From Werner syndrome patient group |
| 17:00-17:15 | From Cockayne syndrome patient group |
| 17:15-17:35 | From Rothmund-Thomson syndrome patient group |
| 17:40-19:10: | **Poster Session** |
| | **Welcome Reception at Hotel Okura** |

## Day2 Feb 17th (Sat)

08:30-09:20 **Plenary Lecture 2**

Chair: Hironori Nakagami (Osaka University, Osaka, Japan)

Werner syndrome: mechanisms and intervention
Vilhelm A. Bohr (National Institute of Aging, Bethesda, USA)

**Session 3 Travel Award Presentations and Mini Presentations of Poster Presenters**

Chairs: Junko Oshima (University of Washington, Seattle, USA)
Minoru Takemoto (International University of Health and Welfare, Narita, Japan)

09:20-11:20 Travel Awardees and All the Poster Presenters

**Room B**

11:30-12:20 **Luncheon Seminar (Sponsored by Novo nordisk Pharma Limited)**

Chair: Shigeki Kuzuhara (Suzuka University of Medical Science, Suzuka, Japan)

Werner Syndrome Research in Japan: For the Patient and Aging Science
Koutaro Yokote (Chiba University, Chiba, Japan)

**Patients and Families Meeting (in Japanese)**

Chairs: Kazuki Kobayashi (Asahi General Hospital, Chiba, Japan)
Kinue Kuzuta (Chiba University, Chiba, Japan)

09:20-11:20 From Patient group of Werner, Cockayne, Rothmund-Thomson syndromes,Researchers and Doctors

**Session 4 Rothmund-Thomson Syndrome, Mitochondrial Dysfunction and Aging**

Chairs: Lisa L. Wang (Texas Children's Hospital, Baylor College of Medicine, Houston, USA)
Hideo Kaneko (Nagara Medical Center, Gifu, Japan)

12:30-13:00 Update on Clinical Studies of Rothmund-Thomson Syndrome and RECQL4 Disorders
Lisa L. Wang (Texas Children's Hospital, Baylor College of Medicine, Houston, USA)

13:00-13:30 Role of RECQL4 in genome maintenance
Deborah L. Croteau (National Institute on Aging, Bethesda, USA)

13:30-14:00 Mitol dependent ubiquitylation of RECQL4 prevents its function as an accessory factor for mitochondrial DNA replication
Sagar Sengupta (National Institute of Immunology, New Delhi, India)

14:00-14:30 Roles of mitochondrial ubiquitin ligase MITOL in mitochondrial dynamics and aging-related diseases
Shigeru Yanagi (Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan)

**Problem Oriented Sessions for Patients (in Japanese)**

Chair: Kazuki Kobayashi (Asahi General Hospital, Chiba, Japan)

13:00-13:20 Diabetes mellitus and metabolic syndrome
Yoshiro Maezawa
(Chiba University, Chiba, Japan)

13:20-13:40 Skin ulcer and its prevention
Seiichiro Motegi
(Gunma University, Maebashi, Japan)

13:40- Good shoes for your health
Meister Karsten Rieche
(Nature's Walk, Chiba, Japan)

| | |
|---|---|
| 14:30-15:20 | **Plenary Lecture 3** |
| | Chair: Yasuhiro Furuichi (GeneCare Research Institute, Kamakura, Japan) |
| | Genome-Wide promotome and enhancerome analysis based on CAGE technology and its application to the identification of cancer biomarkers<br>Yoshihide Hayashizaki (Riken, Wako, Japan) |
| | **Session 5 Aging related Diseases and Hutchinson Gilford Progeria Syndrome (Co-sponsored by Daiichi Sankyo Company, Limited)** |
| | Chairs: Vilhelm A. Bohr (National Institute of Aging, Bethesda, USA)<br>Kenji Ihara (Oita University, Oita, Japan) |
| 15:40-16:10 | Mitotic death of cancer cells by gene silencing of RECQ helicases: a preferential sensitivity of ovarian cancer cells to RECQL1-siRNA<br>Yasuhiro Furuichi (GeneCare Research Institute, Kamakura, Japan) |
| 16:10-16:40 | Role of Akt in skeletal muscle in anti-aging<br>Kohjiro Ueki (Tokyo University and National Center for Global Health and Medicine, Tokyo, Japan) |
| 16:40-17:10 | Immunometabolic regulation of cardiac homeostasis and heart failure in aging<br>Ichiro Manabe (Chiba University, Chiba, Japan) |
| 17:10-17:40 | The clinical characteristics of Asian patients with classical-type Hutchinson-Gilford progeria syndrome<br>Kenji Ihara (Oita University, Oita, Japan) |
| 17:40-18:30 | **Special Lecture:** |
| | Programs and Progress towards Treatments and The Cure For Children with Progeria |
| | Leslie B. Gordon (Brown University, Providence, USA) |
| | **Photo Session** |
| | **Dinner at Takaraya** |

| Day3 Feb 18th (Sun) | |
|---|---|
| 08:00-08:50 | **Morning Seminar (Sponsored by MSD K.K.)**<br>Chair: Yoichi Nabeshima (Foundation for Biomedical Research and Innovation, Kobe, Japan)<br>Roles and mechanisms of cellular senescence in aging and cancer<br>Eiji Hara (Osaka University, Cancer Institute, Osaka and Tokyo, Japan) |
| 09:00-09:50 | **Plenary Lecture 4**<br>Chair: Katsuo Sugita (Chiba University, Chiba, Japan)<br>Genome maintenance protects from aging and cancer: the impact of nutrition<br>Jan H. J. Hoeijmakers (Erasumus University, Rotterdam, Netherland) |
| | **Session 6 Xeroderma Pigmentosa and Cockayne syndrome**<br>Chairs: Chikako Nishigori (Kobe University, Kobe, Japan)<br>Jan H. J. Hoeijmakers (Erasumus University, Rotterdam, Netherland) |
| 09:50-10:20 | Coordinated DNA damage recognition by xeroderma pigmentosum gene products<br>Kaoru Sugasawa (Kobe University, Kobe, Japan) |
| 10:20-10:50 | The present status of Xeroderma pigmentosum in Japan-evaluation of symptoms by severity scale score<br>Chikako Nishigori (Kobe University, Kobe, Japan) |
| 10:50-11:20 | Very mild Japanese Cockayne syndrome (type-IV) cases with a N-terminal truncation mutation in the ERCC6 / CSB gene<br>Tomoo Ogi (Nagoya University, Nagoya, Japan) |
| | **Session 7 Can iPS cells be Future Therapeutics?**<br>Chairs: Koji Eto<br>(Center for iPS Cell Research and Application, Kyoto and Chiba University, Chiba, Japan)<br>Guanghui Liu (Institute of Biophysics, Chinese Academy of Science, Beijing, China) |
| 11:30-12:00 | Using stem cell and gene editing techniques to study and treat aging-associated disorders<br>Guanghui Liu (Institute of Biophysics, Chinese Academy of Science, Beijing, China) |
| 12:00-12:30 | In vitro modeling of bilateral progressive hearing loss with human iPSC technology: Cellular pathology and drug discovery<br>Masato Fujioka (Keio University, Tokyo, Japan) |
| 12:30-13:00 | Patient-specific iPS cells for neural disease modeling and drug screening<br>Wado Akamatsu (Juntendo University, Tokyo, Japan) |
| 13:00-13:10 | **Closing Remarks**<br>Koutaro Yokote (Chiba University, Chiba, Japan) |

Abstracts

## Plenary Lecture 1

## The Werner Syndrome: Past, Present & Future

George M. Martin

Univ. of Washington, Seattle, WA, USA

Otto Werner first described this syndrome in 1904 while a German medical student. His thesis dissertation has been translated to English by Holger Hoehn (Adv. Exp. Med. Bio., 1982). My own involvement occurred around 1960, when I was asked by the late Charles Epstein to review the pathology of Werner Syndrome (WS), the results of which became part of a 1966 review (PMID: 5327241). A 1961 fellowship with Prof. Guido Pontecorvo at Glasgow Univ. aimed at utilizing primary cultures of human diploid fibroblasts for the mapping of autosomal loci via mitotic combination was frustrated by what became known as the Hayflick limit. Those efforts, however, led to a major confirmation of that phenomenon in 1970 (PMID: 5431223); that publication also documented striking limitations of the growth of such cells from WS patients. The first international meeting on WS was held in Kyoto in 1982. In 1996, the WS gene was mapped to the short arm of chromosome 8, with major contributions by both Japanese (M. Goto, Y. Furuichi & colleagues) and our Seattle group, which later that year cloned the gene (WRN, a member of the RecQ family of helicases). Junko Oshima was a co-first author on that publication. There followed research led by Wil Bohr and others that provided evidence of an important role of WRN in DNA replication, repair, transcription and recombination.

As of this writing, some 1,039 publications on various aspects of WRN are listed on PubMed. The University of Washington's International Registry of Werner Syndrome (http://www.wernersyndrome.org/registry/registry.html) is now collaborating with Japanese colleagues. Our Registry has registered some 50 cases of suspected WS who did not have any detectible *WRN* mutations. We have operationally characterized these as cases of Atypical WS. Strikingly, with one exception (a lipodystrophy, PMID: 27868354), all of their mutations impacted genomic instability (see, e.g., PMID: 29105242). Those observations strongly support the proposition that genomic instability is a fundamental mechanism of biological aging.

Looking ahead, I would like to open a discussion concerning priorities for future research on WS and related disorders. First and foremost, we must greatly enhance our efforts on translational research. I know that our Japanese colleagues are very aware of the need to break the cycle of intractable lower limb ulcers, amputations and impaired mobility in WS patients. Given the striking advances in genomic editing, could we engineer isogenic cultures from epidermis and dermis to restore normal WRN functions and use such cultures to heal ulcerations? Using the methods of somatic cell genetics, could we discover alleles at various loci that act to suppress the phenotypic consequences of *WRN* mutations, seek to understand the underlying mechanisms, and use that knowledge to develop pharmacological interventions? Second, should we devote more attention to the discovery of potential impacts of environmental agents upon the health of *WRN* heterozygotes? Third, should we take Ray Monnat's advice and pursue research on the impacts of polymorphic variants of *WRN* upon a wide range of common disorders (PMID: 29146545)? Fourth, should we be paying more attention to Grandpa George and his colleagues, who argue that not enough attention is given to the discovery of Antigeroid Syndromes (see, e.g., PMID: 26931459)? Finally, I would appreciate critical comments from my colleagues concerning the possibility that stochastic age-related variegations in the expressions of RecQ and other loci of relevance to the pathobiology of aging might play roles in either the accelerations or ameliorations of aging phenotypes (see, e.g., PMID: 16027353, PMID: 19732045, PMID: 21963385).

## Plenary Lecture 2

## Werner syndrome: mechanisms and intervention

Vilhelm A. Bohr

National Institute on Aging, NIH, USA

Werner syndrome is a debilitation disease that in many ways reflects an accelerated aging process. It is caused by a mutation in WRN gene. WRN is a very interesting protein with many functions in DNA and cellular maintenance. It has a number of biochemical properties: DNA helicase, exonuclease, ATPase, and strand annealer. It is interesting and challenging how these properties work together in preventing aging and cancer and WRN also interacts with many proteins and participates in several pathways. It has a major role in DNA repair, participating in different pathways, which I will discuss (1).

There is also emerging evidence for a role of WRN in mitochondrial health. Mitochondria are the energy stations in cells and are essential for organismal function. Using human cells and nematode animal models we find that WRN plays a role in the important process that discards damaged mitochondria, called mitophagy. Mitophagy is required to maintain a healthy pool of mitochondria and this process can be enhanced by the use of specific interventions. Some of these are without side effects and may help the patients in the future.

1: Shamanna RA, Croteau DL, Lee JH, Bohr VA. Recent Advances in Understanding Werner Syndrome. F1000Res. 2017 Sep 28;6:1779. Review. PubMed PMID: 29043077.

## Plenary Lecture 3

### Genome-Wide promotome and enhancerome analysis based on CAGE technology and its application to the identification of cancer biomarkers

Yoshihide Hayashizaki

Director of RIKEN Preventive Medicine and Diagnosis Innovation Program (PMI)

In the past 15 years, nearly 1000 scientists from various research fields have taken part in the FANTOM Consortium to analyze genomic data produced with their original technologies, including a series of full-length cDNAs technologies and, more recently, expression profiling by CAGE (cap-analysis gene expression). The consortium has provided the most comprehensive mouse cDNA collection and extensive maps of the transcriptome containing promoters, enhancers, as well as the transcription regulation network.

With CAGE, a technology for comprehensive mapping and quantification of transcription starting sites in a single-nucleotide resolution, the consortium started to produce new type of data—transcription regulation network. The next-generation sequencers produce sufficient number of reads to analyze networks. Consequently, this study revealed a complex interaction of transcription factors.

The FANTOM consortium subsequently started 5$^{th}$ phase project to provide genome-wide transcriptome maps covering wide variety of human cell type by using CAGE. The consortium produced the largest mammalian transcriptome atlas by identifying more than 180,000 novel promoters. The co-expression analysis revealed that the CAGE expression profile reflects biology of cell based on their function and developmental lineages. With this findings we aimed at identification of cancer prognosis prediction. Currently some projects proceeded to validate obtained biomarkers candidates, including the lung cancer sub-type biomarkers and the lymph node metastasis biomarkers for the uterine body cancer.

Previously, it had been shown that enhancers are broadly marked by bidirectional transcription by another research groups. In the FANTOM data, we found that such enhancer-derived RNAs are expressed with a strong tissue and cell specificity. This led to the creation of a map of 44,000 active enhancers. Further analysis revealed a strong association between 63 common diseases and genetic variants at enhancers, indicating a potential of enhancer region as a new target of disease biomarker exploration.

In 2013, we started Preventive Medicine and Diagnosis Innovation Program, which aims at medical application of these omics technology, data and knowledge. Functional omics analysis is widely applicable in personalized and translational medicine among many practice areas. The program is developing a comprehensive medical care system that includes rapid diagnosis system, a new electric medical record that helps physicians make a decision on drug usage and dosage based on patients' genomic data.

Also, with FANTOM data set we aimed at identification of cancer prognosis prediction. Currently some projects proceeded to validate obtained biomarkers candidates, including the lung cancer sub-type biomarkers and other sort of cancers.

## Plenary Lecture 4

## Genome maintenance protects from aging and cancer: the impact of nutrition

Wilbert Vermeij[1,2)], Dick Jaarsma[3)], Akos Gyenis[1.4)], Joris Pothof[1)] and Jan H.J. Hoeijmakers[1.3.4)]

1)Dept. of Molecular Genetics, Erasmus MC, Rotterdam, The Netherlands
2)Princess Máxima Pedriatic Oncology Center, Utrecht, The Netherlands
3)Dept. of Neurosciences, Erasmus MC, Rotterdam, The Netherlands
4)CECAD, University of Cologne, Germany

The molecular basis of aging(-related diseases), including cancer, is one of the main unsolved questions in biology. Aging appears remarkably plastic: e.g. suppressing insulin signalling extends lifespan in worms, flies and mice. On the other hand, virtually all premature aging syndromes in man provide strong links with genome instability. We have generated mouse models which strikingly mimic human DNA repair deficiency disorders, including xeroderma pigmentosum (XP) and Cockayne syndrome (CS) carrying defects in nucleotide excision and transcription-coupled repair and displaying cancer predisposition and accelerated aging respectively. The intimate connection between persisting DNA damage and many features of aging is supported by dose-response relationships and by specific DNA repair pathways linked with specific segmental aging features. E.g. *Ercc1*$^{\Delta/-}$ mice defective in 4 repair pathways show extensive premature multi-morbidity in virtually all post-mitotic and proliferative tissues limiting lifespan to 4-6 month. Simultaneously, these mice exhibit an anti-aging 'survival response', which suppresses growth and enhances maintenance, resembling the longevity response induced by dietary restriction (DR), providing a link between DNA damage and the insulin signaling control of aging. Interestingly, subjecting the progeroid, dwarf mutants to actual (30%) DR tripled remaining lifespan, and drastically retarded numerous aspects of accelerated aging, with the neuronal system benefitting disproportionally. E.g. DR animals retained 50% more neurons in the neocortex and maintained full motoric function, delaying motor decline >20(!)-fold. Repair-deficient *Xpg*$^{-/-}$ mice also showing many premature aging symptoms responded similarly. The DR response in *Ercc1*$^{\Delta/-}$ mice resembled wt DR including (further) reduced insulin signaling. Interestingly, *ad libitum Ercc1*$^{\Delta/-}$ liver expression profiles showed declining expression of long genes, consistent with genome-wide accumulation of stochastic, transcription-blocking lesions, which affect long genes more than short ones. Similar aging-related transcriptional stress was discovered in human brain profiles, demonstrating relevance for normal aging in humans. DR in repair-deficient mice alleviated this decline, indicating that DR prolongs genome function. We found DR to reduce spontaneous DNA damage load, explaining the strong response of DNA repair deficient progeroid mice to DR. We will present phenotypes of conditional DNA repair models targeting aging to selected organs and connections with the unfolded protein response and proteinopathies (Alzheimer's and Parkinson diseases). Our findings strengthen the link between DNA damage and aging, establish *Ercc1*$^{\Delta/-}$ mice as powerful model for identifying interventions to promote healthy aging, reveal untapped potential for reducing endogenous damage, provide new venues for understanding the molecular mechanism of DR, and indicate a counterintuitive DR-like therapy for progeroid syndromes and DR-like interventions for preventing neurodegenerative diseases.

## Special Lecture

### Programs and Progress towards Treatments and The Cure For Children with Progeria

Leslie B. Gordon

Medical Director, The Progeria Research Foundation
Professor of Pediatrics Research, Warren Alpert Medical School of Brown University and Department of Pediatrics, Hasbro Children's Hospital, Providence, RI
Department of Anesthesia, Boston Children's Hospital and Harvard Medical School, Boston, MA

Hutchinson-Gilford Progeria Syndrome (HGPS, Progeria) is an extremely rare, uniformly fatal, segmental premature aging disease in which children die of the consequences of premature atherosclerosis leading to heart attacks and strokes at an average age of 14.6 years. Over the past 18 years, the field of Progeria has gone from almost complete obscurity, to HGPS gene mutation discovery, an exponential increase in annual peer-reviewed research publications, a first-ever clinical drug trial and subsequent publication of the first treatment for HGPS, the identification of children with Progeria worldwide, and a highly active ongoing search for new treatments and the cure. This presentation will review the natural history of disease in Progeria, describe The Progeria Research Foundation's essential and generalizable programs that have led to key advancements towards treatments and cure, and discuss the challenges that HGPS and other rare diseases must address in developing concrete, morbidity-relevant, objectively measurable clinical biomarkers for successful assessment of whether a drug has influenced disease.

## Morning Seminar

### Roles and mechanisms of cellular senescence in aging and cancer

Eiji Hara

Department of Molecular Microbiology, Research Institute for Microbial Diseases, Osaka University

Due to improvements in healthcare and lifestyle over the past decades, human life expectancy has increased dramatically and people are now living longer, especially in developed countries. Ironically, however, extended lifespan has resulted in a startling rise in the incidence of various intractable diseases, such as cancer. To have a meaningful impact on the continued healthcare and well-being of this aging population, there is an urgent need for increased understanding of the molecular mechanisms underlying aging-associated intractable diseases, such as cancer. Cellular senescence is the state of essentially irreversible cell cycle arrest that can be induced by a variety of potentially oncogenic stimuli, such as telomere erosion, oxidative stress or activation of certain oncogenes and is therefore considered to act as an important tumor suppression mechanism. However, emerging evidence indicates that senescent cells may also promote deleterious side effects including chronic inflammation and cancer promotion. It is therefore quite possible that accumulation of senescent cells in vivo may contribute to aging –associated intractable diseases. Here, I discuss the molecular events associated with these two faces of cellular senescence, focusing on the pro-inflammatory side effects. I believe that a better understanding of the molecular mechanisms involved will lead to new strategies for the prevention of aging and aging-associated diseases.

## Luncheon Seminar

## Werner Syndrome Research in Japan: For the Patient and Aging Science

Koutaro Yokote

Department of Medicine, Chiba University Graduate School of Medicine

Werner syndrome (WS) is an autosomal recessive disorder caused by mutations in the RecQ helicase, *WRN*. WS is known for aged appearance and early onsets of age-related disorders starting after adolescence. Those symptoms include the graying and loss of hair, cataracts, skin atrophy, diabetes mellitus, atherosclerosis and malignancies. Due to the presence of a founder mutation, WS is more prevalent in Japan compared to the Western countries as well as other Asian countries. With a support of the Japanese Ministry of Health, a research group led by Dr. Etsuro Ogata at the University of Tokyo made a diagnostic criteria for WS in 1984 based on clinical signs and growth capacity of dermal fibroblasts. Dr. Makoto Goto at the Otsuka Hospital and Drs. Seijiro Mori and Shunichi Murano at the Chiba University further refined the clinical features focusing on the inflammation and metabolic derangement.

A breakthrough was achieved by identification of the causal gene, *WRN*, by an international team of Drs. Junko Oshima and George Martin at University of Washington in collaboration with Dr. Tetsuro Miki at Osaka University. Dr. Yasuhiro Furuichi at GeneCare Institute played a pivotal role in the elucidation of *WRN* functions and its regulations. Meanwhile, clinical managements of WS particularly in the aspect of diabetes and dyslipidemia have been improved over time, leading to the extension of patients' life spans by several years. However, the mechanisms underlying premature aging symptoms remain unclear partly due to absence of appropriate animal models, and the patients were left behind from the development of curative therapy.

In 2009, a new WS research consortium was organized with a support of the Ministry of Health, Labour and Welfare to carry out a nation-wide survey in Japan together with systematic review of literature. According to the survey data, revised diagnostic criteria including genetic examination and the management guideline were published in 2012. In 2010, an advocacy association of WS patients and families was established for the first time, enabling active exchange of information between patients and researchers. With tremendous efforts by the association, WS was designated as "Nanbyo (intractable disease)" by the Japanese government in 2015, earning financial support for the medical care. Cockayne syndrome, Rothmund-Thomson syndrome and Bloom syndrome are now also supported by the Japanese government as Nanbyo. The Japanese Werner syndrome registry was also initiated in 2015 supported by the AMED (Agency for Medical Research and Development) with expectation to provide valuable clinical data in the near future. In addition to Werner syndrome, a nation-wide survey on Hutchinson-Gilford progeria syndrome was performed recently, which clarified the characteristics of Asian patients.

Advancement of novel technologies may shed light to the mechanistic insights into WS. Inducible pluripotent stem (iPS) cells established from WS fibroblasts by Dr. Akira Shimamoto and other groups will be a promising tool to identify molecular details underlying the disease. We have also obtained several lines of patient iPS cells derived from the peripheral blood using Sendai virus to minimize genomic damage, expected to be useful in a variety of assays. We hope that Werner syndrome research will proceed into the new era leading to cure of the disease and to contribute to the understanding of human aging in general.

## Session 1 Clinical Features and Genetics of Werner Syndrome

### Lessons from Werner syndrome :A biased view from a rheumatologist

Makoto Goto

Department of Rheumatology, Nerima-Hikarigaoka Hospital

{Background} Human ageing is a dynamic life process, beginning after programmed development and maturation, followed by an increasing chance of diseases and death. Human ageing is globaly programmed and finely modulated by a summation of environmental insults such as infections, mutations, life-style damages and chronic diseases. Historical ideas on ageing so-far proposed could be summarised that ageing is a decreasing adaptation ability to homeostatic changes associated with diminishing cell number, and lower threshold against environmental attacks than the previous life stage, thus leading to an organismal death. Mechanistic theories on ageing suggested that with normal ageing, functions of most tissues/organs are declining because of 1) loss of cell number due to telomeric attrition in mitotic cells and tissue injury, 2) loss of nutritional /energy supply due to atherosclerosis and mitochondrial dysfunctions, 3) somatic mutations due to radiation and metabolic damages, 4) deposition of bioundegradable substances such as lipofuscin and amyloid fibrils, especially in post-mitotic cells/organs due to inflammation and autophagy dysfunctions. What does drive human ageing is still a complete mystery. However, the recent accumulating evidences propose an important rookie driver of minimal level of chronic inflammation on human ageing, coined as inflammageing.
{Clinical observations} When does ageing begin? There are possibly three ideas; 1) ageing begins immediately after birth in tandemly with growth and maturation, and 2) ageing starts immediately after teenage growth spurt. 3) ageing comes after menopausal stage in female, or similar age in male. I would like to select the second opinion.
How does ageing record or recognize? Characteristic ageing phenotypes in general population usually claimed are 1) gray hair/alopecia, skin atrophy, skin pigmentation, and wrinkles, 2) cataracts, presbyopia and dry eye, 3) hoarseness, dry mouth, toothlessness and dysphagia, 4) senile dementia, 5) hearing loss, 6) osteoporosis, sarcopenia, central obesity, kyphosis and arthritis, 7) gynecomastia ( male ), prostate hypertrophy ( male ), menopause ( female), and hypogonadism 8) diabetes mellitus, hyperlipidemia, hypertension, renal dysfunction and atherosclerosis, 9) decreased immunity, and malignancy. The patients with WS usually show the underlined signs and symptoms described above at relatively earlier age than usual. In addition to the normal ageing phenotypes, WS patients characteristically manifest 1) intractable skin ulcers, 2) skin sclerosis and painful corns on the sole of feet, 3) subcutaneous calcification, especially along with Achilles tendons, 4) mesenchymal malignancy such as melanoma and rare sarcomas. These connective tissue symptoms are rarely observed in normal ageing. Interestingly, the hierarchical deterioration pattern of body systems in WS have been rapidly changing, in correspond to the rapid pathophysiological changes in general ageing population.
{Clinical interventions} As a rheumatologist and a gerontologist, I have paid special attention to inflammatory signs and symptoms in WS from the beginning of my WS research. 1) As well documented, chronic inflammation may contribute the formation of age-related pathologies such as atherosclerosis, diabetes mellitus, central obesity, osteoporosis, Alzheimer disease, sarcopenia and cancer. 2) Pro-inflammatory proteins such as high sensitivity CRP, MMP-9, a variety of cytokines such as IL-4,6,15, GM-CSF, and TNF-$\alpha$,soluble FasL are significantly elevated in normal ageing and WS sera. 3) Average life-span has lengthened with the recent popularity of coloric restriction/ low carbohydrate diet in general population, leading to a reduction in inflammation. 4) Recent Lancet/NEJM papers reported a successful treatment of human atherosclerosis and a reduction of lung cancer incident by anti-IL-1 antibody, canakinumab.
{Summary & conclusion} Long-term and detailed survey on the WS case reports and general population indicates a significant change of ageing phenotypes and suggests an important contribution of the stochastic environmental changes over genetics in the general population and even in WS. Accumulating data concerning to inflammation suggest an important role of 'inflammageing' to make-up the age-associated pathologies in normal ageing and also in the premature ageing syndrome: Werner syndrome.

## Session 1 Clinical Features and Genetics of Werner Syndrome

### Genomic clues to Werner syndrome disease pathogenesis and cancer risk

Raymond J. Monnat, Jr.

Departments of Pathology and Genome Sciences, University of Washington, Seattle, WA 98195, USA

RECQ helicase proteins play key roles in DNA metabolism and genome stability assurance in virtually all organisms. Heritable loss of function mutations in the human *RECQ* helicase genes *WRN*, *BLM* and *RECQL4* cause the cancer predisposition syndromes Werner syndrome, Bloom syndrome and Rothmund-Thomson syndrome.

Positional cloning of the disease-associated *RECQ* helicase genes and the cloning of two related genes, *RECQ*L and *RECQL5*, has enabled a diversity of new approaches to understand physiologic roles of RECQ helicase proteins and the mechanisms that drive disease pathogenesis and cancer risk following the loss of RECQ function.

My talk will focus on the use of genomic data to understand WRN protein function, and the mechanisms that promote Werner syndrome disease pathogenesis and cancer risk. The starting point for this presentation will be *WRN* gene structure, and the spectrum of *WRN* mutations that have been associated with Werner syndrome or linked to other disease states or predispositions.

I will place these results against a backdrop of human population genetic variation in the *WRN* gene, and more recent work to test several explicit hypotheses: that DNA sequences with a propensity to form G-quadruplex (G4) structures are physiologic substrates for *WRN* action in human cells; that mtDNA alterations may be driving WS or WS-associated disease risk; and that the genomics of cancers arising in WS patients have unique genomic features. This last topic will draw on work performed as part of the TCGA DNA Damage Repair Analysis Working Group that will be published with the TCGA PanCancerAtlas Project in 2018.

In closing, I will reflect upon the most interesting—or to my thinking the most potentially revealing—directions for future work on Werner syndrome and the other RECQ helicase deficiency syndromes.

This work has been supported over the past two decades by a US National Cancer Institute Program Grant Award P01 CA77852, by US National Heart, Lung and Blood award R01 HL120948 and by a Nippon Boehringer Ingelheim Sponsored Research Agreement as part of the Virtual Research Institute of Aging Research Program between 2001 - 2004.

## Session 1 Clinical Features and Genetics of Werner Syndrome

### International Registry of Werner Syndrome: Search for progeroid syndrome mutations and mechanisms

Junko Oshima

Department of Pathology, University of Washington, Seattle, WA
Department of Medicine, Chiba University, Chiba, Japan

The International Registry of Werner Syndrome (University of Washington, Seattle, WA; www.wernersyndrome.org) was established in 1988 with the original purpose of collecting classical Werner syndrome (WS) cases for positional cloning of the *WRN* gene (Science 1996; 272:258). Patients are referred from all over the world for the molecular diagnosis of Werner syndrome. Those who do not carry *WRN* mutations were operationally categorized as “atypical Werner syndrome” (AWS). Our Registry has expanded its scope from WS to the search for causative mutations and mechanisms responsible for the broader range of progeroid syndromes. As of November 2017, the International Registry has accumulated clinical data and cryopreserved biological materials from 172 WS patients with documented *WRN* mutations and 102 AWS cases. A combination of next generation sequencing, SNP arrays and candidate gene sequencing have successfully identified novel mutations in subsets of AWS cases. Those AWS loci highlight major roles in DNA damage repair and response: *LMNA* (nuclear structure and chromatin interaction)(Lancet 2003; 362); *POLD1* (DNA polymerase delta) (Hum Mut 2015; 36:1070), *SPRTN* (recruitment of translesional DNA polymerase eta)(Nat Genet 2014; 24:1239); *ERCC4* (nucleotide excision repair)(Hum Mutat, in press), *MDM2* (an inhibitor of p53)(J Clin Invest 2017;127:3598); and *SAMHD1* (regulation of dNTP pools)(Am J Med Genet 2014; 164A:2510). Cases of a *BSCL2* mutation responsible for Seip syndrome (Am J Med Genet A 2017;173:471)as well as mosaic trisomy 8 were also identified. These findings continue to support the concept of genomic instability as a major mechanism of biological aging.

## Session 2 Bloom Syndrome and Stem Cell Aging in Skin

### NSMCE2 is required for the generation of sister chromatid exchanges at collapsed replication forks

Nathan A. Ellis, Kelvin W. Pond, Mary K. Yagle, Christelle de Renty

University of Arizona Cancer Center, Tucson, AZ, USA

The functions of the BLM helicase in replication fork stabilization, fork rescue, and homologous recombination repair are regulated by sumoylation, but the E3 SUMO ligase that catalyzes this post-translational modification remains unknown. To gain insight into which SUMO E3 ligase performs this function, we performed siRNA-mediated knockdowns of NSMCE2, PIAS1, and PIAS4 in a U2OS cell line that expresses a histidine-tagged SUMO2 followed by nickel-NTA pull-downs and Western blot analysis with antibodies to BLM and to RANGAP and SUMO2 as pull-down controls. Treatment of cells with hydroxyurea (HU) for 24 hours results in a nearly eightfold increase in SUMO2-BLM levels relative to untreated cells. Analysis of the levels of SUMO2-BLM in HU-treated and untreated cells revealed that knockdown of NSMCE2 and PIAS4 results in an approximately 65% reduction of SUMO2-BLM compared to control knockdown in HU-treated cells whereas knockdown of PIAS1 had no effect on SUMO2-BLM levels. Based on these results, we suggest that there is hierarchical control of BLM sumoylation by E3 ligases.

Studies in yeast had implicated the *NSMCE2* ortholog *MMS21* in regulation of the *BLM* ortholog *SGS1*. We previously found that expression of a *BLM* with mutations in the sumoylation acceptor sites in Bloom syndrome cells causes an increase in the levels of focal RPA but a decrease in the levels of focal RAD51. Thus, we initially predicted that cells in which NSMCE2 was knocked down would have similar phenotypes. On the contrary, NSMCE2-deficient cells exhibit a twofold increase in levels of focal RAD51 and severely decreased levels of focal RPA in HU-treated cells. Recruitment of BLM to stalled forks was also severely reduced. Analogous to results from yeast, sumo-binding sites on BLM very likely mediate its recruitment to stalled forks by NSMCE2 auto-sumoylation. With lower levels of BLM and higher levels of RAD51 at stalled forks, we predicted higher levels HU-induced sister chromatid exchange (SCE); but again, contrary to expectation, NSMCE2-deficient cells exhibit a defect in HU-induced SCE.

To investigate this RAD51-related paradox, we measured the levels of DSBs that accumulate after release from HU block in NSMCE2-deficient compared to control knockdown cells by pulsed-field gel electrophoresis. The rationale for this experiment is as follows. Prolonged treatment of cells with HU induces replication fork collapse, that is, replication forks are unable to resume DNA synthesis after release from the HU block but must be rescued by replication forks initiated at adjacent dormant origins in order to complete DNA replication. Rescue of collapsed forks is associated with the formation of double strand breaks (DSBs), and we hypothesize that HU-induced SCEs are generated during subsequent DSB repair. Consistent with this hypothesis, NSMCE2-deficient cells exhibit a defect in the generation of DSBs. Focal RAD51 levels remain high throughout S and G2 phases in NSMCE2-deficient cells whereas focal RAD51 is lost during S phase in normal cells. RAD51 accumulation at collapsed forks may be resolved during collapsed fork rescue and/or during repair of DSBs. The defect in DSB formation in NSMCE2-deficient cells is associated with two to threefold increases in the levels of ultra-fine bridge formation and anaphase bridges during mitosis and in micronuclei formation and 53BP1 foci in the subsequent G1 relative to normal cells. These data indicate that NSMCE2-deficient cells have a defect in completion of DNA synthesis. Our interpretation of these data is that excessive accumulation of RAD51 in NSMCE2-deficient cells blocks the convergence of active forks with collapsed forks, preventing rescue of collapsed replication forks. As a corollary of these findings, we conclude that an important source of SCEs is derived from the repair of DSBs during collapsed-fork rescue.

## Session 2 Bloom Syndrome and Stem Cell Aging in Skin

### Altered Nucleolar Trafficking of the Blm Helicase in the Mouse Reduces Size, Increases Tumor Susceptibility and Genome Instability, and Facilitates Aging

Michael McIlhattan, Max Fernandez, Samir Acharya, Vincenzo Coppola, Joanna Groden

Department of Cancer Biology and Genetics, The Ohio State University, Columbus, OH, 43210 USA

Ribosomal DNA (*rDNA*) stability is a critical determinant of cellular and organismal growth. Dysregulation of *rDNA* stability, affecting *rDNA* recombination, replication and transcription, is associated with reduced *rRNA* expression, ribosomes and protein synthesis, as well as genome-wide instability, cellular transformation and senescence. The recQ-like BLM helicase, mutated in an inherited form of dwarfism and cancer susceptibility, localizes to the nucleolus and is required for appropriate *rDNA* stability, transcription and DNA repair. Our studies show that BLM includes two serines within a highly conserved carboxy-terminal nuclear localization sequence that are critical for dynamic nucleolar trafficking of BLM where it facilitates *rRNA* transcription through direct interactions with RNA polymerase I and topoisomerase 1. CRISPR/*Cas9*-directed gene editing has generated two homozygous murine models with these two conserved amino acids substituted by aspartic acids or alanines, and Blm proteins that are invariably excluded (DD) or constitutively localized (AA) within the nucleolus. Both isoforms retain biochemical functions and nuclear localization. $Blm^{DD/+}$ and $Blm^{DD/DD}$ mice are smaller than their wild-type littermates at 8, 12 and 16 weeks of age, and develop significantly more adenomas than littermates when crossed to the $Apc^{Min/+}$ model of intestinal tumor formation. Finally, $Blm^{DD/DD}$ mice in comparison to wild-type littermates display signs of premature aging by one year, with weight loss, changes in fur growth and pigmentation, and alterations of *rDNA* copy number. These data provide a mechanistic understanding of how loss of nucleolar Blm affects organismal growth, genome stability and cancer susceptibility, and test how maintenance of the ribosomal genome may impact lifespan.

## Session 2 Bloom Syndrome and Stem Cell Aging in Skin

### Stem cells orchestrate hair follicle aging program

Emi K. Nishimura

Tokyo Medical and Dental University, Japan

Tissues and organs undergo structural and functional declines due to aging, yet the underlying mechanisms involved and whether the tissue aging is programed or not have been poorly understood. Hair thinning and graying, the typical aging phenotypes in mammals, are prominently found in many cases of segmental progeroid syndromes including Rothmund-Thomson syndrome and Werner syndrome. The hair follicle is a mini-organ of the skin that is specialized to grow pigmented hair. Hair follicle stem cells (HFSCs) and melanocyte stem cells (McSCs) reside in mammalian hair follicles to sustain the cyclic growth of pigmented hair during each hair cycle. To understand the mechanisms of tissue aging and the involvement of genomic instability, we have studied the physiological aging-associated changes in murine and human hair follicles, the involvement of genomic instability in the changes and the underlying mechanisms. In vivo fate analysis of stem cells in naturally aging hair follicles revealed that those stem cells undergo specific fate changes through the proteolysis of hemidesmosomal transmembrane collagen (COL17A1) upon DNA damage response, thereby causing aberrant differentiation and depletion of those stem cells and the resultant typical hair aging phenotypes in a stepwise manner. Furthermore, we found that the expression of those aging phenotypes can be prevented by the forced expression of COL17A1 in HFSCs. These results demonstrate the existence of a stem cell-centric aging program as the core to orchestrate tissue aging and the involvement of genomic instability in COL17A1 proteolysis and the resultant fate changes of stem cells. In this symposium, I will introduce the new concept of stem cell division program for aging and will discuss the role in tissue/organ aging and progeroid syndromes.

## Session 4 Rothmund-Thomson Syndrome, Mitochondrial Dysfunction and Aging

### Update on Clinical Studies of Rothmund-Thomson Syndrome and RECQL4 Disorders

Lisa L. Wang

Department of Pediatrics, Division of Hematology/Oncology, Texas Children's Hospital, Baylor College of Medicine, Houston, TX 77030

RECQL4 belongs to the family of RECQ DNA helicases that as a whole play important roles in DNA metabolism and maintenance of genomic stability. The human syndromes associated with three of these RECQ genes-WRN, BLM and RECQL4- have overlapping clinical features, including a strong predisposition to cancer. However, they are also clinically distinct from one another in other features such as the specific types of cancers that develop or the predominance of diseases associated with premature aging, likely reflecting the multifunctional nature of these proteins and the distinct roles they play among the different syndromes.

This talk will focus Rothmund-Thomson Syndrome which is caused in most cases by pathogenic variants in the *RECQL4* gene, as well as two allelic disorders RAPADILINO Syndrome and Baller-Gerold Syndrome which are also associated with *RECQL4*. Updates will be provided on clinical and translational research aimed at understanding genotype-phenotype associations based on data collected through the Rothmund-Thomson Syndrome Registry at Baylor College of Medicine in collaboration with the Rothmund-Thomson Syndrome Foundation, and will highlight the importance of partnership between academic research and family support groups in the study of rare disorders. Emphasis will be placed on efforts to understand the molecular basis of skeletal conditions that are frequently seen in patients with Rothmund-Thomson Syndrome.

## Session 4 Rothmund-Thomson Syndrome, Mitochondrial Dysfunction and Aging

### Role of RECQL4 in genome maintenance

Deborah Croteau

RECQL4 belongs to the RecQ family of DNA helicases that participates in DNA metabolism. Like Werner and Bloom, mutations in RECQL4 are associated with cancer-prone syndromes. RECQL4 is deficient in Rothmund-Thomson Syndrome and plays a major role in DNA replication and DNA repair. Recently, we have sought to describe its role in double strand break repair (DSB). DSB repair is a tightly regulated process that is critical for the faithful maintenance of genomic integrity. RECQL4 , promotes both major DSB repair pathways: non-homologous end joining (NHEJ) and homologous recombination (HR). I will be discussing how RECQL4 interacts with various protein players in NHEJ and HR, and how this differs across the cell cycle. Using in vitro and cellular assays combined with fluorescence microscopy, we have delineated how RECQL4 modulates DSB repair pathway choice by differentially regulating NHEJ and HR in a cell cycle-dependent manner. I will also describe how posttranslational modifications on RECQL4 modify its biochemical properties and the implications for these findings. Further understanding of the pathways and processes that RECQL4 participates in will help us understand why RECQL4 mutations promote cancer.

Lu H, et al. Cell cycle-dependent phosphorylation regulates RECQL4 pathway choice and ubiquitination in DNA double-strand break repair. Nat Commun. 2017
Dec 11;8(1):2039. doi: 10.1038/s41467-017-02146-3. PubMed PMID: 29229926.

This research was supported by the Intramural Research Program of the NIH, National Institute on Aging.

## Session 4 Rothmund-Thomson Syndrome, Mitochondrial Dysfunction and Aging

### Mitol dependent ubiquitylation of RECQL4 prevents its function as an accessory factor for mitochondrial DNA replication

Mansoor Hussain[1], Himanshi Agarwal[1], Ullas Kolthur-Seetharam[2], Sagar Sengupta[1]

1. National Institute of Immunology, New Delhi, India
2. Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, India

Mutations in RECQL4 lead to Rothumund Thomson Syndrome (RTS) characterized by predisposition to cancer and alterations in the aging process. RECQL4 belongs to the RecQ family of helicases which participates in mitochondrial DNA (mtDNA) replication by acting as an accessory factor to mitochondrial polymerase, Pol $\gamma$ A/B2. We found that RECQL4 is ubiquitylated in the tissues of aged mice, which causes its enhanced turnover. The enhanced degradation of RECQL4 is due to its interaction and subsequent K6-linked ubiquitylation by the mitochondrial E3 ligase Mitol at two specific lysine residues. Mitol mediated ubiquitylation of RECQL4 decreases its binding to Tom20, thereby diminishing its entry into the mitochondria. Consequently non-ubiquitylated RECQL4, which preferentially enters the mitochondria, can enhance Pol $\gamma$ A dependent polymerization and exonuclease activity. Once inside the mitochondrial matrix, RECQL4 is phosphorylated by mtPKA and thus becomes a substrate of Lon protease for its subsequent proteolysis. RTS patient mutants are hyper-ubiquitylated by Mitol which prevents its entry into the mitochondria and consequent function as an accessory factor for mtDNA replication. These results indicate that Mitol dependent ubiquitylation controls the entry of RECQL4 into the mitochondria and thereby its function during mtDNA replication.

## Session 4 Rothmund-Thomson Syndrome, Mitochondrial Dysfunction and Aging

### Roles of mitochondrial ubiquitin ligase MITOL in mitochondrial dynamics and aging-related diseases

Shigeru Yanagi

Tokyo University of Pharmacy and Life Sciences

Mitochondria are remarkably dynamic organelles that constantly migrate and frequently undergo fusion and fission events. A disruption in mitochondrial dynamics leads to a functional deterioration of mitochondria, resulting in a variety of diseases including neurodegenerative disorders and various aging-related diseases. We previously identified a mitochondrial ubiquitin ligase MITOL, which belongs to the membrane-associated RING-CH E3 ubiquitin ligase (MARCH) family (also known as MARCH5). MITOL is four integral mitochondrial outer membrane protein and a catalytic domain, called RING domain, is exposed to the cytosol. MITOL is ubiquitously expressed in almost all cells and tissues. We previously reported that MITOL plays an important role in the regulation of mitochondrial dynamics including mitochondrial morphology, transport and interaction with ER. First identified substrate for MITOL is Drp1, a mitochondrial fission factor. We found that MITOL ubiquitnates Drp1 and promotes proteasomal degradation of Drp1. Therefore, when Mitol gene was specifically deleted by tamoxifen-inducible Cre-loxP system from MEFs, Drp1 was rapidly accumulated on the mitochondria and induced mitochondrial fission. Excessive Drp1 accumulation has been shown to cause mitochondrial damages. Indeed, MITOL knockout enhanced mitochondrial ROS and this enhancement was partially rescued by Drp1 inhibitor, suggesting that MITOL knockout enhanced mitochondrial ROS partially via Drp1 toxicity. Actually, $\beta$-gal positive senescent cells were extensively increased by MITOL knockout and these senescent cells decreased by treatment of Drp1 inhibitor. Thus, MITOL regulates cellular senescence partially via Drp1. Recently, we generate several tissue specific MITOL-conditional knockout mice because whole MITOL knockout mice showed embryonic lethality. Interestingly, these mice reveal typical aging phenotypes. In this symposium, we discuss the role of MITOL in mitochondrial dynamics and aging-related diseases, and finally suggest that MITOL is a potential target for anti-aging drug therapy.

## Session 5 Aging related Diseases and Hutchinson Gilford Progeria Syndrome

### Mitotic death of cancer cells by gene silencing of RECQ helicases: a preferential sensitivity of ovarian cancer cells to RECQL1-siRNA

Yasuhiro Furuichi

GeneCare Research Institute Co. Ltd.

The RecQ helicase family involved in DNA repair consists of RECQL1, WRN, BLM, RTS and RECQL5. Previously, we reported that gene silencing of RECQL1 and WRN by cognate siRNAs induces a mitotic death in checkpoint-defective cancer cells but not in normal cells [1, 2]. To investigate this cancer cell-specific killing, we studied five helicases in various cancer cells. All RecQ helicases are highly expressed in the JFCR39 cell panel while RECQL1 expression is relatively low in cells from ovarian cancers (OCs). However, the mitotic death by RECQL1-siRNA was surprisingly outstanding in OC cells. The data imply that RECQL1 showing low expression is excellent target for cancer chemotherapy in OC, controversial perhaps to a generally accepted notion that highly expressed genes are assumed to be cancer drivers, and thus are excellent anticancer-drug targets. To confirm the therapeutic effect of RECQL1-siRNA in OC cells, we investigated four subtypes; Serous, Clear, Mucinous, and Endometrial OC cells [3]. The results were spectacular in that all OC cells were killed by RECQL1-siRNA within 96~120 hours of culture. In particular, Clear OC cells, most notorious as drug resistant, were proven sensitive. These results convince us that the Clear OC cell, although a serious problem now in gynecology, will be controlled by DNA repair systems, particularly by inhibiting the activity of RECQL1 helicase [4].

References:

(1)Futami *et al*. Induction of mitotic cell death in cancer cells by small interference RNA suppressing the expression of RecQL1 helicase: *Cancer Sci*. 99, 71–80 (2008).
(2)Futami *et al*. Anticancer activity of RecQL1 helicase siRNA in mouse xenograft models: *Cancer Sci*. 99,1227-1236 (2008).
(3)Sanada *et al*. RECQL1 DNA repair helicase: a potential therapeutic target and a proliferative marker against ovarian cancer: *PLoS One*. (2013) 9:e72820. doi: 10.1371/journal.pone.0072820.
(4)K. Futami and Y. Furuichi: (Review article) RECQL1 and WRN DNA repair helicases: Potential therapeutic targets and proliferative markers against cancers. *Fronties in Genetics*. (2015) doi:10.3389/fgene,

## Session 5 Aging related Diseases and Hutchinson Gilford Progeria Syndrome

### Role of Akt in skeletal muscle in anti-aging

Kohjiro Ueki

Diabetes Research Center, Research Institute, National Center for Global Health and Medicine

Aged people often show decreased skeletal muscle volume and impaired its function, namely sarcopenia, one of the major risk factors of diabetes and other diseases. Although age-dependent changes in insulin/IGF-1 signaling, mitochondrial functions and autophagy are thought to be involved in the development of sarcopenia, the precise mechanism still remains unclear. Since Akt is the key signaling mediator of insulin/IGF-1 signaling and appears to regulate mitochondrial function and autophagy, we have generated skeletal muscle specific Akt1 and Akt2, two major isoforms of Akt, double knockout mice using MLC1f-Cre mice (MLC-DKO mice). MLC-DKO mice grow normally and show normal glucose tolerance until 8 weeks of age despite the almost complete shutdown of signaling downstream of Akt. However, MLC-DKO mice quite rapidly exhibit decreased muscle volume and functions, especially in fast twitch muscle, with systemic insulin resistance in an age-dependent fashion, compared to the control mice, indicating the development of premature sarcopenia in MLC-DKO mice. In muscle of MLC-DKO mice, mitochondrial functions and biogenesis are impaired presumably due to a defect in the process of mitophagy, at least in part. Interestingly, MLC-DKO mice also show osteopenia even before they show decreased muscle volume. Finally, these mice significantly live shorter compared to the control mice. Additional knockout of FoxO1 and FoxO4, the major isoforms of FoxO transcription factors in skeletal muscle, almost completely rescues most of the phenotypes of MLC-DKO mice. These data demonstrate that loss of Akt signaling in muscle accelerates premature aging associated with sarcopenia, osteopenia, insulin resistance and shorter lifespan. Thus, maintaining Akt activity in muscle which can be achieved by increased insulin sensitivity is important to protect age-related diseases such as diabetes, metabolic syndrome and locomotive syndrome, and to prolong healthy lifespan.

## Session 5 Aging related Diseases and Hutchinson Gilford Progeria Syndrome

### Immunometabolic regulation of cardiac homeostasis and heart failure in aging

Ichiro Manabe

Department of Disease Biology and Molecular Medicine, Chiba University Graduate School of Medicine

Heart failure is a complex clinical syndrome characterized by cardiac function that is insufficient to meet systemic demand. The prevalence of heart failure is rapidly increasing partly due to aging society so that heart failure is now considered as a global pandemic. In addition to abnormalities intrinsic to the heart, dysfunction in other organs and systemic factors greatly affect the development and consequences of HF. In particular, nearly half of chronic HF (CHF) patients also have chronic kidney disease (CKD), which increases their rate of cardiovascular mortality, suggesting cardiorenal linkage via mechanisms still poorly understood. We found that pressure overload in the heart activates renal collecting duct (CD) epithelial cells via sympathetic nerves. Within the kidneys, activated communication between CD cells, tissue macrophages and endothelial cells leads to secretion of CSF2, which in turn stimulates cardiac-resident Ly6C$^{lo}$ macrophages essential for the myocardial adaptive response to pressure overload. We show that CD-specific deletion of the transcription factor *Klf5*, renal sympathetic denervation or adrenergic beta2 receptor blockade/deletion disrupts the renal response to cardiac pressure overload. Our results clearly demonstrate that dynamic interplay between the heart, brain and kidneys is necessary for proper adaptation to cardiac stress, and highlight the novel homeostatic functions of tissue macrophages and the sympathetic nervous system. We also found that macrophages play a key role in aging-associated changes in cardiac homeostasis.

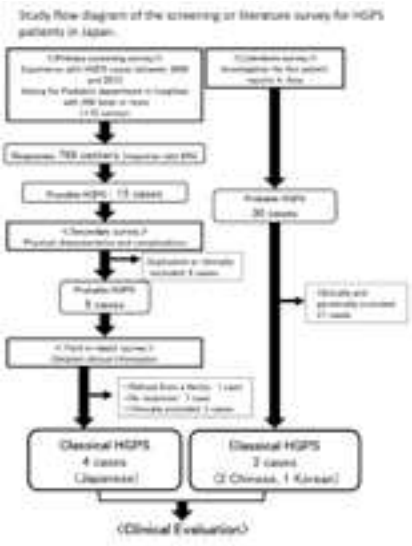
## Session 5 Aging related Diseases and Hutchinson Gilford Progeria Syndrome

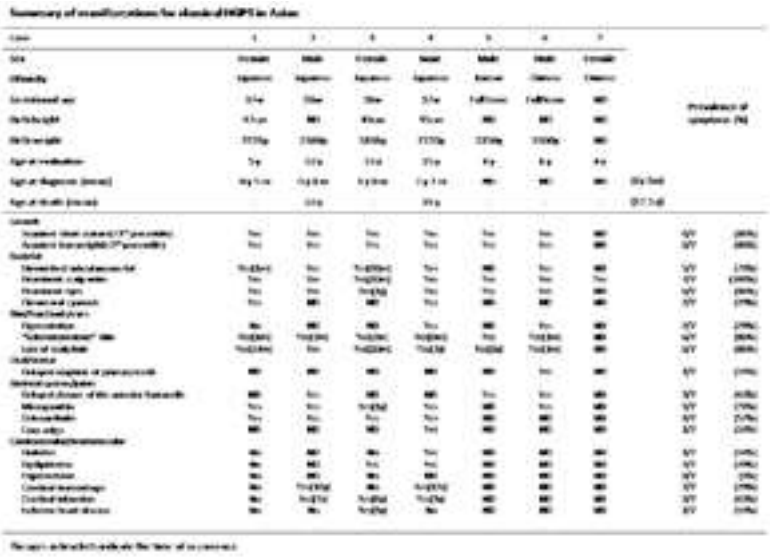
### The clinical characteristics of Asian patients with classical-type Hutchinson-Gilford progeria syndrome

Ihara K, Sato-Kawano N, Takemoto M, Okabe E, Yokote K, Matsuo M, Kosaki R

Department of Pediatrics, Oita University Faculty of Medicine

Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare genetic disorder that shows a characteristic progeria phenotype. We conducted a questionnaire survey of 1173 tertiary hospitals in Japan and reviewed the academic reports, to identify the characteristics of Asian patients with classical HGPS. As a result, four Japanese patients were identified; this was estimated to account for approximately two-third of the prevalence in Japan. Three Asian patients who had definitively been diagnosed with classical HGPS were identified in the literature; in total, the clinical characteristics of seven patients were evaluated. Most of the clinical phenotypes of Asian patients were essentially similar to those of patients of other ethnicities, such as sclerodermatous skin, growth failure, loss of scalp hair or severe complications of cardiovascular and cerebral ischemic disease. In conclusion, to circumvent or minimalize severe vascular complication, an early diagnosis, careful observation and, promisingly, new intervention with farnesylation inhibitors may improve the prognosis of classical HGPS patients.





## Session 6 Xeroderma Pigmentosa and Cockayne syndrome

### Coordinated DNA damage recognition by xeroderma pigmentosum gene products

Kaoru Sugasawa

Biosignal Research Center, Kobe University, 1-1 Rokkodai-cho, Nada-ku, Kobe, Hyogo 657-8501, Japan

Xeroderma pigmentosum (XP) is an autosomal recessive disorder, clinically characterized by cutaneous photosensitivity and a marked predisposition to skin cancer. In addition to such skin symptoms, some, but not all, of the patients with XP manifest progressive neurological degeneration. Eight genetic complementation groups have been identified for XP, and seven of them (XP-A through G) are associated with defects in a major DNA repair pathway, nucleotide excision repair (NER). NER can remove from the genomic DNA an extremely broad range of DNA lesions, which can be induced by various environmental agents such as ultraviolet (UV) irradiation and chemical carcinogens as well as by endogenously produced reactive oxygen species and other metabolites.

It is crucial to understand how the XP gene products cope with diverse DNA lesions and thereby protect us from the threats caused by them. In the mammalian NER subpathway operating throughout the genome, the heterotrimeric complex containing the XPC protein (XPC-RAD23-CETN2) initiates the repair reaction by recognizing sites of DNA damage, and this depends on detection of disrupted or destabilized base pairs, rather than lesions themselves, within the DNA duplex. Most DNA lesions processed by NER associate with a relatively large helix distortion that locally destabilizes base pairs; however, UV-induced cyclobutane pyrimidine dimers (CPDs) retain hydrogen-bonding with opposite purines, and thereby easily escape direct detection by XPC. This problem is partially overcome by the DDB1-DDB2 (XPE) complex, which exhibits unprecedented binding affinity and specificity for UV-induced photolesions and thereby promotes recruitment of XPC.

On the other hand, our cell-free NER assays revealed that the presence of mismatched bases in 5' side of CPD substantially enhances repair efficiencies of the photolesions. We proposed the model that XPC first interacts with unpaired normal bases within the 'undamaged' strand and then loads the XPD ATPase/helicase in TFIIH onto the 'damaged' strand. The presence and location of a lesion can be finally verified by XPD, when it translocates along the DNA strand in 5'-3' direction and stalls at sites with abnormal DNA chemistry. Notably, the helicase activity and the damage verification function of TFIIH are markedly enhanced by the presence of XPA. Therefore, the tripartite XPC-TFIIH-XPA complex would be responsible for a final decision to proceed with the repair process toward dual incisions by the two structure-specific endonucleases, ERCC1-XPF and XPG. On the other hand, TFIIH contains another ATPase subunit XPB, which is essential for both basal transcription and NER; however, precise roles of the two ATPase subunits in NER still remain to be fully understood. Based on the biochemical results, the sophisticated molecular machinery will be discussed, which ensures versatility, efficiency, and accuracy of the NER system.

## Session 6 Xeroderma Pigmentosa and Cockayne syndrome

### The present status of Xeroderma pigmentosum in Japan-evaluation of symptoms by severity scale score

C Nishigori [1], E Nakano [1], Kunisada M [1], T Ueda [2], M Tsujimoto[1], Fujita T [3], R Ono[1]

1. Divison of Dermatology, Kobe University Graduate School of Medicine ,Kobe, Japan
2. Divison of Neurology, Kobe University Graduate School of Medicine ,Kobe, Japan
3. Division of Otolaryngology, Kobe University Graduate School of Medicine ,Kobe, Japan

In Japan the frequency of Xeroderma pigmentosum (XP) is about 1:22000 and this figure is much higher than that in other countries. XP-A, manifesting the severest cutaneous and neurologic symptoms accounts for 50 %. As XP-A patients experience severe sunburn usually at the first sun exposure after birth, early diagnosis for XP-A has been encouraged. We performed a nationwide survey on XP to determine the present status of XP in Japan. The distribution of complementation groups in Japan was considerably different from that in other countries; a higher frequency in XP-A and followed by variant type and XP-D. The frequency of skin cancers in patients with XP-A has decreased, and these skin cancers have been occurring in much older people than those previously observed. It indicates the early diagnosis and strict sun protection has effective in the prevention of skin cancer. On the other hand because the life span of patients with XP-A is much longer than before, an appropriate way to support their neurological ability becomes increasingly important. We also created a tentative scale for classifying the severity of XP, and we evaluated the neurological symptoms of XP-A using this severity scale. Our classification correlated well with patients' age, and the genotype-phenotype was observed on the severity scale score, suggesting that it may be useful and feasible in clinical practice to assess the progression of symptoms of each patient with XP and evaluate the effects of treatment in the future. In order to evaluate the effect of treatment drug, mice model is also useful. Previously *Xpa*-KO mice were not considered to display XP-A neurologic symptoms. However, we found that threshold of ABR in *Xpa*-KO mice was much higher than that of the wild type at the age of 38-40 weeks. It might also give us a good tool for evaluating the treatment drug for XP-A.

## Session 6 Xeroderma Pigmentosa and Cockayne syndrome

### Very mild Japanese Cockayne syndrome (type-IV) cases with a N-terminal truncation mutation in the *ERCC6* / *CSB* gene

Nan JIA, Yasuyoshi OKA, Chaowan GUO, Yuka NAKAZAWA, Tomoo OGI

Department of Genetics, Research Institute of Environmental Medicine (RIeM), Nagoya University, Japan 464-8601

Transcription coupled nucleotide excision repair (TC-NER) is a crucial DNA repair system that removes transcription-blocking DNA lesions from actively transcribed genes. Cockayne syndrome (CS) is a representative genetic disorder associated with TC-NER deficiency, displaying growth retardation, progressive neurological abnormalities, and photosensitivity. CS patients are classified into types I-IV based on their onset and clinical severity: type-I, classic form; type-II / COFS, severe form, also known as Cerebro-Oculo-Facio-Skeletal syndrome; type-III, milder form; type-IV, very mild adult-onset form. Most CS patients have defects in the *ERCC8 (CSA)* or *ERCC6 (CSB)* genes and have substantial loss of TC-NER activity; however, no clear genotype-phenotype relationship has been elucidated.
Intriguingly, *CSB*-Arg77* homozygous truncation mutation leads to further mildest phenotype, UV-sensitivity syndrome (UVSS), and the patients only display mild freckles and photosensitivity with no devastating clinical manifestation. The *CSB*-Arg77* homozygous pathogenic mutation was first identified by Prof. Kiyoji Tanaka's group (Osaka University, Japan) from a Japanese adolescent UVSS patient (UVS1KO) with no developmental or neurological abnormalities (Horibata et al., 2004 *PNAS*). In the report, CSB protein expression was studied and found to be undetectable in the patient, concluding that the CSB N-terminal truncation causes null mutation and elicits milder clinical pictures than most of other CSB amino acid substitutions or nonsense mutations occurring in the middle to the C-terminal regions of the protein.

In our laboratory, we are collaborating with domestic / oversea pediatric neurologists and molecular biologists, and performed genetic screening of ~200 CS / UVSS cases. We recently identified additional Japanese very mild late onset CS-type IV / UVSS patients with the Arg77* pathogenic mutations in the *CSB* gene. Our research revealed distinct DNA damage-dependent RNA polymerase IIo (RNAP IIo) modification and degradation dynamics in CS-I, CS-IV and $UV^{S}S$ patients, suggesting that the milder clinical pictures in CS- IV / $UV^{S}S$ cases can be explained by faster degradation / removal of stalled RNAP II from DNA damage sites.

## Session 7 Can iPS cells be Future Therapeutics?

### Using stem cell and gene editing techniques to study and treat aging-associated disorders

Guang-Hui Liu

National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, 100101, China

Hutchinson-Gilford progeria syndrome (HGPS) and Werner syndrome (WS) are two human premature aging disorders with features that closely recapitulate the characteristics of human aging. Mutations in *LMNA* and *WRN* genes lead to aberrant splicing product progerin and protein loss in HGPS and WS, respectively. Study on how genetic alteration leads to the cellular and organismal phenotypes of premature aging will provide clues to the molecular mechanisms underlying physiological aging and facilitate our understanding of the molecular pathways contributing to healthy aging. We have generated induced pluripotent stem cells (iPSCs) from fibroblasts obtained from patients with HGPS, Parkinson's disease (PD), Amyotrophic lateral sclerosis (ALS), Fanconi Anemia (FA), and Xeroderma pigmentosum (XP). Further, using targeted gene correction technique, we successfully corrected the mutated *LMNA* in HGPS-iPSCs, mutated *LRRK2* in PD-iPSCs, mutated *FANCA* in FA-iPSCs, and mutated *SOD1* and *FUS* in ALS-iPSCs. Finally, by using targeted "knock-out" and "knock-in" techniques, we generated WS-, FA-, PD-, and Glioblastoma multiforme (GBM)-specific human stem cells with relevant pathogenic mutations. Upon differentiation of these disease-specific pluripotent stem cells to specific somatic cell types, the latter recapitulated aging/disease-associated and tissue-specific phenotypic defects. Altogether, these studies provide important platforms for studying aging/disease mechanisms and developing new therapies.

## Session 7 Can iPS cells be Future Therapeutics?

### *In vitro* modeling of bilateral progressive hearing loss with human iPSC technology: Cellular pathology and drug discovery

Masato Fujioka

Department of Otorhinolaryngology-Head and Neck Surgery,
Keio University School of Medicine

Human iPSC technology was first reported in 2007 by Drs. Takahashi & Yamanaka and has been broadly applied in medical science, especially for modeling diseases in many different organs. Particularly, careful inspection of cellular phenotypes may discover novel, and sometimes unexpected, pathological mechanisms, leading to the discovery of new modalities of candidate therapeutics by screening drugs in diseased cells derived from patients' iPSCs.
We have been using the hiPSC technology for inner ear research to understand its cellular biology, physiology and pathophysiology and to exploit them for the translational research by performing screening previously developed clinically usable compounds; We are attempting eventually to reposition their usage to the deafness. In this presentation, I will first start with showing how to induce cochlear cells efficiently and present, as an example, our data regarding cellular phenotypes of cochlear cells derived from patients of Pendred syndrome/DFNB4 (PDS/DFNB4), most frequent syndromic hereditary hearing loss in Japan: The results clearly indicate that the cellular degeneration with intracellular aggregates of mutant protein is involved in its pathogenesis and that an autophagy inducer rapamycin and metformin may relieve their cellular susceptibility. (Hosoya et al, *Cell Reports*, 2017). Second, two modes of model, acute and chronic changes in the cochlear cells derived from either healthy or deaf individuals will be shown, together with the data in changes of aging markers; These results suggest the relevance of hiPSC-based *in vitro* model for recapitulating chronic disorders at the cellular level featuring accelerated aging in hearing. Lastly, I would briefly introduce our near-future perspective of the clinical trials for PDS/DFNB4 with a drug found by iPSC-based drug discovery. Because there is no appropriate animal model recapitulating symptoms of PDS/DFNB4 patients in hearing, the trial may open a new era of drug development: Human iPSCs-based *in vitro* model may offer a platform for the fast-track, reliable and rational approach of the drug development by translating patients' cellular phenotypes into the bedsides' clinical therapeutics.

## Session 7 Can iPS cells be Future Therapeutics?

## Patient-specific iPS cells for neural disease modeling and drug screening

Wado Akamatsu

Center for Genomic and Regenerative Medicine
Juntendo University

Induced pluripotent stem cells (iPSC) can be used for the study of neurodegenerative disease such as Parkinson's disease (PD). We have recently showed that T-cell-derived iPSCs could be used for neural disease modeling with a robust differentiation protocol and the regional identity of neural stem cells could be controlled by adding small molecules during neural induction. This culture system could be a useful tool to elucidate the mechanism underlying the subtype specificity of neurological disease phenotypes. However, iPSC-derived neurons require long-term in vitro cultivation to exhibit disease-specific phenotypes of late onset neurodegenerative disorders. To find chemicals that accelerate *in vitro* maturation and differentiation of iPSC-derived neurons, we screened 400 inhibitor compounds by using a lentiviral synapsin-GFP reporter and found a compound that accelerated differentiation and maturation of iPSC-derived neurons. We also found that this compound accelerated the phenotypes of late onset familial PD, PARK4. Large aggregation of H2AX was observed in the nucleus of dopaminergic neurons treated with this inhibitor. These results suggested that this compound also induced aging in dopaminergic neurons by suppression of DNA repair mechanism. By using these protocol and patient-specific neurons derived from iPSCs, we performed high throughput pharmaceutical drug screening for juvenile Parkinson's disease and found several potential candidates that improve impaired mitophagy and cell death in the patient cells. In this session, I would like to introduce our recent advances in neural disease modeling and drug screening using patient-specific iPSCs.

Abstracts

# Poster Presentations P38 - P70

## P-001

### A case of Werner syndrome complicated with isolated adrenocorticotropic hormone deficiency

Yusuke Baba[1], Kana Ide[1], Kazuki Kobayashi[1], Yoshiro Maezawa[1], Minoru Takemoto[2], Tomoaki Tanaka[3], Koutaro Yokote[1]

1. Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine
2. International University of Health and Welfare
3. Department of Molecular Diagnosis, Chiba University Graduate School of Medicine

Werner syndrome (WS), a representative progeria, is an autosomal recessive disease due to mutations of WNR gene encoding a RecQ DNA helicase. Endocrinological abnormalities such as hypothyroidism or hypogonadism sometimes complicate to WS, but there are no previous reports of WS with central adrenal insufficiency.

The patient is a 42-year-old woman. Around age 27, she felt general malaise and was diagnosed with depression, but antipsychotic drugs did not improve her symptom. At age 36, she developed cataracts in both eyes. At age 39, her right second toe was amputated because of compressive changes in the bones and ischemia. During those periods, scleroderma had also progressed. In addition to these characteristic symptoms, type 4 homozygous mutation in WNR gene was identified and then she finally diagnosed with WS at age 42. Additionally, central adrenal insufficiency was also suspected because both level of plasma adrenocorticotropic hormone (ACTH) and serum cortisol was extremely low (1.1 pg/ml and 6.4 μg/dl, respectively).

The patient was of small physique and demonstrated an obvious soft tissue calcification as typical symptom of WS, while she did not have vocal abnormalities or bird-like face. As results of endocrinological evaluation, the patient demonstrated primary hypogonadism; however, her ACTH and cortisol levels were within normal ranges. A pituitary gland stimulation test also did not reveal any evident abnormalities including in ACTH-cortisol responsiveness. However, a growth hormone releasing peptide-2 stimulation test and an insulin tolerance test demonstrated significantly low ACTH-cortisol responsiveness. According to these results, the patient was diagnosed with hypothalamic isolated ACTH deficiency. Her general malaise was improved by treatment with 15 mg/day of hydrocortisone.

In addition to hypothyroidism or hypogonadism, adrenal insufficiency including isolated ACTH deficiency should be also considered as the candidate for the cause of general malaise or depressed mood complicated to WS.

## P-002

### Abnormal fat tissue distribution contributes to glucose dysmetabolism in Werner syndrome patients or Cockayne syndrome patient with diabetes

Aiko Hayashi[1], Minoru Takemoto[2], Yoshiro Maezawa[1], Kotaro Yokote[1]

1. Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine
2. International University Of Health and Welfare

Werner syndrome (WS) and Cockayne syndrome (CS) belong to progeria syndrome.
Both syndromes share premature symptoms that develop chronologically. Although CS patients occasionally develop diabetes, WS is often complicated with diabetes. Because the pathophysiology of diabetes in either WS or CS is largely unknown, we analyzed glucose metabolism in detail in both syndromes.
To understand the mechanism of pathogenesis of hyperglycemia, changes of hormone levels were analyzed after consumption of a test meal in 3 WS patients with diabetes (mean age 57 years, all females, A1c mean 7.6%). The meal was Calorie Mate (500 g; calorific level: carbohydrate:fat:protein = 60:26:14). A 75-g glucose tolerance test (GTT) was administered to one CS patient with diabetes (age 24 years, male, A1c 8.6%). Subcutaneous and visceral fat areas were evaluated by computed tomography (CT). The effects of sitagliptin, a dipeptidyl Peptidase-4 inhibitor, on glycemic control were also examined in all cases.
Both WS and CS patients showed high insulin resistance (IR; mean fasting immunoreactive insulin (IRI): 28 IU/mL, mean HOMA-IR: 7.6 for WS; fasting IRI: 16.8, HOMA-IR: 5.4 for CS). The meal test and GTT revealed that post hyperglycemia with the paradoxical pattern of postprandial glucagon secretion was present in both syndromes. WS showed the accumulation of visceral fat (mean 142 cm2), whereas CS showed fewer subcutaneous and visceral fat areas (54.4 and 13.7 cm2), respectively. Sitagliptin effectively ameliorated postprandial hyperglycemia in WS, but did not work effectively in CS. CS needed the addition of pioglitazone to increase fat tissue and ameliorate blood glucose metabolism.
Patients with WS are reportedly insulin-resistant due to the accumulation of visceral fat. Paradoxical pattern of postprandial glucagon secretion contribute to the development diabetes in WS, whereas lipodystrophic-like conditions contribute to IR and glucose dysmetabolism in CS. Nevertheless, abnormal fat tissue distribution seemed contributing to development of diabetes in both syndrome.

## P-003

### A case of Werner syndrome with diabetes mellitus in which liraglutide was effective for improvement of glycemic control

Masashi Yamamoto, Masanori Fujimoto, Shintaro Ide, Hiromi Inoue, Ko Ishikawa, Kazuki Kobayashi, Minoru Takemoto, Koutaro Yokote

Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine

**Aims**
Werner syndrome (WS) is an autosomal recessive progeria syndrome caused by mutations in the WRN gene, which encodes a RecQ DNA helicase. They typically demonstrate advanced accumulation of visceral fat and insulin resistance, which often leads to comorbid diabetes mellitus (DM). So far we have reported that pioglitazone, peroxisome proliferator-activated receptor γ agonist or sitagliptin, dipeptidyl peptidase-4 inhibitor was effective for treating DM in WS, but there are no reports on the use of liraglutide, GLP-1 analogs for them.
**Material and methods**
The patient was a 51-year-old man who demonstrated some progeroid symptoms since around age 20 and then was diagnosed as WS with DM. At admission, 56 units of short-acting insulin, 44 units of long-acting insulin and 100mg of sitagliptin per day was administered for the treatment. HbA1c was maintained around 6.0%, but he was sometime suffered from hypoglycemia. He also demonstrated visceral fat accumulation with liver dysfunction indicating fatty liver.
**Key Results**
We altered sitagliptin to 0.9 mg of liraglutide and 1500mg of metformin under gradual titration, which finally lead to substantial decrease of insulin (only 14 units of long-acting insulin was remained) and to less frequency of hypoglycemia without deterioration of glycemic control. In addition, liver dysfunction was improved with the decrease of visceral fat, and even the vascular endothelial function evaluated by flow mediated dilation test was also improved from 7% to 10% by the alteration of treatment.
**Conclusion**
Therefore, we thought that liraglutide, in addition to reducing blood glucose level, may serve as the totally effective treatment for DM in WS via pleiotropic effects.

## P-004

## A case of Werner syndrome with a heterozygous point mutation of exon 9 of WNR gene

Shigeatsu Hashimoto[1], Koji Hasegawa[1], Aki Watanabe[2], Yoshiro Maezawa[2], Minoru Takemoto[3], Koutaro Yokote[2]

1. Department of Endocrinology, Metabolism, Diabetology and Nephrology, Fukushima Medical University Aizu Medical Center
2. Clinical Cell Biology and Medicne Chiba University Graduate School of Medicine
3. International University of Health and Welfare Graduate School of Medicine

Case presentation; A 30-year-old Japanese woman was referred to our department with complaints of delayed healing of the hallux valgus surgery. This patient had had menarche at the age of 11 with regular menstrual cycles. She was underwent surgery of bilateral cataracts and left hallux valgus, at the age of 24 and 28, respectively. She was pointed out cutaneous thinning and delayed healing of surgery, and was suspected of Cushing's syndrome by an orthopedist in charge of the surgery of hallux valgus. No family history of diabetes mellitus and/or malignant neoplasma was elicited.
Physical examination revealed that the patient was lean and slender extremities (sarcopenia) , 150 cm tall and weighed 39.5 kg (BMI 17.56, waist circumference 70 cm) with a high pitched hoarseness, dyed hair for whole gray hair, atrophic and tight skin and bird-like countenance. However, she had no Cushingoid appearance, such as moon face, Buffalo hump, striae cutis, or truncal obesity. Her menstruation occurred regularly every 28 days.
Diagnosis of Werner syndrome: She had typical physiological sign of Werner syndrome, described above, except Achilles tendon calcification. Her plasma fasting glucose(PG), immune-reactive insulin(IRI) and HbA1c were 104 mg/dL, 38.4 μIU/mL, 5.5%, respectively. An 75g oral glucose tolerance test showed normal glucose tolerance with high vassal value of and an exaggerated insulin response (fasting; plasma glucose (PG):104 mg/dL, (IRI) 38.4 μIU/mL, 120 min; PG:127, IRI 898). Plasma lipid profiles, liver function and renal function were normal. Basal value of plasma Cortisol and ACTH were normal and were suppressed by overnight 1mg dexamethasone suppression test, by which Cushing's syndrome be ruled out. Basal plasma levels of GH and IGF-1 and GH response to GHRP were normal. Basal plasma levels of LH, FSH, estradiol and progesterone and LH and FSH response to GnRH were also normal. Dual-energy X-ray absorptiometry showed normal bone mineral density (1.12, YAM 102%), diminished skeletal muscle of extremities, and relative abdominal adiposity. Diminished skeletal muscle volume was due to neither GH deficiency, nor hypogonadism. No evidence of atherosclerosis was obtained with the examination of carotid artery ultrasound examination and cardio ankle vascular index. Gene testing revealed a point mutation of one allele of WNR, c.1105C>T (exon 9).
Discussion and Conclusion; Werner syndrome has been recognized as a rare inherited progeroid syndrome caused by a mutation in the WRN gene. However, aging acceleration in this case was heterogeneous by organs. Aging phenomena, such as cataract, skin hardening, decrease of subcutaneous tissue, gray hair, and reduction of limb skeletal muscle were observed, but not in bone, gonad function and arteriosclerosis at the present time. Insulin resistance might be due to diminished skeletal muscle and relative increase of adiposity. Werner syndrome could be considered as a model for sarcopenia due to accelerated aging of skeletal muscle. Almost cases of Werner syndrome were cased homozygous, or compound heterozygous mutation of WNR gene, however single heterozygous mutation was revealed in this case. Unknown gene mutation might be exist. Further examination of WNR gene analysis should be continued.

**P-005**

## The First Japanese woman of mandibular hypoplasia, deafness, progeroid features, and progressive lipodystrophy (MDPL) syndrome caused by a de novo mutation in the POLD1 gene.

Kunihisa Kobayashi[1], Haruka Sasaki[1], Kumiko Yanagi[2], Satoshi Ugi[3], Kumiko Okubo[1], Yuji Tajiri[4], Hiroshi Maegawa[3], Atsunori Kashiwagi[5], Tadashi Kaname[2]

1. Fukuoka University
2. National Center for Child Health and Development
3. Shiga University of Medical Science
4. Kurume University
5. Kusatsu General Hospital

**[Background]** MDPL syndrome (MIM #615381) is a novel subtype of progeroid syndrome with lipodystrophy, carrying genetic mutations in *POLD1* encoding DNA polymerase delta 1. To date, only 21 cases with *POLD1*-related MDPL syndrome have been reported worldwide and its natural course and underlying mechanism for pathophysiology has not been fully understood.

**[Case]** A 46-year-old (currently)Japanese female with congenital partial lipodystrophy, mandibular hypoplasia,sensorineural deafness, and short stature including various progeroid features. She was born to non-consanguineous parents at full term gestation with normal birth appearance (weight 3.65kg, height 49.5cm). Skin pigmentation and deafness during infancy, fat-loss on the limbs and growth retardation around the age of 12 month were noticed. At 14 years of age, she was admitted to the university hospital due to short stature and muscle cramp of her lower extremities. She had progeroid features with symmetrical fat loss (predominantly her face and extremities), excessive accumulation of visceral/intraperitoneal fat, skin atrophy, and short stature (weight 28.6kg, height 139.2cm, both < 3SD) despite hyperphagia. An oral glucose tolerance test showed normal glucose tolerance with increased insulin resistance (HOMA-IR 5.3). She developed oligo-/amenorrhea since she was 19 years old. Pituitary-adrenal axis including GH and IGF-1 was within normal. At 21 years of age, she had non-alcoholic steatohepatitis, dyslipidemia, impaired glucose tolerance (HbA1c 5.7%, HOMA-IR 6.1). At 33 years of age, she developed diabetes mellitus (HbA1c 5.0%, HOMA-IR 6.1), acanthosis nigricans and polycystic ovary syndrome. Euglycemic hyperinsulinemic glucose clamp study showed severe insulin resistance (glucose infusion rate 4.32 mg/kg/min: normal 7.4 ± 0.5 ). Low plasma levels of leptin (3.6ng/ml) and adiponectin (2.4μg/ml) were also observed. Since she was 25, thiazolidinediones (troglitazone, pioglitazone) was administered. During administration, visceral fat tissue was markedly increased, while subcutaneous fat tissue was slightly increased. At the age 45 years, abdominal computed tomography showed that visceral and subcutaneous fat area at umbilical plane were 301.5 and 20.4cm$^2$, respectively. Glucose and lipid metabolism, indexes of insulin resistance, and liver function were significantly improved. However, there were no satisfactory changes in the affected lipoatrophic regions. She had been suspected as congenital partial lipodystrophy with atypical progeroid syndrome or a novel subtype of progeria lipodystrophy based on observations spanning a 30-year period; however, repeated genetic testing did not identify any causative mutation related to various types of congenital partial lipodystrophy and/or mandibular dysplasia A/B including *LaminA/C*, *ZMPSTE24*, and others (i.e., *LMNB2*, *PPARG*, insulin receptor, *IRS-1*, *Cav-1*, *Cav-2*, *BSCL2*, *AGPAT2*, *CICDEC*, *PTRF*, *AKT-2*, and *PLIN-1*). Therefore, we carried out whole exome sequencing. We identified a *de novo* mutation in exon 15 (p.Ser605del) of the *POLD1* gene by using both whole exome and Sanger sequencing analyses. This mutation was not found in her parents and healthy brother. To our knowledge, this is the first identified sporadic/isolated Japanese woman of MDPL syndrome.

**[Conclusion]** Our results provide further evidence that mutations in POLD1 are responsible for MDPL syndrome and serve as a common genetic determinant across different ethnicities and helpful information for understanding of mechanism of this ultrarare intractable disease.

## P-006

## A novel deletion mutation of the WRN gene identified in a Japanese patient with Werner syndrome

Tsuneo Nakajima[1], Shuko Takeda[2], Yasushi Takeya[1], Ken Sugimoto[1], Kouichi Yamamoto[1], Minoru Takemoto[3], Tomohiro Katsuya[2], Hironori Nakagami[4], Koutaro Yokote[3], Ryuichi Morishita[2], Hiromi Rakugi[1]

1. Department of Geriatric and General Medicine, Graduate School of Medicine, Osaka University
2. Department of Clinical Gene Therapy, Graduate School of Medicine, Osaka University
3. Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine
4. Department of Health Development and Medicine, Osaka University Graduate school of Medicine

Werner syndrome (WS) is a rare autosomal recessive disorder characterized by premature aging with a high incidence in Japan.WS is caused by a mutation in the *WRN* gene, which encodes a nuclear protein with both endonuclease and helicase activities and WRN protein is known to be involved in DNA repair and stability.
Here, we report a novel deletion mutation at nucleotide 407 in exon 5 of *WRN* gene. The patient was a 60-year-old man, who was the oldest of three siblings and whose parents were consanguineous. He was suspected of having WS due to premature aging signs, including graying and thinning of scalp hair, bilateral cataracts, short stature, and soft tissue calcification.
This mutation results in a frameshift that introduces a premature stop codon in exon 5, which is within the exonuclease domain. This mutation is predicted to result in the elimination of the nucleic localization signal, presumably precluding functional interactions in the nucleus, like other *WRN* gene mutations.
The patient lacked the skin and voice alterations typical for patients with WS. Notably, he suffered recurrent cerebrovascular events, which is a rare manifestation of WS. These results indicate that the clinical phenotypes of WS are remotely related to *WRN* mutation types. Although detailed mechanisms linking this mutation to the characteristic clinical symptoms remain unknown, the atypical presentations of the patient with this novel *WRN* mutation may give insight into the pathogenesis of WS.

## P-007

### Confirmation of Biallelic WRN Mutations in Newly Identified Japanese Werner Syndrome Patients

Yoshiro Maezawa[1], Hisaya Kato[1], Minoru Takemoto[1,2], Aki Watanabe[1], Masaya Koshizaka[1], Takahiro Ishikawa[1], Forough Sargolzaeiav[3], Masafumi Kuzuya[4], Hiroshi Wakabayashi[5], Koutaro Yokote[1], Junko Oshima[1,3]

1. Department of Clinical Cell Biology and Medicine, Chiba University, Graduate School of Medicine, Chiba, Japan
2. Department of Medicine, International University of Health and Welfare, Narita, Japan
3. Department of Pathology, University of Washington, Seattle, WA, USA
4. Department of Community Healthcare & Geriatrics, Nagoya University Graduate School of Medicine, Nagoya, Japan
5. Okayama City General Medical Center, Okayama, Japan

Werner syndrome is a rare autosomal recessive disorder characterized by the systemic accelerated aging. It is caused by the pathogenic variants of the WRN gene that encodes a nuclear helicase. In this report, we describe four newly identified Werner syndrome cases among those referred to the Japanese Werner Consortium, Chiba University, Japan. Three of these cases were compound heterozygotes of the Japanese founder mutation, c.3139-1G>C, and novel null pathogenic variants, c.1587G>A, c.2448+1G>A, or c.3233+1G>T. One was a compound heterozygote of the Japanese founder mutations and an amino-acid substitution variant, c.1720G>A, p.G574R, previously reported in an European patient. The identification of the second p.G574R case with classical WS features further confirm the pathogenic nature of this variant. For the case with c.3233+1G>T, we determined the phase of two disease-causing mutations and demonstrated that they are on the different chromosomes. This assay would be particularly important for those cases with ambiguous clinical diagnosis.

## P-008

## Generation of novel Werner syndrome model mice -Wrn/Recql5 double-deficient mice-

Shuichi Shibuya[1,2], Kinue Iizuka[1], Koutaro Yokote[2], Takahiko Shimizu[1,2]

1. Department of Advanced Aging Medicine, Chiba University Graduate School of Medicine
2. Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine

Werner syndrome (WS) is progeroid syndrome, which characterized by multiple features such as cataract, skin ulcer, diabetes mellitus, atherosclerosis, and malignancy resulted in premature death. WS is caused by the recessive mutation of *WRN* gene, a member of the RecQ family of DNA helicases. Three RecQ helicase genes, *WRN*, *BLM*, and *RECQL4*, among five RecQ helicase genes are associated with progeroid syndrome, suggesting that loss of helicase activity might cause genomic instability and age-related pathologies. However, WRN-deficient mice showed no premature aging symptoms and death. Therefore, we cannot develop therapeutic strategy for WS *in vivo*. Accumulating evidence has indicated that RECQL5 protein physiologically interacts with WRN protein and that *Wrn/Recql5* double-knockdown, but not single-knockdown, induced DNA damage and suppressed cell proliferation, suggesting that WRN and RECQL5 proteins play co-operative and complementary roles in cellular function [1]. In the present study, to establish novel WS model mice showing WS-characteristic phenotypes, we separately targeted at Ex6 of *Wrn* gene and Ex3 of *Recql5* gene as editing sites for CRISPR/Cas9 system to additively reduce helicase activity in cells. By sequencing analyses, we identified flame-sift mutation alleles of *Wrn* and *Recql5* genes that approximately 178 and 141 amino acid residues as prematurely terminated proteins, respectively, in *Wrn* or *Recql5* editing alleles. Western blot analysis also confirmed that no full-length WRN and RECQL5 proteins expressed in *Wrn* or *Recql5* mutant cells. We are generating *Wrn/Recql5* double-deficient mice by crossing with *Wrn* and *Recql5* mutant mice, and are analyzing premature aging phenotypes of *Wrn/Recql5* double-deficient mice at adult age.

1. Popuri V. *et al*. *Nucleic Acids Res* **2013**, 41, 881-899.

**P-009**

Poster Award

## Mouse model of Werner syndrome exhibits cardiac remodeling and resistance to diabetes

Takahiro Kamihara, Yasuko K. Bando, Haruya Kawase, Kazuyuki Nishimura, Remina Yasheng, Toyoaki Murohara

Department of Cardiology, Nagoya University Graduate School of Medicine.

[INTRODUCTION]
[Introduction]: Aging is one of the primary cause of heart failure, particularly due to diastolic dysfunction. Werner syndrome is a premature aging disorder caused by dysfunction of the DNA-helicase-regulatory protein (WRN). However, there is little information whether they may have any specific myocardial remodeling and vulnability for heart failure due to progeria. Interestingly, previous reports suggest that a mouse model lacking part of the helicase domain of the murine Wrn homologue causes the impaired glucose metabolism via augmentation of p53 signaling with concomitant cardiac fibrosis.
AA substitution of human WRN at position 577 (K577M) has been reported to abolish the ATPase and helicase activities, but not the exonuclease activity (Gray et al. 1997; Huang et al. 1998). This substitution in mice resulted in reduced replicative potential in skin fibroblast. However, it remains unclear whether the AA substitution at K577M that may result in cardiac remodeling.
We thus examined the role of K577 mutation of WRN in myocardial phenotyope in mice.
[HYPOTHESIS]
[Methods]: Transgenic mice expressing human WRN with a putative dominant-negative mutation (K577M-WRN; WRN-KD) were evaluated in terms of #1) cardiac function by cardiac catheterization and echocardiography, #2) cardiac remodeling in terms of myocardial hypertrophy and fibrosis. To address the potential vulnerability for diabetes, cardiac remodeling, comprehensive gene screening was performed by use of DNA microarray of myocardium.
To assess the potential vulnerability for diabetes in the WRN-KD mice, we tested the impact of diabetic stress induced by high-fat/high-sucrose (HF/HS) diet (WRN-KD-dm and CON-dm).
[RESULTS]
[Results]: WRN-KD exhibited increase in heart weight with enhanced cardiac fibrosis and hypertrophy. Diastolic left-ventricular (LV) function was markedly impaired, whereas their systolic LV function remained unchanged. Cardiac p53/ERK axis in the WRN-KD was markedly augmented. The DNA microarray analysis revealed that the WRN-KD-ad heart exhibited the upregulation of 253 genes as compared to CON-ad, of which 51 genes were overlapped in those CON-dm. WRN-KD-ad exhibited the more than quadruple upregulation of 16 genes. KEGG ontology revealed that the 2 specific genes were hypertrophy related genes (Myh7, Klkb11), 2 genes were fibrosis related genes (Fgf21, Ctgf) and 2 genes were of p53 signaling and apoptosis related signaling.
The casual glucose level was higher in WRN-KD fed with normal chow (WRN-KD-ad) than CON-ad. Unexpectedly, the intraperitoneal glucose loading test (ipGTT) revealed that the glucose metabolism of WRN-KD-ad was similar to those control counterparts (CON-ad).
[CONCLUSION]
We first reported the evidence regarding the WRN-mutant mice by AA-mutation in terms of changes in glucose metabolism and cardiac remodeling. The K577M mutation of WRN is responsible for cardiac hypertrophy and fibrosis via ERK/p53 axis, however, it is independent of glucose metabolism.

## P-010
## EVIDENCE FOR AN EPIGENETIC CAUSE OF AGING IN MICE

Motoshi Hayano[1,5], Elias Levy Itshak Salfati[1], John Apostolides[3], Jae-Hyun Yang[1], Michael S. Bonkowski[1], Luis A. Rajman[1], Sachin Thakur[1], Neha Garg[1], Ana-Maria Balta[1], Sarah Mitchell[2], Andrea Di Francesco[2], Rafael deCabo[2], Shelley L. Berger[3], Andreas R. Pfenning[3], Philipp Oberdoerffer[4] and David A. Sinclair[1]

1. Glenn Labs for the Biological Mechanisms of Aging, Department of Genetics, Harvard Medical School, Boston, MA 02115, USA
2. Gerontology Branch, NIA/NIA, Bethesda, MD, 21224 USA
3. Carnegie Mellon University, Pittsburgh, PA 15213, USA
4. The Mouse Cancer Genetics Program, Center for Cancer Research, NCI-Frederick, USA
5. Keio University, School of Medicine, Tokyo 160-0016, Japan

If there are highly conserved causes of aging in diverse species, they are likely to be caused by a breakdown in the maintenance of biological systems that are inherently difficult to maintain throughout life. Chromatin, the DNA-protein complex that stabilizes the genome and dictates gene expression, is one of the most difficult structure a cell has to maintain over a lifetime. Studies in budding yeast have pointed to changes in chromatin organization and gene expression as a main contributor to ageing in that species, raising the possibility that similar processes underlie ageing in more complex organisms. In mammals, numerous epigenetic changes have been noted during aging, including DNA methylation and histone modification and gene expression changes. Changes in chromatin are also known to precede metabolic diseases, neurodegeneration, and cancer, and the underlying basis of cellular senescence undoubtedly has a chromatin component. However, the mechanism that drives these epigenetic changes and whether they contribute to aging is still debated. In mammals, evidence is accumulating that the relocation of chromatin factors in response to DNA damage is a major upstream cause of the gene expression changes that occur during aging (the “RCM” hypothesis). We have developed a novel model called the “ICE mouse” (for inducible changes in epigenetics) that allows us to induce a few DSBs in non-coding regions of the mouse genome within all tissues, then switch the system off and monitor the effects on tissues and age-related physiology. Consistent with the RCM hypothesis, ICE mice exhibit metabolic changes, decreased bone density, muscle and brain function, cataracts, skin aging, and frailty, among other effects consistent with aging. RNA-seq and ChIP-seq experiments indicate that lipid metabolism and inflammation pathways are involved and that chromatin is altered in specific ways. These experiments are consistent with epigenetic change driven by DNA repair processes as an upstream cause of aging in mammals. Further work will assess whether this process can be slowed or reversed using known and novel agents.

**P-011**

Poster Award

## Progerin impairs vascular smooth muscle cell growth via the DNA damage response and pro-inflammatory pathways

Daisuke Kinoshita[1], Takashi K Ito[2], Ippei Shimizu[3,4], Yohko Yoshida[3,4], Atushi Iwama[1], Toshiya Hayano[5], Tohru Minamino[3]

1. Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University
2. Department of Cardiovascular Medicine, Chiba University Graduate School of Medicine
3. Department of Cardiovascular Biology and Medicine, Niigata University Graduate School of Medical and Dental Sciences
4. Division of Molecular Aging and Cell Biology, Niigata University Graduate School of Medical and Dental Sciences
5. Department of Biomedical Sciences, College of Life Sciences, Ritsumeikan University

Hutchinson-Gilford Progeria Syndrome(HGPS) is a fatal premature ageing disease. The common cause of death in HGPS is atherosclerosis caused by the depletion of vascular smooth muscle cell(VSMCs). The genetic cause of HGPS is a mutated form of lamin A called progerin. However, little is known about the molecular mechanisms by which progerin causes the smooth muscle depletion and atherosclerosis in HGPS patients.

To elucidate the mechanisms, we conducted an interactome and transcriptome analysis. Progerin induction caused cell death specifically in VSMCs, while the viability and cell growth of vascular endothelial cells and fibroblasts remained unaffected. Through the interactome analysis using mutant forms of lamin A involved in progeroid syndromes, we found that progerin could not bind to proteins related to the DNA damage response, including DNA-dependent protein kinase (DNA-PK).We also found that forced expression of progerin in vascular smooth muscle cells led to activation of DNA-PK and cellular growth arrest, while knockdown of DNA-PK attenuated this. Deletion of p53 also improved the inhibition of cell growth due to forced expression of progerin. Microarray analysis revealed that a repertoire of pro-inflammatory cytokines were up-regulated in progerin-expressing VSMCs, such as 1375-fold induction of MMP3 gene.

These data suggest that the DDR and pro-inflammatory pathways may contribute to VSMC degeneration and atherosclerosis in HGPS patients.

## P-012

Travel Grant

## NAD+ restores WRN deficiency-induced stem cell exhaustion

Evandro F Fang[1], Vilhelm A Bohr[2]

1. Department of Clinical Molecular Biology, University of Oslo and Akershus University Hospital, 1478 Lörenskog, Norway
2. Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, Baltimore, MD 21224

Werner syndrome (WS) is an autosomal recessive accelerated aging disorder caused by mutations in the gene encoding the WRN protein. As a member of the human five RecQ DNA helicases, WRN plays a major role in the maintenance of genomic stability through its helicase activity and endonuclease activities1. Major early aging phenotypes on the WS patients include gray and loss of hair, juvenile cataracts, bird-like face, and skin/muscle atrophy2. WRN patients also show significant metabolic abnormalities as exemplified by abnormal glucose levels, insulin-resistant diabetes, and dyslipidemia, leading to atherosclerotic vascular diseases which are major causes of death of the patients2. Mitochondria play fundamental roles in cell metabolism, survival, and stem cell rejuvenation3. In vitro studies indicate WRN deletion-induced exhaustion of the mesenchymal stem cells may contribute to the aging features in WS4. However, a role of WRN in stem cell maintenance in vivo as well as the contribution of stem cell dysfunction in AD pathology is largely elusive.
We hypothesized that WRN mutation induces mitochondrial dysfunction which leads to stem cell exhaustion, and finally exacerbating WS. To test this hypothesis, we used a wrn-1 C. elegans model which recapitulates major WS features, including short lifespan and genomic instability. Compared with the age-matched isogenic N2 worms, the wrn-1 C. elegans showed mitochondrial dysfunction and less stem cells in the germline. NAD+ is a coenzyme in cellular bioenergetics and adaptive stress responses. NAD+ plays major roles in cell metabolism, mitochondrial maintenance, and stem cell rejuvenation, while NAD+ reduction is emerging as a fundamental feature of aging that may predispose to many premature aging diseases5. Interestingly, NAD+ was decreased in both WS patient cells and wrn-1 animals, and NAD+ replenishment improved both lifespan and healthspan as well as stem cell numbers in the wrn-1 worms. To verify WRN-deficiency induced stem cell exhaustion is evolutionarily conserved, we involved a WRN Drosophila system. Consistently, there was an impaired proliferation of gut stem cells in WRN Drosophila compared with WT controls, and NAD+ supplementation almost completely restored the proliferation potency of the WRN gut stem cells. In conclusion, our cross-species data consistently support mitochondrial dysfunction and compromised stem cell rejuvenation in WS, possibly due to NAD+ depletion. Our study indicates NAD+ depletion is likely a universal feature of aging, and highlights a novel therapeutic potential for this currently incurable disease.

1 Shamanna, R. A. et al. WRN regulates pathway choice between classical and alternative non-homologous end joining. Nat Commun 7, 13785, doi:10.1038/ncomms13785 (2016).
2 Takemoto, M. et al. Diagnostic criteria for Werner syndrome based on Japanese nationwide epidemiological survey. Geriatr Gerontol Int 13, 475-481, doi:10.1111/j.1447-0594.2012.00913.x (2013).
3 Fang, E. F. et al. Nuclear DNA damage signalling to mitochondria in ageing. Nat Rev Mol Cell Biol 17, 308-321, doi:10.1038/nrm.2016.14 (2016).
4 Zhang, W. et al. Aging stem cells. A Werner syndrome stem cell model unveils heterochromatin alterations as a driver of human aging. Science 348, 1160-1163, doi:10.1126/science.aaa1356 (2015).
5 Fang, E. F. et al. NAD+ in Aging: Molecular Mechanisms and Translational Implications. Trends Mol Med, doi:10.1016/j.molmed.2017.08.001 (2017).

**P-013**

**Poster Award**

## Recapitulation of premature senescence and tumor predisposition phenotype in Werner syndrome specific iPSCs

Hisaya Kato[1], Yoshiro Maezawa[1], Yasuo Ouchi[2], Naoya Takayama[3], Masamitsu Sone[3], Takahiro Ishikawa[1], Masaya Koshizaka[1], Katsunori Fujii[4], Atsushi Iwama[5], Koji Eto[3.6], Akira Shimamoto[7], Hidetoshi Tahara[7], Koutaro Yokote[1]

1. Department of Clinical Cell Biology and Medicine, Graduate School of Medicine, Chiba University
2. Department of Mucosal Immunology, Graduate School of Medicine, Chiba University
3. Department of Regenerative Medicine, Graduate School of Medicine, Chiba University
4. Department of Pediatrics, Graduate School of Medicine, Chiba University
5. Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University
6. Center for iPS Cell Research and Application, Kyoto University
7. Department of Cellular and Molecular Biology, Graduate School of Biomedical & Health Sciences, Hiroshima University

Overview

Werner syndrome (WS) is an autosomal recessive disorder caused by a mutation in the WRN gene, a member of the RecQ helicase family, and presents aging-like symptoms in young age as well as a variety of malignancies including nonepithelial tumors. We experienced a male WS-patient with typical symptoms who died of myelodysplastic syndrome (MDS) at the age of 64. We induced pluripotent stem cells (iPSCs) from fibroblasts of this patient and one of the cell lines acquired the p53 gene mutation.

Case report

The patient's parents were second cousins. He was normal at birth, was an active child, but frequently developed clavuses at the sole from 13 years old. At the age of 21, he underwent surgery due to bilateral cataract. He showed difficulty in vocalization, impaired glucose tolerance and liver dysfunction around 28, then subsequently diagnosed as WS by clinical symptoms. A genome sequencing revealed heterozygous mutations (c.1105 C>T and c.3139-1 G>C) in the WRN gene. At 30, all the hair became gray and he began to be afflicted by the pain of elbows, knees, and soles. Refractory ulcers with severe pain appeared on the left elbow, both ankles, and Achilles tendons at age 45. Diabetes worsened and insulin therapy was started at 53. When he was 55, leg ulcers caused osteomyelitis, and both legs were eventually amputated. He was diagnosed with MDS from pancytopenia at age 64 and was treated with blood transfusion, but died six months later from diagnosis.

Basic research

In order to clarify the mechanisms of premature aging and tumorigenesis seen in WS patients, we tried to recapitulate the phenotype using iPSCs. We established several iPSCs from skin fibroblasts of this patient. After long-term culture (P (passages) > 80), two iPSCs (#2 and #13 WS-iPSC) were differentiated into mesenchymal stem cells (MSCs) and their growth potential was evaluated. As expected, MSCs derived from one WS-iPSC (#2 iMSC) showed early growth arrest at P10. However, interestingly, MSCs derived from the other WS-iPSC (#13 iMSC) were able to proliferate over a long time (P>27). The #2 iMSC displayed high expression of p53 and p21, whereas #13 iMSC exhibited low expression of p21 although p53 was highly expressed. Since p21 is considered to be a downstream target of p53, these results suggested malfunction of p53 in #13 iMSC. Indeed, a genome sequencing analysis revealed that p53 heterozygous loss-of-function mutation (c.527 G>T) was acquired during long-term culture of the #13 WS-iPSC.

Discussion

The c.527 G>T mutation in the p53 gene has been reported in over 200 malignant tumor cases, not only in epithelial malignancies but also nonepithelial tumors. WRN protein and p53 have many interactions, and the absence of WRN protein enhances p53 expression. In our study, one of the WS-iPSCs successfully recapitulated the replicative senescence, and the other showed enhanced proliferation that was reminiscent of tumorigenesis. These findings suggest the usefulness of disease-specific iPSCs in the study of WS, and also indicate the possibility that p53 genomic locus is specifically susceptible to the DNA damage in WS cells and that might explain the link between premature aging and tumor predisposition in WS.

**P-014**

Travel Grant

## Generation of human stem cells resistant to cellular aging and oncogenic transformation

JING QU

State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences Room. 1 Beichen West Road, Chaoyang District, Beijing, 100101, P. R. China

Stem cell therapy holds great promises for mitigating stem cell exhaustion or dysfunction associated with human diseases and aging, thereby restoring the balanced cellular homeostasis and tissue repair capacity. However, challenges including premature senescence-associated functional attrition and neoplastic transformation of transplanted stem cells cast doubts on their clinical applications.

Recent advances in targeted genome editing have greatly facilitated the development of stem cell replacement therapy. Precise manipulation of human genome allows production of healthy autologous tissue stem cells via targeted correction or disruption of disease-causing mutation(s) in patient-derived stem cells. Even though, it remains unexplored whether it's possible to generate human stem cells via targeted genome editing harbouring more robust regenerative capacity and minimized risk of tumorigenesis over normal stem cells.

In our current study, we found that a single base change in the human NRF2 locus conferred human mesenchymal stem cells (hMSCs) resistance to both cellular senescence and oncogenic transformation. Genetic modified hMSCs exhibited a sustained proliferative lifespan as evidenced by the absence of key aging hallmarks after long-term cell culture, as well as enhanced regenerative ability following in vivo transplantation. Additionally, we also demonstrate the effectiveness of the Genetic Enhancement toward thwarting oncogene-induced tumorigenesis, minimizing safety concern for stem cell replacement therapy. Our study provides the proof-of-concept of genetic enhancement of human stem cells, a strategy that may hold tremendous potential towards providing superior and safer stem cell replacement therapy.

**P-015**

**Poster Award**

## Treating aging model of Werner syndrome specific induced pluripotent stem cells by CRISPR/Cas9 systems

Gakuto Yozu[1,2], MD, Shinsuke Yuasa[1], MD, PhD, Shugo Tohyama[1], MD, PhD, Tomohisa Seki[1], MD, PhD, Minoru Takemoto[3], MD, PhD, Koutaro Yokote[3], MD, PhD, Hiroyuki Daida[2], MD, PhD, Keiichi Fukuda[1], MD, PhD

1. Department of Cardiology, Keio University School of Medicine
2. Department of Cardiovascular Medicine, Juntendo University Graduate School of Medicine
3. Division of Diabetes, Metabolism and Endocrinology, Chiba University Hospital

***Backgrounds:*** Werner syndrome (WS) is a rare autosomal recessive disorder characterized by premature onset of several aging-associated diseases, such as atherosclerosis, diabetes, cancer, and early death. The aging phenotypes of WS resemble to those of normal aging. WS is caused by mutations in *WRN* gene belonging to the RecQ DNA helicase which plays a role in genomic stability. But some of WS phenotypes are hardly explained by genomic instability. Thus, we aimed to model WS by patient-specific induced pluripotent stem cells (iPSCs) to decipher the novel pathway to accelerate aging.

***Methods and Results:*** We sampled T lymphocytes from a patient with WS. Then we transduced with Yamanaka factors (*OCT4*, *SOX2*, *KLF4*, and *MYC*) by Sendai virus, and iPSC colonies were derived. We confirmed that WS-iPSCs expressed pluripotent markers, could differentiate into all three germ-layer derived tissues, and retained a normal karyotype. We could culture WS-iPSCs over 2 years with pluripotent status. Then, we differentiated WS-iPSCs into fibroblast-like cells. WS-iPSC-derived fibroblast-like cells (WS-iPSC-fibroblasts) showed characteristics of cellular senescence, the poorly proliferation rate and increased positive cells of $\beta$-galactosidase activity and $\gamma$-H2AX a marker for DNA damage, subjected to cellular stress substance. Singled WS-iPSC-fibroblasts showed other characters of excessive blebbing of plasma membrane and increased apoptosis. WS-iPSC-fibroblasts also showed the decreased migration capacity in wound healing assay. We performed microarray analysis, WS-iPSC-fibroblasts reproduced the global gene expression pattern of physiological aging. The phenotype of human diseases would be associated with many genomic factors including responsive gene mutation, accessory gene mutations and polymorphism. To elucidate whether *WRN* mutation would be responsible for the phenotype of WS-iPSC-fibroblasts, we corrected the gene mutation in *WRN* by homologous recombination using clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 systems. Among the 60 clones, 14 clones showed heterozygosity (WT/MT; WS-Hetero) and 3 clones showed homozygosity (WT/WT; WS-Homo). We confirmed both of recombinant corrected-WS-iPSCs expressed pluripotent markers and differentiated into fibroblast-like cells (WS-Hetero-and WS-Homo-iPSC-fibroblasts). The proliferation rate was increased in WS-Hetero-and WS-Homo-iPSC-fibroblasts. The incidence of blebbing positive cells and apoptotic cells were also decreased in WS-Hetero-and WS-Homo-iPSC-fibroblasts. Delayed wound healing in WS-iPSC-fibroblasts also rescued in WS-Hetero-and WS-Homo-iPSC-fibroblasts Gene expression pattern regarding with aging in WS-Hetero-and WS-Homo-iPSC-fibroblasts is also shifted to that in WT-iPSC-fibroblasts.

***Conclusion:*** We modeled aging phenotypes by WS-specific iPSCs. And we successfully treated such phenotypes by CRISPR/Cas9 systems.

**P-016**

Poster Award

## Establishment of in vitro Bloom syndrome model using BS-iPSC

Kazuki Ohuchi[1,2], Chizuru Kawase[2], Michinori Funato[2], Junko Seki[2], Yasuhiro Ikawa[3], Akihiro Yachie[3], Hideaki Hara[1], Hideo Kaneko[2]

1. Molecular Pharmacology, Department of Biofunctional Evaluation, Gifu Pharmaceutical University
2. Department of Clinical Research, National Hospital Organization, Nagara Medical Center
3. Department of Pediatrics, Kanazawa University

【Background and Purpose】 Bloom syndrome (BS) is a rare autosomal recessive inherited disease characterized by genomic instability, leading to various complications including cancer. The genetic abnormality in BS is caused by the mutation of BLM gene, which is responsible gene of RECQL3 helicase. The deletion of BLM causes a high number of sister-chromatid exchange (SCE) which is a diagnostic indicator of BS. To our knowledge in vitro BS system which reflects the patient's condition could not be developed. In the report, we tried to generate iPS cell derived from the patient with BS (BS-iPSC) and report the abnormality of the cortical neuron phenotype for the first time. 【Methods】 We analyzed a patient with BS, who is 24-years-old female and has the complication with T cell lymphoma and diabetes mellitus. In this study, we generated the BS-iPSC from the patient' s skin fibroblasts and inducted the cortical neuron according to the previous report (Kondo T et al., 2013, Cell Stem Cell) and the axon was stained by TUJ1 antibody (marker of axon). 【Results】 The frequency of SCE in iPSC of our patient was 10 times higher than that of control individual. In addition, the axon length of cortical neuron derived from BS-iPSC was shorter than that of control individual (201B7), 【Conclusion】 In this report, we succeeded in generating the in vitro BS model using BS-iPSC which reflects the feature of pathological condition, suggesting that BS might have the disorder in the development of cortical neuron.

**P-017**

Travel Grant

## Immunodeficiency and radiosensitivity in Bloom's Syndrome

Michiel Schoenaker[1], Miriam van der Burg[2], Hanna Ijspeert[2], Corry Weemaes[1]

1. Department of Pediatrics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands
2. Department of Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam, The Netherlands

Bloom's syndrome (BS) is a genetic disorder, caused by mutations in the BLM gene. This gene codes for BLM protein, which is a DNA helicase involved in DNA repair. Clinically BS is characterized by an immunodeficiency and a predisposition to both haematologic and solid malignancies (with toxicity after radiotherapy). Since DNA repair is crucial for recovery after DNA damage and the development/ maturation of the T and B cells, both processes could reveal parts of the pathophysiology of BS. Therefore we studied both T and B cell subsets, and the radiosensitivity of fibroblasts derived from BS patients to study the possible defect in DNA repair.
We performed immune phenotyping of the B and T cell subsets and analyzed somatic hypermutations (SHM) and class switch recombination (CSR) in detail by analyzing IGHA and IGHG transcripts using next generation sequencing. We irradiated the fibroblast of 10 BS patients to quantify DNA damage and the function of DNA repair in BS patients.
The absolute numbers of the T and B cells were relatively low, but still in the normal range of the healthy controls. Remarkably, all BS patients studied had a high percentage (20-80%) of CD4+ and CD8+ effector memory T cells. The process of SHM seems normal, however the Ig subclass distribution was not normal, since the BS patients had more IGHG1 and IGHG3 transcripts, The longitudional serum immunoglobulin levels were low, and the patients had increased number of infection, although they did not have severe or opportunistic infections.
The radiosensitivitiy in BS fibroblasts is still under investigation and we will present the first results at the congress.
In conclusion, the immune system of BS seems only mildly affected. The function of DNA repair after radiation is not clarified yet, but could be of great clinical value in the context of radiation.

## P-018

## DNA unwinding mechanism of human RecQ helicases WRN and BLM

Ken Kitano

Graduate School of Biological Sciences, Nara Institute of Science & Technology

The RecQ family DNA helicases Werner syndrome protein (WRN) and Bloom syndrome protein (BLM) play a key role in protecting the genome against deleterious changes. In humans, mutations in these proteins lead to rare genetic diseases associated with cancer predisposition and accelerated aging.
WRN and BLM are distinguished from other helicases by possessing signature tandem domains referred to as the RQC (RecQ C-terminal) and HRDC domains. Although the precise function of the HRDC domain remains unclear, structural studies of WRN and BLM have visualized a central role for the RQC domain in recognizing, binding and unwinding DNA at branch points [1-6]. In particular, a prominent hairpin structure (the $\beta$-wing) within the RQC winged-helix motif acts as a scalpel to induce the unpairing of a Watson–Crick base pair at the DNA duplex terminus.
I review the unique DNA-unwinding mechanism of WRN and BLM, and then present the latest docking simulation with a Holliday junction [1]. The model offers an explanation for the efficient branch migration activity of WRN and BLM toward recombination and repair intermediates.
References
[1] Kitano, K. (2014). *Front. Genet.*, 5, 366.
[2] Kitano, K. (2014). *J. Crystallogr. Soc. Jpn.*, 56(2), 133-138. (in Japanese)
[3] Kim, SY., Hakoshima, T., Kitano, K. (2013). *Sci. Rep.*, 3, 3294.
[4] Kitano, K. et al. (2010). *Structure*, 18(2), 177.
[5] Sato, A. et al. (2010). *J. Biochem.*, 148(4), 517.
[6] Kitano, K. et al. (2007). *J. Biol. Chem.*, 282(4), 2717.

**P-019**

Travel Grant

## Determining the impact of RECQL4 mutations on normal homeostasis and cancer predisposition

Wilson Javier Castillo-Tandazo[1,2], Monique F Smeets[1,2], Carl R Walkley[1,2]

1. St. Vincent's Institute, Fitzroy, Vic, 3065 Australia
2. Department of Medicine, St. Vincent's Hospital, University of Melbourne, Fitzroy, Vic, 3065 Australia

Studies of rare human cancer pedigrees have provided enormous insight into the pathogenesis of both familial and sporadic human cancer. A rare familial cancer predisposition syndrome is Rothmund-Thomson Syndrome (RTS). This autosomal recessive disorder is characterised by poikiloderma, skeletal dysplasias, premature aging, and is associated with elevated rates of malignancies, in particular osteosarcoma and haematological cancers. Mutations in *RECQL4* have been defined as causative in RTS. We have previously described a conditional knockout allele of *Recql4,* and obtained a number of relevant findings: Firstly, germ-line null alleles of *Recql4* caused early embryonic lethality. Secondly, when *Recql4* was acutely deleted in adult animals, the mice rapidly succumbed to a fully penetrant bone marrow failure. Thirdly, the deletion of *Recql4* in the proliferative osteoblast progenitor population resulted in mice with shorter bones and reduced bone volume, but not in the development of osteosarcoma. All these phenotypes were the result of the complete deletion of *Recql4*. However, mutations in patients with RTS appear to result in a disabled, but not absent, RECQL4 protein. Therefore, we have now established four distinct point mutant (PM) *Recql4* alleles (K525A, G522EfsX43, R347* and M789K), in addition to compound heterozygous animals (eg Recql4 R347*/K525A), which map closely to mutations reported in RTS patients. To determine the effect of germ-line expression of the mutant *Recql4* alleles, mice are continuously assessed for phenotypes associated with RTS and malignancies. Amongst cell types affected in RTS, osteoblasts appear to be very sensitive to mutations of *RECQL4*. To understand this, we crossed the point mutant alleles to the established *Osterix1*-GFP-Cre (*Osx*-Cre) *R26*eYFP *Recql4*$^{fl/fl}$ line to generate cohorts of *Osx*-Cre *R26*eYFP *Recql4*$^{fl/PM}$ animals. As the cohorts of each genotype age, osteosarcoma incidence is being assessed, and tibias are collected for analysis of bone development. Lastly, to assess the requirement of RECQL4 in primary cells and cell line models, we have isolated primary osteoblasts and established immortalised haematopoietic cell lines from *R26*-CreER *Recql4*$^{+/+}$, *R26*-CreER *Recql4*$^{fl/+}$, and *R26*-CreER *Recql4*$^{fl/PM}$. These cell types are of special interest given the high incidence of osteosarcoma and haematologic cancers in RTS patients. In conclusion, this study has the potential to determine the effects of *RECQL4* mutations, in addition to provide a critical understanding of the role of *Recql4* in developmental biology and cancer development.

**P-020**

Travel Grant

## Mitol dependent ubiquitylation of RECQL4 prevents its function as an accessory factor for mitochondrial DNA replication

Mansoor Hussain[1], Himanshi Agarwal[1], Ullas Kolthur-Seetharam[2], Sagar Sengupta[1]

1. National Institute of Immunology, New Delhi, India
2. Department of Biological Sciences, Tata Institute of Fundamental Research, Mumbai, India

Mutations in RECQL4 lead to Rothumund Thomson Syndrome (RTS) characterized by predisposition to cancer and alterations in the aging process. RECQL4 belongs to the RecQ family of helicases which participates in mitochondrial DNA (mtDNA) replication by acting as an accessory factor to mitochondrial polymerase, Pol $\gamma$ A/B2. We found that RECQL4 is ubiquitylated in the tissues of aged mice, which causes its enhanced turnover. The enhanced degradation of RECQL4 is due to its interaction and subsequent K6-linked ubiquitylation by the mitochondrial E3 ligase Mitol at two specific lysine residues. Mitol mediated ubiquitylation of RECQL4 decreases its binding to Tom20, thereby diminishing its entry into the mitochondria. Consequently non-ubiquitylated RECQL4, which preferentially enters the mitochondria, can enhance Pol $\gamma$ A dependent polymerization and exonuclease activity. Once inside the mitochondrial matrix, RECQL4 is phosphorylated by mtPKA and thus becomes a substrate of Lon protease for its subsequent proteolysis. RTS patient mutants are hyper-ubiquitylated by Mitol which prevents its entry into the mitochondria and consequent function as an accessory factor for mtDNA replication. These results indicate that Mitol dependent ubiquitylation controls the entry of RECQL4 into the mitochondria and thereby its function during mtDNA replication.

## P-021

### Malfunction in protective mechanisms via annexin A2 extracellular release against oxidative stress in Cockayne syndrome (CS) patient-derived cells

Kazuko Kita[1], Katsuo Sugita[2], Atsushi Kaneda[1]

1. Department of Molecular Oncology,Graduate School of Medicine, Chiba University
2. Department of Clinical Medicine, Faculty of Education, Chiba University

Purpose: Cockayne syndrome (CS) is a rare hereditary multisystem disease characterized by neurological and development impairment and premature aging. Cultured cells from CS patients (CS cells) are defective in nucleotide excision repair (NER) function for ultraviolet light (UV)-induced DNA damages. However, CS patients do not present increased cancer risk as expected in DNA repair defective syndromes and show some progeroid features. Recently CS cells are suggested to be hypersensitive to a variety of oxidizing agents not only UV light. We examined the sensitivity to cell death, the repair activity of DNA damages and extracellularly released proteins in CS cells after exposure to oxidative stress.
Methods: We used two CS cell lines, CS3BES (CSA defective) and CS1ANS (CSB defective), the human cervical cancer cell line HeLa cells, and the human fibroblastic cell line RSa. Cells were exposed to oxidative stresses, such as X-ray irradiation and hydrogen peroxide treatment, and the sensitivity to cell death was examined using the colony survival assay and MTT assay. DNA lesions were analyzed using the comet assay. Proteins released into media from cells exposed to oxidative stress and intracellular reactive oxygen species (ROS) signal proteins were examined using immunoblotting analysis.
Results: CS3BES and CS1ANS cells showed higher sensitivity to cell death induced by X ray and hydrogen peroxide than HeLa and RSa cells. Furthermore, after exposure to the stresses the levels of DNA damage were higher, or repair activity was lower in CS3BES cells when compared with HeLa cells. In HeLa cells, extracellular release of annexin A2 was greatly enhanced via ROS-activated p38 MAPK signal pathway after low-dose X-ray radiation. Cells treated with annexin A2-containing media released from irradiated cells acquired radioresistance. However, in CS cells neither the oxidative stress-induced annexin A2 release nor radioresistance by media from irradiated cells was observed.
Conclusions: The present results clearly show that the two CS cell lines are vulnerable to oxidative stress. Malfunction in the protective mechanism against oxidative stress, in which extracellular release of annexin A2 via the ROS-activated signal is involved, might be related to the vulnerability.

## P-022

### Radiation-hypersensitive genetic disorder: AT-LD and roles of responsible gene, MRE11

Junya Kobayashi[1,2], Kasumi Kawamura[1,2], Fei Qi[1,2], Qingmei Meng[1,2], Shinya Matsuura[3], Kenshi Komatsu[1]

1. Radiation Biology Center, Kyoto University, Japan
2. Department of Interdisciplinary Environment, Graduate School of Human and Environmental Sciences, Kyoto University, Japan
3. RIRBM, Hiroshima University, Japan

Ataxia Telangiectasia (AT) is radiation-hypersensitive genetic disorder, showing chromosomal instability, radio-resistant DNA synthesis, immuno-deficiency and predisposition to malignances. The product of responsible gene, ATM phosphorylates several factors for cell cycle checkpoints. Nijmegen breakage syndrome (NBS) and AT-like disorder (AT-LD) also show similar phenotypes to A-T. Their responsible gene products, NBS1 and MRE11 contribute to ATM activation through physical interaction among them. Such interaction in DNA damage responses must be related with the similarity of phenotypes among AT, NBS and ATLD patients. However, the remarkable neurodegeneration phenotype (progressive cerebellar) in AT and ATLD patients is not observed in NBS patients. Recently, it was clarified that ATM could also be activated with ROS (reactive oxidative species), suggesting that the role of ATM in oxidative stress response is related with the neurodegeneration in A-T and AT-LD. Therefore, we are focusing on AT-LD patients to identify unknown MRE11 function, related with neurodegeneration.
Recently, we found novel Japanese AT-LD patient, possessing the substitution of 47th alanine in MRE11 to valine. As the 47th alanine is wildly conserved in mammal, we investigated the role of this region in MRE11-related responses. We noticed that the Japanese patient cells reduced ATM and ATR-related phosphorylation. Furthermore, the GFP-tagged MRE11-A47V did not localize into nucleus and MRE11-A47V, seemed not to bind with NBS1, suggesting that nuclear localization of MRE11 dependently on NBS1 might be important for ATM and ATR activation. Although such DNA damage-related responses occurred in nucleus, we also clarified cytoplasmic role of MRE11. We will also introduce such cytoplasmic functions of MRE11.

**P-023**

## Rothmund-Thomson syndrome and Baller-Gerold syndrome in Japan

Hideo Kaneko, Michinori Funato

Department of Clinical Research Nagara Medical Center

(Background) Rothmund-Thomson syndromae (RTS) is a rare autosomal genetic disorder characterized by poikiloderma and radial aplasia/hypoplasia. Baller-Gerold syndrome (BGS) is a RTS-related disease characterized by radial aplasia/hypoplasia and craniosynostosis. The causative gene for RTS and BGS encodes RECQL4, which belongs to the RecQ helicase family. (Method) To understand RTS and BGS patients in Japan, a nationwide survey was conducted. (Results and Conclusion) Nationwide survey identified 10 RTS patients and 2 BGS families and 3 BGS patients. All the three BGS patients showed radial defects and craniosynostosis. In one patient who showed a dislocated joint of the hip and flexion contracture of both the elbow joints and wrists at birth, a homozygous large deletion in the RECQL4 gene was identified. This is the first reported case of BGS in Japan caused by RECQL4 gene mutation.

**P-024**

Travel Grant

## Osteoporosis and fracture risk in Rothmund-Thomson syndrome

Linchao Lu[1], Felicia Cao[1], Steven A. Abrams[2], Allison Tam[1], Weidong Jin[1], Brian Dawson[1], Roman Shypailo[1], Hao Liu[1], Brendan Lee[1], Sandesh C.S. Nagamani[1,3], Lisa L Wang[1,3]

1. Baylor College of Medicine, Houston, TX 77030, USA
2. Dell Medical School at the University of Texas at Austin, Austin, TX 78712, USA
3. Texas Children's Hospital, Houston, TX 77030, USA

**Background:** Rothmund-Thomson Syndrome (RTS) is an autosomal recessive disease caused by germline mutations in the DNA helicase gene *RECQL4* in the majority of cases. RTS is characterized by a skin rash called poikiloderma and features of accelerated aging, including alopecia, cataracts, osteopenia, and increased cancer risk. The purpose of this study was to investigate the prevalence of osteoporosis in a cohort of RTS patients and to systematically evaluate factors contributing to this phenotype.
**Methods:** We enrolled 29 RTS subjects with (n=20) and without (n=9) *RECQL4* mutations, and assessed areal bone mineral density (aBMD) using dual-energy X-ray absorptiometry, bone remodeling markers, and calcium kinetics parameters. In addition, a mouse model of RTS that closely mimics the skeletal defects in RTS was used to investigate mechanisms underlying the low bone mass phenotype.
**Results:** We measured aBMD at various body sites for 22 individuals (13 pediatric and 9 adult). Compared to age matched reference values, median Z-scores (interquartile range) for aBMD at all sites were significantly lower ($P < 0.01$), while the proportion of individuals with a Z-score less than -2.0 (osteoporosis) was significantly increased in RTS subjects. There was at least one fracture reported in 45% of children (9/20) and 67% (6/9) of adults. Among the patients with fracture history, two-thirds (10/15) reported two or more fractures. In addition, *RECQL4* mutation status was statistically associated with number of fractures, suggesting RTS patients with *RECQL4* mutations have increased risk of multiple fractures.
Compared to reference values, RTS patients had no significant changes in markers of calcium homeostasis (serum calcium, vitamin D, and parathyroid hormone) or calcium metabolism as measured by calcium labeling. Measurement of bone remodeling markers showed no significant change in levels of collagen N-telopeptide (NTX), a marker of bone resorption, while the bone formation marker osteocalcin, was found to be low in 26% of RTS patients, indicating that osteoblast activity could be defective in RTS. To further study this, we used our RTS mouse model with inactivated *Recql4* in skeletal progenitor cells ( *Prx1-Cre$^{tg/+}$; Recql4$^{fl/fl}$* ) , to examine postnatal bone homeostasis. μCT showed that *Prx1-Cre$^{tg/+}$; Recql4$^{fl/fl}$* mice had > 50% reduction in trabecular bone mass and cortical bone area, in line with the low aBMD seen in RTS patients. Bone histomorphometric analyses showed that, while there was no significant change in the osteoclast number and function, *Prx1-Cre$^{tg/+}$; Recql4$^{fl/fl}$* mice had significantly reduced osteoblast number and osteoblast surface. There was also > 50% reduction in the surface of newly formed bone (osteoid) in mutant mice, suggesting that reduced osteoblast number and function lead to the low bone mass in these mice.
**Conclusions:** Our data demonstrate that RTS patients have low bone mineral density and increased risk of osteoporosis. In addition, *RECQL4* status is associated with increased risk of multiple fractures, and decreased osteogenesis contributes to the low bone mass phenotype in RTS patients. These findings are important for the establishment of standard of care for low bone mass in RTS, and will aid in developing effective managements of osteoporosis in RTS.

**P-025**

**Poster Award**

## Establishment of a care system aimed at improving QOL of Patients with Hutchinson-Gilford progeria syndrome in Japan

Nanae Kawano[1], Minoru Takemoto[2], Koutaro Yokote[3], Muneaki Matsuo[4], Rika Kosaki[5], Kenji Ihara[1]

1. Department of Pediatrics, Oita University Faculty of Medicine
2. Department of Diabetes, Metabolism and Endocrinology, School of Medicine, International University of Health and Welfare
3. Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine
4. Department of Pediatrics, Faculty of Medicine, Saga University
5. Division of Medical Genetics, National Center for Child Health and Development

**Introduction**: Hutchinson-Guildford syndrome (HGPS) is an extremely rare genetic disorder that shows a characteristic progeria phenotype. The typical symptoms, such as failure to thrive, loss of scalp hair, a small chin, facial features that resemble aged persons, lipodystrophy, sclerodermatous skin and tightened joint ligaments gradually progress after birth. In the later years of life, HGPS patients develop severe, prominent multisystem complications, such as cerebral infarction, coronary artery disease, cardiac valvulopathy and hypertension. The average life expectancy is reported to be approximately 14.6 years old, and it is estimated that there are approximately 350 to 400 children living with HGPS worldwide. We conducted the Japanese national questionnaire survey, and confirmed 10 Japanese patients with HGPS. We then carried out detailed investigation on their clinical characteristics, and created diagnostic criteria for HGPS, which was subsequently registered as a disease of the Specific Pediatric Chronic Diseases in Japan.

**Purpose**: Aging phenomena in HGPS patients appear and progress soon after birth whereas the patient's psychomotor development is usually preserved within almost normal ranges throughout their life. As a result, the patients suffered from lifelong complications related to premature aging, but the social support system has not been established for this orphan disease. In this study, we aim to investigate quality state of the patients with HGPS and to create support system for each of their difficulties. Furthermore, we try to produce extensive manual how to provide better life for the patients. The establishment of HGPS-specific welfare services to deal with both geriatric- and pediatric-specific matters can provide better daily life for patients with HGPS.

**Method (in progress)**: 1. We conduct a current situation survey for QOL of the HGPS patients and their families using Kinder Lebensqualitat Fragebogen (KINDLE), Pediatric Quality of Life Inventory (PedsQL) genetic core scale survey paper. 2. We carry out a questionnaire and interview survey for various professions, such as medical care, education and welfare services for the patients, to extract reliable information about the HGPS patients and their families. 3. Based on the survey, we try to make a provisional handbook for HGPS and then evaluate the applicability of it for the patients with HGPS and their family.

**P-026**

## Sclerotic skin lesion as an initial manifestation of Hutchinson-Gilford Progeria syndrome

Muneaki Matsuo[1], Nanae Kawano[2], Minoru Takemoto[3], Koutaro Yokote[4], Rika Kosaki[5], Kenji Ihara[6]

1. Department of Pediatrics, Faculty of Medicine, Saga University
2. Department of Pediatrics, Almeida Memorial Hospital
3. Department of Diabetes, Metabolism and Endocrinology, School of Medicine, International University of Health and Welfare
4. Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine
5. Division of Medical Genetics, National Center for Child Health and Development
6. Department of Pediatrics, Oita University Faculty of Medicine

Hutchinson-Gilford Progeria syndrome (HGPS) is a rare premature aging syndrome caused by a mutation of *LMNA* gene. We conducted questionnaire survey for the tertiary hospitals in Japan and reviewed academic reports in Japan, to identify the characters of Japanese patients with HGPS. By the stepwise questionnaire survey, we finally registered 8 patients and analyzed skin lesions on Japanese HGPS patients. We also collected 45 cases of HGPS from the literature, and compared those skin findings with those of Japanese cases. Sclerotic skin lesions were observed in all 8 patients, whereas 37 out of 45 cases (82.2%) in the literature had the sclerotic skin. Most commonly involved skin region was abdomen, then lower extremities. The age of initial manifestation of the skin lesion was a median age of one month (at birth to 18 months), while in the literature it was a median age of one month (at birth to 8 months). Histopathological finding showed normal or atrophic epidermis with thick collagen bundles under it. Alopecia was also common finding, and it appears at a median age of 24 months (10 to 48 months). Since skin change of HGPS appears early in life, it is important to notice it as an initial manifestation of HGPS.

## P-027

## Diabetes patients with Japanese Werner syndrome exhibit high incidence of cancer

Ohnishi S, Fujimoto M, Oide T, Nakatani Y, Tsurutani Y, Koshizaka M, Mezawa M, Ishikawa T, Takemoto M, Yokote K

Division of Diabetes, Metabolism and Endocrinology,Department of Internal Medicine, Chiba University Hospital

Werner syndrome (WS), a representative type of progeroid syndrome, is caused by mutation of the WNR gene (*WNR*). Since WS patients often have diabetes and malignant tumors, we initiated a nation-wide epidemiological survey in Japan to understand the current relationship between diabetes and malignant tumors among WS patients. We sent 6000 questionnaire survey sheets to hospitals with more than 200 beds and detailed clinical data for 171 cases were obtained. The WS patients were divided into the following 2 groups: patients with diabetes (n = 94) and those without diabetes (n = 55). We examined the correlation among epithelial tumors (cancer) and non-epithelial tumors and diabetes using the chi-square test. The morbidity rate of cancer and non-epithelial tumors were 41.5% and 70.7%, respectively. There was no significant difference in the morbidity rates of non-epithelial tumors in patients with or without diabetes. However, the prevalence of cancer was significantly higher in patients with diabetes (14.9%) than in those without (3.6%). The prevalence of non-epithelial tumors and cancer are known to be similar in WS patients, although non-epithelial tumors are seldom observed in the general population. The underlying mechanism for the high prevalence of non-epithelial tumors in patients with WS is unknown. It has recently been reported that expression of *WNR* was suppressed in cancer tissues from patients without WS, probably due to the epigenetic changes in the promoter region of *WNR*. This indicates that *WNR* might act as a tumor suppressor. Diabetic patients are prone to developing malignant tumors, and insulin resistance has been proposed as one of the reasons for the high prevalence of cancer in diabetic patients. Diabetes with WS is usually caused by high insulin resistance, which may be one of the contributing factors for cancer development but not for non-epithelial tumors. This might be significant for understanding the mechanism of cancer and for the development of new therapeutic methods in the future.

**P-028**

## Physician-initiated clinical study of limb ulcers for diabetes patients and Werner syndrome treated with a novel peptide, SR-0379

Hironori Nakagami[1], Ken Sugimoto[2], Takahiro Ishikawa[3], Yoichi Takami[2], Minoru Takemoto[3], Masaya Koshizaka[3], Hideki Hanaoka[4], Xiang Jing Yao[5], Koutaro Yokote[3], Hiromi Rakugi[2]

1. Department of Health Development and Medicine, Osaka University
2. Department of Geriatric and General Medicine, Osaka University Graduate School of Medicine
3. Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine
4. Clinical Research Center, Chiba University Hospital
5. Department of Medical Innovation, Osaka University Hospital

SR-0379 is a novel functional peptide that exerts anti-microbial actions during wound healing, making it an ideal drug to prevent infection. To evaluate the safety, efficacy and pharmacokinetics of SR-0379 for the treatment of leg ulcers, a physician-initiated, phase I/IIa, first-in-patient clinical study was designed. A multi-center, double-blind, randomized clinical study was conducted from October 2015 to September 2016. The inclusion criteria for severe leg ulcers were 1) diabetes or critical limb ischemia and 2) wound size less than 6 cm in diameter. Twelve patients were randomized into 4 groups and administered 0.02, 0.1, or 0.5% SR-0379 or placebo treatment on skin ulcers once per day for 28 days. Peptide efficiency was evaluated by determining the rate of wound size reduction as a primary endpoint at 4 weeks after the first treatment compared with the pretreatment wound size. As a secondary endpoint, the DESIGN-R score index, time to wound closure, and the 50% wound size reduction ratio were also evaluated. The safety of SR-0379 was evaluated during the study period. In the evaluation of efficiency, the skin ulcer reduction rates at the last evaluation were 44.73% for the 0.02% SR-0379 group, 68.25% for the 0.1% group, and 71.61% for the 0.5% group, compared with 9.95% for the placebo group. Six adverse events were reported in 4 patients, of which one occurred in the placebo group, and causal relationships were denied for all 6 events. One patient in the 0.1% group was diagnosed with Werner Syndrome, and his ulcer, which was on the heel, was drug-resistant; however, it demonstrated a 34.92% size reduction. One patient in the 0.02% group with an ulcer in the lateral malleolus was also diagnosed with Werner Syndrome. Treatment with SR-0379 resulted in a 61.45% size reduction. Treatment with SR-0379 for chronic leg ulcers was safe, well-tolerated, and effective.

**P-029**

## Japanese case of atypical progeroid syndrome/atypical Werner syndrome with heterozygous *LMNA* mutation: Increased susceptibility to oxidative stress-and ultraviolet A-induced apoptosis in fibroblasts

Sei-ichiro Motegi , Osamu Ishikawa

Department of Dermatology, Gunma University Graduate School of Medicine

Atypical progeroid syndrome (APS), including atypical Werner syndrome (AWS), is a progeroid syndrome involving heterozygous mutations in the *LMNA* gene encoding the nuclear protein lamin A/C. We report the first Japanese case of APS/AWS with a *LMNA* mutation (p.D300N). A 53-year-old Japanese man had a history of recurrent severe cardiovascular diseases as well as brain infarction and hemorrhages. Although our APS/AWS patient had overlapping features with Werner syndrome (WS), such as high-pitched voice, scleroderma, lipoatrophy and atherosclerosis, several cardinal features of WS, including short stature, premature graying/alopecia, cataract, bird-like face, flat feet, hyperkeratosis on the soles and diabetes mellitus, were absent. In immunofluorescence staining and electron microscopic analyses of the patient's cultured fibroblasts, abnormal nuclear morphology, an increase in small aggregation of heterochromatin and a decrease in interchromatin granules in nuclei of fibroblasts were observed, suggesting that abnormal nuclear morphology and chromatin disorganization may be associated with the pathogenesis of APS/AWS.
Recently, it has been reported that UVA induced abnormal truncated form of lamin A, called progerin, as well as HGS-like abnormal nuclear structures in normal human fibroblasts, being more frequent in the elderly, suggesting that lamin A may be involved in the regulation of photoageing. Therefore, we examined the sensitivity to cell damage induced by oxidative stress or UVA in fibroblasts from APS/AWS patient. Using immunofluorescence staining and flow cytometry analysis, the amount of early apoptotic cells and degree of intra-cellular reactive oxygen species (ROS) generation were higher in H2O2- or UVA-treated APS/AWS fibroblasts than in normal fibroblasts, suggesting that repeated UV exposure may induce premature ageing of the skin in APS/AWS patients and that protecting against sunlight is possibly important for delaying the emergence of APS/AWS symptoms. In addition, we demonstrated that H2O2-, or UVA-induced apoptosis and necrosis in normal and APS/AWS fibroblasts were enhanced by farnesyltransferase inhibitor (FTI) treatment, indicating that FTI might not be useful for treating our APS/AWS patient.

## P-030

### Evidence creation through nationwide surveillance of the progeria syndrome Werner syndrome and establishment of a case registration system - Werner Syndrome Nationwide Survey and Registry-

Masaya Koshizaka[1], Kazuki Kobayashi[2,3], Takahiro Ishikawa[1,2], Yoshiro Maezawa[1,2], Minoru Takemoto[2,4], Koutaro Yokote[1,2]

1. Chiba University Hospital
2. Chiba University Graduate School of Medicine
3. Asahi General Hospital
4. International University of Health and Welfare

<Background>
In order to solve the clinical questions regarding with the adult progeria, Werner Syndrome (WS), and establish high-quality evidence that contributes to the revision of clinical practice guidelines of WS, we have had the nationwide survey and have established the Werner Syndrome Registry. By the survey and registry, we could reveal the current disease profile, the natural history, and prognosis of WS in Japan.
<Methods>
① Nationwide survey: We had the nationwide survey of WS. We had sent questionnaires to 7888 doctors, whose specialty were endocrinology, collagen disease, geriatrics, ophthalmology, dermatology, plastic surgery or orthopedic.
② Werner Syndrome Registry: Registry was conducted for facilities that reported diagnosed cases. We analyzed data from the registry, during the past year as pilot data.
<Results>
① We have received 3154 answers. The return rate was 40%. There were 116 cases diagnosed, 51 cases of suspicious cases, and 153 cases of past hospital visit. Orthopedic doctors, plastic surgeon and dermatologist reported most of patients. There was no regional bias in the distribution of the current patient in Japan, suggesting that patients are widely present in Japan.
② The average age of cases was 51 years old. The life expectancy extended compared with before. The mean onset age was 24.3 years old, but the mean diagnosed age was 42.5 years old. There was a discrepancy from onset to diagnosis. It suggested that there are chances of early intervention. The average height and weight were lower than Japanese average, and BMI was low. It is said that WS patients have central obesity, but the average abdominal circumference is not large. The physique is originally small.
Regarding the major signs, there were some surely caused symptoms such as hair change, cataract, calcification of soft tissues, but some were not surely caused. We found glycolipid abnormality and hypertension in majority abnormality cases, but that atherosclerotic diseases were a few. Consanguineous marriage rate was about 60%. One quarter of cases had foot amputation. Compared with the previous survey, skin ulcer and history of angina or myocardial infarction and history of obstructive arteriosclerosis have decreased. For the treatment of diabetes, DPP-4 inhibitors and pioglitazone were often used.
The grip strength, which is one of the diagnostic criteria of Asian sarcopenia by the Asian Working Group for Sarcopenia (AWGS), was also significantly lower than 16.9 kg for males and 9.1 kg for females (<26 kg and <18 kg for thresholds). Walking speed was also 0.8 m/sec on average. Total skeletal muscle mass (SMI) was 4.0 kg/m2 on average for males, 3.9 kg/m2 for females (6.87 kg/m2 and 5.46 kg/m2 for thresholds of diagnostic criteria of sarcopenia).
<Conclusions>
We can clarify current disease profile, natural history and prognosis of WS in Japan through the results of this research and improve patient data accuracy and use effectively, and improve medical system for WS. We will be able to rescue patients who do not have appropriate medical treatment and support patient's prognosis improvement and reintegration by improving the quality of medical treatment.

**P-031**

## Quantification of Skin Stiffness in Werner Syndrome to Establish Prevention Management of Foot Ulcers

Yoshitaka Kubota, Hideyuki Ogata, Yoshihisa Yamaji, Takafumi Tezuka, Yoshitaro Sasahara, Shinsuke Akita, Nobuyuki Mitsukawa

Department of Plastic Surgery, Chiba University Hospital

**Introduction**

Ulcers on lower extremities reduce quality of life in patients with Werner syndrome. There are differences in the etiology of foot ulcers between patients with Werner syndrome and those with critical limb ischemia caused by hypertension or diabetes. Sclerosis of foot skin is one of the most chief causes of foot ulcers in patients with Werner syndrome. Severe sclerosis of skin of lower extremity, especially in foot and distal one third of the leg, exists in almost all patients with Werner syndrome. Sclerosis of foot skin produces multiple disorders such as, pain, tylosis, deformity, and ulcers. Tyloses in the foot of patients with Werner syndrome sometime lead to osteomyelitis through the skin ulcer and destruction of cortical bone. To prevent foot ulcers and osteomyelitis in patients with Werner syndrome, management of foot focusing on skin sclerosis is essential. However, objective measurement of skin stiffness in clinical practice is difficult. SkinFibroMeter® (Delfin Technologies, Kuopio, Finland) is recently appeared handy, non-invasive equipment for measurement of skin stiffness. We compared objective skin stiffness of foot between patients with Werner syndrome and normal control using the new equipment.

**Patients and Methods**

We measured skin stiffness of the foot of the patients with Werner syndrome using SkinFibroMeter®. As a control, the foot of normal persons matched by age were selected. We measured three points in a feet. First point was the dorsum of the feet, between 1st and 2nd metatarsal bone. Second point was the instep as a non-weight bearing portion of the plantar. Third point was a center of the calcaneal area as a weight bearing portion of the plantar. Measurement using SkinFibroMeter® was performed five times and an average was taken on each points.

**Results**

Seven patients with Werner syndrome were measured. Measurement using SkinFibroMeter® were performed without any problems in both patients with Werner syndrome and normal controls. In dorsum of the foot, the values measured by SkinFibroMeter were significantly higher in the patients with Werner syndrome compared to normal controls ($0.17 \pm 0.07$ vs $0.02 \pm 0.01$ kg•m / s2, $P < 0.01$). In the instep, SkinFibroMeter values showed similar tendency ($0.09 \pm 0.04$ vs $0.04 \pm 0.01$ kg•m / s2, $P = 0.04$). In the calcaneal area, values were significantly higher in higher in the patients with Werner syndrome compared to normal controls ($0.55 \pm 0.05$ vs $0.12 \pm 0.01$ kg•m / s2, $P < 0.01$). SkinFibroMeter values of the calcaneal area were higher compared to the dorsal foot and the instep.

**Conclusion**

Quantifying of skin stiffness using newly developed SkinFibroMeter can clearly show the specific characteristics of the foot of the patients with Werner syndrome. To establish the management of foot conditions basing on the objective measurement of skin stiffness can be beneficial for the patients with Werner syndrome.

## P-032

## Revision of the management guideline for Werner syndrome

Minoru Takemoto[1], Yoshitaka Kubota[2], Seijiro Mori[3], Kazuhisa Tsukamoto[4], Masafumi Kuzuya[5], Koutaro Yokote[6]

1. Department of Medicine, Division of Diabetes, Metabolism and Endocrinology, International University of Health and Welfare, Chiba, Japan
2. Department of Plastic, Reconstructive, and Aesthetic Surgery, Chiba University Graduate School of Medicine, Chiba, Japan
3. Center for the Promotion of Clinical Investigation, Tokyo Metropolitan Geriatric Hospital, Tokyo, Japan
4. Department of Internal Medicine, Teikyo University, Tokyo, Japan
5. Department of Geriatrics, Medicine in Growth and Aging, Program in Health and Community Medicine, Nagoya University Graduate School of Medicine, Nagoya, Japan
6. Department of Clinical Cell Biology and Medicine, Chiba University Graduate School of Medicine, Chiba, Japan

Werner syndrome (WS), also known as an adult progeria, is an autosomal recessive disorder caused by a mutation in the WRN gene encoding a RecQ-type DNA helicase. The diagnostic criteria for WS were revised in 2012 based on the results of a national epidemiological study conducted from 2009 to 2011 after an interval of 26 years. We also collated our experiences of treating patients with WS and studies from the literature that described the management strategies for disorders accompanying WS, such as refractory skin ulcer, dyslipidemia, infection, and diabetes. Then, we released the management guideline for WS in 2012.
To revise our treatment guidelines for WS, we put up five clinical questions as follows:
1. Management strategy for refractory ulcer
2. Management strategy for dyslipidemia, and hepatosteatosis
3. Management strategy for sarcopenia
4. Management strategy for diabetes,
5. Management method for osteoporosis
Systematic reviews have been conducted, and evidences for each treatment have been collected according to the guidelines of the Medical Information Network Distribution Service. WS is a rare disease; therefore, it is almost impossible to perform placebo-controlled double-blind study, because of which we were unable to obtain good and sufficient evidences for each management. However, experiences of WS treatments should be collected and shared worldwide. At the conference, we will present the recent progress in revising the management guidelines for WS.

**P-033**

## Chromatin accessibility dynamics reveal decrease of DNA motif sequences of transcription factors during macrophage aging

Naoki Osato

Department of Bioinformatic Engineering, Graduate School of Information Science and Technology, Osaka University, Osaka, Japan

The functional activity of innate immunity cells including natural killer cells, macrophages and neutrophils reduces during aging. To prevent the reduction of cellular activities during aging, it would be useful to understand their molecular mechanisms, which are still unclear. The expression level of mRNAs of transcription factors is relatively low, compared with other genes. Thus, the expression level of mRNAs of transcription factors may not change significantly during aging. Previously in our epigenomic analyses, we found that DNA motif sequences of transcription factors in open chromatin regions or regions overlapped with histone modifications of enhancer marks such as H3K27ac and H3K4me1, significantly altered during cell differentiation and changes of environmental conditions [1].
Here I compared young human macrophages with aged ones using DNA motif sequences of transcription factors in open chromatin regions of young and aged cells. Experimental data of open chromatin regions by DNase-seq was obtained from a public database of Blueprint project, which aim to collect various experimental data of immune cells. Experimental data of open chromatin regions of macrophages of six people were separated into three pairs consisting of young and aged cells, which were about 20 years apart in age. I examined statistically significant differences of DNA motif sequences in open chromatin regions between young and aged cells of the three pairs. About 14,000 DNA motif sequences including redundant sequences were collected from various databases and papers.
To find common features of DNA motif sequences of transcription factors in the three pairs of young and aged macrophages, I compared DNA motif sequences in the three pairs and found that DNA motif sequences of transcription factors significantly decreased in the three pairs of young and aged macrophages in common. However, DNA motif sequences of transcription factors increased in the three pairs in common were not observed. The functions of transcription factors of which DNA motif sequences decreased were associated with chromatin interactions, inflammatory and metabolic disorders, proinflammatory signals, and development. Transcription factors of unknown functions in macrophages were also observed. As for chromatin interactions, the DNA binding motif sequences of CTCF and cohesins (RAD21 and SMC3) were decreased in open chromatin regions of aged macrophages, implying that chromatin interactions and gene expression changed. The DNA motif sequences of RXRA and INF1 involved in inflammatory were decreased in aged macrophages, which would affect the activity of macrophages.
The analyses of chromatin accessibility revealed that DNA motif sequences of transcription factors in open chromatin regions decreased during macrophage aging. Molecular mechanisms to form closed and open chromatin structures would be associated with macrophage aging.

**References**

1. Kurotaki D*, Osato N*, Nishiyama A*, Yamamoto M*, et al. Essential role of the IRF8-KLF4 transcription factor cascade in murine monocyte differentiation. Blood. 2013; 121:1839-1849. * equal contribution