Form 3

Report for Joint/Usage Research Program for Endocrine/Metabolism (Fiscal Year 2021)

Date：2021/4/30

To Director of Institute for Molecular and Cellular Regulation, Gunma University

Principal Applicant			
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We report on the results of joint research in fiscal 2020 as below.

（Program No.　　）

1．Research Title	Golgi stress as a source of aging
2．Purpose and Significance of the research project	Anti-aging research has been conducted from various perspectives. This study proposes a new aging mechanism that has not been tried so far, suggests an anti-aging strategy based on this, and seeks to discover related materials. In detail, attention is paid to the aging of the Golgi apparatus, which acts as a major pathway of senescence-associated secretory phenotype (SASP), which is seen when cells are aged. We reveal whether the aging of the Golgi apparatus progresses during aging, and confirms the adverse effects on cells caused by aging of the Golgi apparatus. Furthermore, factors involved in Golgi aging are identified, and related genes/proteins are discovered to reveal the mechanism of Golgi aging. Based on the Golgi aging research method found in this way, we present a method for screening anti-aging materials, discover active ingredients, and observe toxicity and healthy lifespan extension in animal models. The ultimate goal of these studies is to contribute to extending the healthy lifespan of mankind.
3．Period of The Program	April 1, 2021 ～ March 31, 2022

4．Project Members

Name	Age	Gender	Institution/Department	Position	Role
(Principal Applicant) Hantae, Jo	37	M	Ajou University, Department of Biological Sciences	Research Professor	Project director
(Research Collaborators) Byungsun, Cha	29	M	Ajou University, Department of Biological Sciences	Ph. D. Candidate	Researcher

※If additional space is required, attach a separate sheet.

5．Collaborative Researcher of IMCR	Name of the Laboratory	Developmental Biology and Metabolism	Name	Ayako Fukunaka

9. Research Plans

● Presenting a mouse / human Golgi aging model
-Golgi apparatus-related protein changes in aging mouse / human skin and liver: WB (Western blotting) and tissue staining analysis using GM130 antibody and TGN46 antibody
- Analysis of Golgi apparatus-related genes in aging mouse / human skin and liver: Q-PCR (Quantitative PCR) analysis using Arf4, Uap1/1, Hsp47, Mt1 primers
-Golgi apparatus-related genetic changes in passage-aged human fibroblasts: analysis of organelle-related genes in Golgi cells through transcriptome analysis
-Identification of aging-related genes using transcriptome data generated from multiple aging models. Specifically, published in Nature in 2020, produced and published single-cell transcriptome data from 23 tissues and organs in mouse models of different stages of aging. From this data through systems biology techniques, the expression dynamics of genes constituting the Golgi apparatus network and how Golgi stress changes in each tissue according to the aging process are traced (A single-cell transcriptomic atlas characterizes aging tissues in the mouse, Nature, 2021).
- Development of a Golgi apparatus prediction machine learning model using expression information of Golgi apparatus-related genes. To this end, key predictive variables for model development are selected from the transcriptome data of the model with reduced Golgi function from the open database, and the aforementioned single-cell transcriptome data are used as training and cross-validation data. The developed model was finally verified for its performance by utilizing the transcript data of other aging diseases that were published.
-Golgi apparatus protein changes in passage-aged human fibroblasts: WB analysis using GM130 antibody and TGN46 antibody
-Golgi body lipid changes in passage-aged human fibroblasts: FACS (Fluorescence-activated cell sorting) analysis after staining with BODIPY-TR ceramide
-Golgi body morphological changes in passage-aged human fibroblasts: Confocal microscopy/airy scan analysis using GM130 antibody and TGN46 antibody
-Changes in Golgi apparatus protease function of passage-aged human fibroblasts: WB analysis using CREB3 antibody for S1P-dependent processing of CREB3 after BFA treatment
-Changes in Golgi stress responsiveness of passage-aged human fibroblasts: Analysis of the activation of the Golgi stress-regulating transcription factor CREB3 after induction of Golgi stress with GCA, BFA, Momensine, etc. (WB: CREB3, Q-PCR: Arf4, Uap1/1, Hsp47, Mt1)

● Identification of the mechanism of abnormal polymerization of microtubules due to aging of the Golgi apparatus
-Analysis of microtubule polymerization of senescent cells: After treatment with Nocodazole, the degree of recombination, after fluorescence staining with GM130 antibody, TGN46 antibody, and alpha-tubulin antibody, image analysis using confocal microscopy
-Analysis of microtubule polymerization due to Golgi stress: After Golgi stress induction with GCA, BFA, Momensine, etc., Nocodazole treatment, recombination level, GM130 antibody, TGN46 antibody, alpha-tubulin antibody after fluorescence staining, image using confocal microscopy analyze
-Aging Golgi apparatus microtubule polymerization change: analysis of Golgi apparatus-microtubule complex using Tubulin polymerization assay kit (Cytoskeleton, Inc. BK006P)
-Changes in microtubules in the aged crude perinuclear fraction: WB analysis of proteins using GM130 antibody and TGN46 antibody in the non-solubilized fraction of surfactant NP40
-Changes in nuclear migration of microtubule-dependent epigenetic enzymes in senescent cells: WB analysis using antibodies for p300 and HDAC1/2 proteins that migrated in the nucleus after separating the cytoplasm and nucleus using a sucrose cushion
-Microtubule-dependent changes in nuclear migration of p53 in senescent cells: WB analysis using antibody-based p53 protein after separating the cytoplasm and nucleus using a sucrose cushion
-Changes in nuclear migration of the transcription factor SMAD2/3 involved in collagen production in aging fibroblasts: after TGF-beta treatment, fluorescence staining using SMAD2/3 antibody, and confocal microscopy/airy scan analysis

7．Research results:

Golgi stress increased during aging

To elucidate the effects of aging in the function of the Golgi apparatus of senescent cells, we initially analyzed expression levels of Golgi proteins and proteins that changing during aging. Collagen, MT1 (a cellular zinc indicator), and histone 3 acetylation at lysine placed on position 18 (H3K18ac) and position 27 (H3K27ac) were markedly reduced. However, the expressions of Golgi proteins, GM130 and TGN46 increased during senescence (Figure 1A). In accordance, senescent cells showed a widely spread and loosely stacked trans-Golgi/TGN and ci-Golgi/CGN structures (Figure 1B) with increased volume (Figure 1C), indicating an increased Golgi stress, which was also observed in aged mice skin and liver. Moreover, to evaluate the CREB3-dependent stress response, which requires cleavage of CREB3 protein by zinc metalloprotease S2P in the Golgi, pharmacological Golgi disruption by BFA was induced. In senescent cells, CREB3 cleavage does not progress properly, indicating the Golgi dysfunction (Figure 1D). Considering the loosely packed structure of the Golgi and reduced zinc level in senescent cells, zinc-dependent Golgi stacking proteins GRASP55 and Golgin45 were monitored by confocal microscopy, and shown decreased co-stained area in the fragmented Golgi of senescent cells (Figure 1E, insect F).

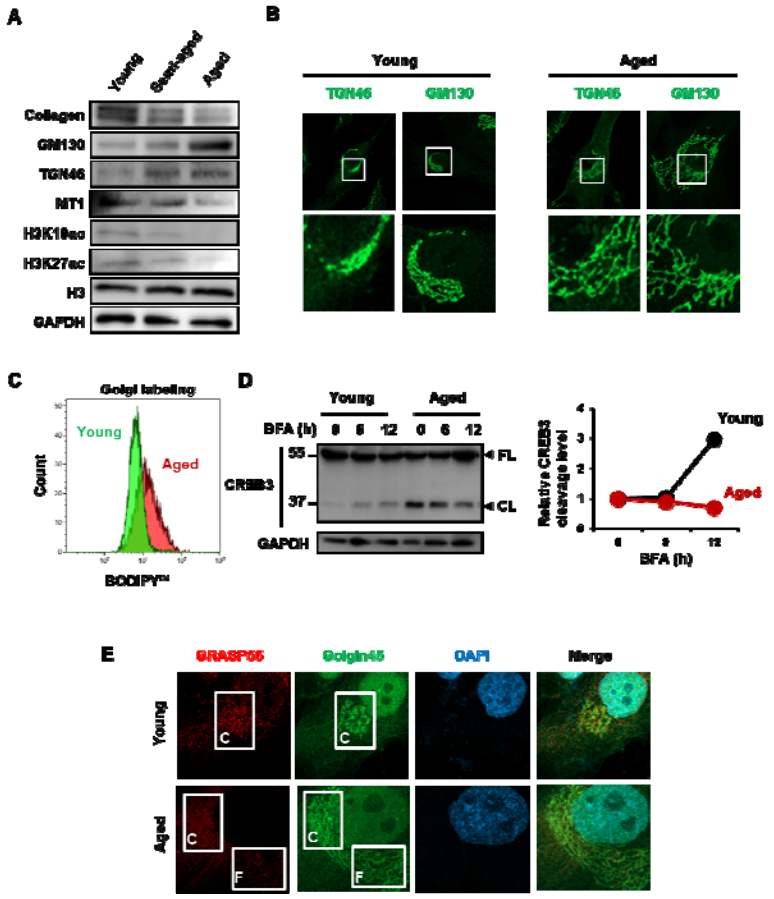

Figure 1. Age-related increase of Golgi stress disturbs Golgi function and stacking.

(A) Western blotting of young (P6), semi-aged (P14) and aged (P32) human primary fibroblasts with each antibody. (B) Immunostaining of young and aged human primary fibroblasts with anti-TGN46 and anti-GM130 antibodies. (C) Golgi volume analysis of young and aged human primary fibroblasts stained with BODIPY™ TR Ceramide and analyzed by FACS. (D) Western blotting of young and aged human primary fibroblasts with anti-CREB3 antibody after BFA treatment (3 μM) for the indicated intervals. FL, Full-length; CL, Cleaved. Right panel indicates relative CREB3 cleavage level. (E) Immunostaining of young and aged human primary fibroblasts with anti-GRASP55 and anti-Golgin45 in young and aged human primary fibroblasts. Nuclei were stained with DAPI. C, Compact, F, Fragmented.

8. Publications and/or Presentations resulting from Joint Research Program with IMCR.
Exchange of information on joint research with faculty members.

①Please describe a list of publications in which the name of the collaborative researcher of IMCR appears and send one paper reprints of each publication to IMCR.
None.

②Please describe a list of publications which include the description that the research is supported by Joint Research Program with IMCR and send one copy of each publication to IMCR.
None.

③Enter the name of the conference, the date of the conference, and the title of the presentation of the conference.(up to 3 cases)
None.

④Implementation status of information exchange with faculty members in charge of joint research.
We keep in touch with Dr. Fukunaka for further study.