

HEALTHY LONGEVITY GLOBAL COMPETITION

2021 Catalyst Awardees

Sponsor: Agency for Medical Research and Development of Japan

A better prediction of onset-risk and onset-age using personal genomes

Principal Investigator	MORISHITA Shinichi
PI Primary Affiliation	Graduate School of Frontier Sciences, The University of Tokyo
Co-Investigator(s)	None

PROJECT DESCRIPTION

The risk of getting a disease and the age of onset vary from person to person. If we can predict the risk and age of onset of disease for each individual, we can take preventive measures in advance to maintain quality of life and realize a healthy society with longevity where the elderly can experience happiness for many years. Statistics that classify the risk of disease by age are useful, but they are an average representation. We want to predict the risk and age of onset of disease for each individual with high accuracy. For this purpose, can we utilize information on all mutations in the personal genome?

For this prediction, we have been developing an algorithm that use single nucleotide variants in personal genomes. Meanwhile, large structural variants could also cause disease; however, they are generally more than 100 bases long and are hard to detect with next-generation short-read sequencing, so they are still largely unexplored. To understand structural variation on the scale of thousands of bases, we have been using long-read sequencing, and we reported tandem repeat expansions associated with benign adult familial myoclonic epilepsy (Nat. Gene. 2018). This extends in an intron with a basic unit of five bases repeated hundreds of time or more. The longer the time, the lower the age of onset, and this is an example where the age of onset can be predicted from the number of repetitions.

We found tandem repeat expansions have a high evolutionary rate and can cause a variety of diseases (Nat. Gen. 2019). In particular, higher order repeats in human centromeres are divergent, and it has been speculated that this is due to the rarity of recombination. Indeed, we found a new linkage disequilibrium that spans the entire centromere (Sci. Adv. 2020). It is intriguing to see how structural variants of centromeres cause chromosomal instability and contribute to disease.

Based on these achievements, we propose and develop a new informatics approach to predict the risk of disease onset and age of onset from personal genomic variants.

Age-dependent trajectory of exosomal protein distribution in healthy aging and Alzheimer's disease

Principal Investigator	HOSHINO Ayuko
PI Primary Affiliation	Tokyo Institute of Technology
Co-Investigator(s)	Lav Varshney; Alexandru Hanganu;

PROJECT DESCRIPTION

Exosomes are small packages of proteins and genetic material that are released into the blood from various cell types and organ systems. They then flow through the blood and into other cells and organs, as a kind of cell-to-cell communication system. It has recently been discovered, however, that the messages carried within exosomes may cause numerous age-related diseases including cancer and Alzheimer's disease (AD). In this work, we aim to characterize the trajectory of how the nature of messages within exosomes change both in healthy aging and in patients that have AD. To do so, we will first use statistical characterizations of age-related changes for the healthy and AD trajectories, and try to interpret the biological significance of specific proteins. Our preliminary data already has shown that the total exosomal protein concentration increases with onset of AD. As a second approach we aim to understand what is happening at a deeper biological level. Taking inspiration from artificial intelligence (AI) approaches in modern natural language processing that use deep learning. The idea is to train a protein language model using large corpora of amino acid sequences to understand the grammar of proteins, and then fine-tune it to classify the cell types and organ systems from where exosomes are coming from. This will allow us to understand the language of the messages in exosomes, as well as the communication paths that exosomes take as they flow through the body. With this understanding, we will be able to explain the biological pathways by which exosomes act, how these pathways evolve with healthy aging, and further to make early predictions for diseases such as Alzheimer's disease.

Clarifying the origins and very early processes of aging in tissue

Principal Investigator	SUSAKI Etsuo A.
PI Primary Affiliation	Graduate School of Medicine, Juntendo University
Co-Investigator(s)	none

PROJECT DESCRIPTION

Clarifying the origin and progression mechanism of aging inside tissue is the most fundamental and vital question in aging research. However, the rareness of such very early aging foci has prevented the identification and investigation of mechanisms so far due to technical limitations. The genetic property of each species essentially determines the aging process. Mutations of genes involved in genome stability cause premature aging syndromes. Therefore, it is plausibly hypothesized that genome information and its instabilities are the determinants or driving forces of the aging process.

To address these questions, we propose two new research designs: 1) identification and analysis of the very early aging foci inside the tissue, and 2) analysis of genomic instability related to the very early aging process inside tissue by newly developed spatial genomics technique. The first idea will be implemented by the applicant's CUBIC cell-omics framework (Susaki+ Cell 2014, Susaki+ Nat Commun. 2020, and others). We have already identified potential very early aging foci (sporadic astrocyte foci in the cerebral cortex) by whole-brain CUBIC immunostaining of senescence-related astrocyte marker (GFAP) in young, physiological mouse brains. We are analyzing their inter-tissue structures and functions. We will further investigate the molecular mechanisms by the recent spatial transcriptomics approach. The second idea can be deployed by integrating our developing technologies for whole-cell spatial addressing and single nuclei collection with current genome sequencing analysis. We will analyze the genomes of the cell groups that comprise the very early aging foci by utilizing this spatial genomics technology.

The above ideas are incredibly innovative and unique, as they are based on our globally acclaimed state-of-the-art technology. We will identify essential perturbation points for intervening the aging process from both young and aged individuals. This project will thus provide unique and effective anti-aging strategies, ensuring the realization of a healthy, longevity society.

Compounds that suppress the aging process through selective autophagy

Principal Investigator	ARIMOTO Hirokazu
PI Primary Affiliation	Graduate School of Life Sciences, Tohoku University
Co-Investigator(s)	none

PROJECT DESCRIPTION

Autophagy is a major degradation system in cells. Best known is its ability to degrade existing proteins into amino acids during starvation. Autophagy also plays a vital role in the protection of the human body by removing invading pathogens and harmful substances such as protein aggregates that accumulate along the aging process. If we can target and remove harmful substances from cells, we would be able to expect to slow down the aging process and reduce the chance of diseases.

Toward this goal, we recently developed a new method named AUTAC (Autophagy-targeting chimera) in 2019. This method uses a combination of a "targeting ligand" to select a degradation target and a "degradation tag", a very unique compound that can trigger autophagy. By changing the targeting ligand appropriately, autophagic degradation of various intracellular substances can be boosted. We believe this is the first example of an autophagy-promoting compound with the ability to "select a degradation target".

In this NAM-HLGC research and development, we will create a variety of AUTAC compounds to realize a long and healthy life. Specifically, we aim to eliminate protein aggregates and dysfunctional mitochondria that accumulate in cells. Since dysfunctional mitochondria are involved in the production of inflammatory cytokines and reactive oxygen species, their removal is expected to inhibit chronic tissue inflammation, neurodegenerative diseases, and cancer. Chronic inflammation is itself a hallmark of aging, and is thought to be a cause of age-related diseases such as cancer and metabolic disorders. In addition, we will analyze the mechanism of AUTAC function to further improve its function.

Development of High Throughput 3D-Patient Cancer Culture System with Anticancer Drug Exposure Through Blood Capillary

Principal Investigator	MATSUSAKI Michiya
PI Primary Affiliation	Graduate School of Engineering, Osaka University
Co-Investigator(s)	KATAYAMA Ryohei

PROJECT DESCRIPTION

Cancer is still unsolved severe disease because of the inter- and intra-tumor genetic heterogeneity. Thus, personalized cancer medicine has been strongly desired to find the best therapeutic strategy for each patient. To achieve it, in vitro expanding technology of the patient cancer cells maintaining their characteristics and genetic information is important for the anticancer drug screening.

We recently found novel technology "sedimentary culture" to develop three-dimensional (3D) cancer-stromal tissues by optimizing physicochemical factors together with biological factors such as blood capillary. The constructed 3D-cancer-stromal tissues were suitable for high-throughput in vitro culture of patient derived cancer cells (PDC) maintaining their characteristics and genetic information. However, there were still big issues about different drug exposure pathway because we could not add drugs to the cultured PDC through blood capillary. Accordingly, the exposed drug concentrations of the 3D-culture were not the same dose as the actual drug exposure to the patients. To the best of my knowledge, there are no reports of in vitro 3D-PDC culture systems to achieve the drug exposure through blood capillary to compare with patient anticancer drug history.

In this research, we propose development of high throughput 3D-PDC culture systems with anticancer drug exposure through blood capillary by combination of our "sedimentary culture" and "open-pored 3D-blood capillary formation in 3D-tissues" technologies. This research will contribute to achieve for the first time the cancer personalized medicine by precise comparison with patient anticancer drug history at same concentration.

Development of in-body tissue-engineered autologous vascular grafts (Biotube)

Principal Investigator	MIYAMOTO Shinji
PI Primary Affiliation	Faculty of Medicine, Oita University
Co-Investigator(s)	NAKAYAMA Yasuhide; YAMBE Tomoyuki; TAJIKAWA Tsutomu; TERASAWA Takeshi; IWAI Ryosuke

PROJECT DESCRIPTION

It's most reassuring if you can use your own tissues for implantation treatment. However, the amount and type of your own tissues available for implantation in the body is limited. In-body tissue architecture (iBTA) is an innovative concept of regenerative medicine technology that allows free preparation of tissues for autologous implantation in the patient's own body. Implantable tissues consisting only of autologous tissues — with no artificial materials — can be obtained by implanting designed molds under the skin of a patient for about one month. When the resulting iBTA-induced tissues are implanted they survive and regenerate within a few months. They are then metabolized and grow as part of the patient's own body. iBTA-induced tissues are self-sustaining over a lifetime: They do not require proven durability in the same sense as does an artificial medical device.

Furthermore, iBTA technology can easily prepare tissues that cannot be completely cured by existing medical technology or cannot be created by the latest regenerative medicine technology. Therefore, iBTA technology can overcome many, even intractable, diseases. It can help many disabled people, many people with congenital irregularities, and can promote better health in many older people. The first goal is to provide the world's smallest-diameter iBTA-induced blood vessel, named Biotube, to rescue patients with chronic limb-threatening ischemia from amputation. After that, we aim to expand the technology to the regeneration of other tissues and to make this technology a general treatment overseas, mainly in developing countries such as Southeast Asia and Africa.

Elucidation of the mechanism of autophagy driven by liquid-liquid phase separation

Principal Investigator	FUJIOKA Yuko
PI Primary Affiliation	Institute of Microbial Chemistry
Co-Investigator(s)	none

PROJECT DESCRIPTION

Life science has entered a new phase to reconsider various phenomena, including autophagy, after discovery of liquid-liquid phase separation in cells. Autophagy is a major intracellular proteolysis pathway and plays an important role in proteostasis, which is essential for our healthy life expectancy. I found first in the world that the site of autophagosome formation, termed the pre-autophagosomal structure (PAS), is in fact a liquid droplet of Atg proteins formed by liquid-liquid phase separation (Fujioka et al., Nature 2020). However, the study was restricted to “early PAS” in budding yeast that is composed solely of the Atg1 complex. Autophagy progression is mediated by the PAS in a mature state, termed “mature PAS”, which contains various Atg proteins and lipid membranes. Moreover, the role of phase separation in the organization of autophagosome formation sites in mammals has remained elusive. This research proposal aims to unveil the molecular functions of the PAS and to answer the question of why the PAS must be a liquid droplet through in vitro reconstitution of mature PAS. Using these knowledges, the function and regulation of the autophagosome formation sites in mammals will also be analyzed. I also found that early PAS transforms into a solid state through liquid-solid phase transition in vitro and that PAS loses its function by solidification caused by loss of phospho-regulation in vivo. Through the analysis of the mechanism and structural basis of how PAS droplets lose the activity by liquid-solid phase transition and how phospho-regulation prevents that, I establish the basis for understanding liquid-liquid phase separation and liquid-solid phase transition regulating various aspects of proteostasis. These knowledges will contribute to understanding the mechanism of formation of pathogenic protein aggregates such as amyloids and

provide a basis for developing preventive and/or curative medicines for neurodegenerative diseases through regulating phase separation and transition that affect both autophagy activity and state of pathogenic proteins.

No autograft: Bone regeneration using carbonate apatite bone replacement

Principal Investigator	NAKASHIMA Yasuharu
PI Primary Affiliation	Faculty of Medicine, Kyushu University
Co-Investigator(s)	ISHIKAWA Kunio

PROJECT DESCRIPTION

Carbonate apatite honeycomb (CO3Ap HC) arterial bone is the first 100% chemically pure artificial bone made from bone mineral, i.e., carbonate apatite. CO3Ap artificial bone is replaced by actual bone with time based on so-called bone remodeling since CO3Ap artificial bone is resorbed by osteoclasts whereas hydroxyapatite (HAp) artificial bone is not resorbed by the osteoclasts and thus is stable at the bone defect. Additionally, CO3Ap artificial bone demonstrate much higher osteoconductivity (a property of bone formation on the artificial bone when implanted adjacent to an existing bone). Due to honeycomb structure, CO3Ap HC artificial bone is useful even for the reconstruction of tubular bone, and bone with haversian systems is regenerated.

Bone related disease are known as one of the key causes of nursing service. At present, autograft or allograft are the golden standard for the reconstruction of bone defect. However, invasion of the healthy site due to collection of autograft or infection risk are indispensable. 100% chemically pure CO3Ap HC artificial bone that has the same or higher function completely solve these problems, and contribute healthy long life. Preventing a sequence of bone fracture, bedridden man, dementia obviously contribute elder person's health and Quality of Life.

CO3Ap HC arterial bone is produced by extruding calcium carbonate (CaCO_3) and binder mixture through honeycomb die, fabricating raw material HC, followed by heat debinding to fabricate CaCO_3 HC. The CaCO_3 HC thus fabricated is immersed in the phosphate salt solution for dissolution-precipitation reaction, where composition of the CaCO_3 HC became CO3Ap maintaining honeycomb structure of the CaCO_3 HC. After optimization of the larger size CO3Ap HC and biological safety test etc., Collaborating company is planning to manufacture and sell CaCO_3 HC.

Patch-type muscle sensing device for early detection of Frailty

Principal Investigator	TAKEI Yusuke
PI Primary Affiliation	National Institute of Advanced Industrial Science and Technology
Co-Investigator(s)	none

PROJECT DESCRIPTION

We propose a patch-type sensor for early detection of frailty, which evaluates muscle quality (muscle mass, muscle strength, muscle fatigue, muscle contraction speed) from a mechanomyogram generated when muscles are contracted by electrical stimulation. This sensor is expected to extend the healthy life expectancy and improve the QOL of the elderly by early detection of frailty, a gradual decline in muscle strength in the elderly, and quantitative evaluation of the process of improving frailty through muscle strength training.

Our proposal uses electrical stimulation to induce highly reproducible muscle contractions, and the contraction is measured by a mechanomyogram. Mechanomyogram is a pressure wave generated by deformation and vibration of muscle contraction. Since it is a mechanical signal, it does not interfere with electrical stimulation signals. This proposal will integrate this sensing mechanism into a patch-type shape with a diameter of less than 5 cm. The realization of this device will enable people to regularly measure and record their muscle quality not only in hospitals but also at home and is expected to contribute to the healthy super-aging society.

This proposal aims to (1) clarify the physical mechanism of how muscle sound changes depending on muscle mass and muscle strength, (2) develop a prototype device for evaluating muscle quality based on the needs of medical institutions, and (3) conduct a demonstration experiment using the prototype device to confirm that it is possible to measure the improvement or decay of muscle strength over two years from 2020 to 2021.

Rewiring inter-organ immuno-metabolic crosstalks in the ageing bone marrow

Principal Investigator	NAKAMURA-ISHIZU Ayako
PI Primary Affiliation	Tokyo Women's Medical University
Co-Investigator(s)	Weiqiang Chen; Celia Martinez Jimenez

PROJECT DESCRIPTION

Ageing is characterised by the progressive decline of physiological and cellular functions. However, little is known about how our different tissues respond to aging and whether every cell in our body has the same susceptibility to age-related disorders. We aim to understand the cross communication between organs during ageing, and study whether the intervention in one organ might help to restore certain functionalities in another organ. Our project especially focuses on the aging in the liver and bone marrow and how the aging process in the two organs interact. We will use cutting-edge technologies to explore the effects of ageing in individual cells using advanced genomics and novel methodologies to model aging in a dish. Our multidisciplinary team will tackle basic open research questions about how and when to intervene with dietary or pharmacological interventions to improve the opportunities of good quality of life during aging (healthy aging).

The Correlation of ectopic calcification signatures in the ageing eye and cardiovascular system

Principal Investigator	MAEKAWA Tomoki
PI Primary Affiliation	Niigata Univeristy
Co-Investigator(s)	Anna Tan Cheng Sim; Ricardo A. Pena-Silva

PROJECT DESCRIPTION

Ageing has contradictory effects on calcium metabolism, causing bone demineralization (osteoporosis) while in blood vessels and soft tissues proinflammatory cytokines activate osteogenesis and biomineralization, resulting in ectopic calcification. Aging is also associated with decreased expression of modulators of inflammation and osteogenesis such as DEL-1 (developmental endothelial locus-1). Ectopic calcification is seen in age-related macular degeneration, a major cause of blindness. Similarly, age-related calcification of coronary and carotid arteries, and heart valves, is major cause of morbidity due to stroke, heart and renal failure. Collectively, these diseases affect up to 9% of the elderly and induce an enormous burden of disease, increasing health care costs worldwide. In vivo non-invasive retinal imaging enables identification of biomarkers of age-related pathological calcification. If noninvasive retinal imaging of calcification in the ageing retina and cardiovascular calcification can be correlated, retinal examination has the potential to detect age-related ectopic calcification and monitor its progression.

We hypothesize that A) retinal imaging can be used to monitor ectopic calcification in the ageing cardiovascular systems, and B) DEL-1 can downregulate proinflammatory, profibrotic and osteogenic processes associated with age-related ectopic calcification.

Our preliminary findings suggest a good correlation between the formation of calcifying nodules in cultures of vascular smooth muscle cells (VSMC) and retinal pigmented epithelial cells (RPEC) in response to aging and inflammation. We also found that recombinant DEL-1 decreases calcification in vitro. Our multidisciplinary team intends to use retinal imaging to identify biomarkers of ectopic calcification, which could be useful for the

screening of early stages of disease in at-risk populations. Additionally, we intend to study recombinant DEL-1, and inducers of DEL-1 (erythromycin), as novel therapeutic targets for ectopic calcification, not only in the retinal and cardiovascular systems, but also in other inflammatory and metabolic disorders, associated with ageing.

The mechanism for the functional decline of aged neurons by comprehensive analysis of chromatin structure

Principal Investigator	KISHI Yusuke
PI Primary Affiliation	Graduate School of Pharmaceutical Sciences, The University of Tokyo
Co-Investigator(s)	None

PROJECT DESCRIPTION

Prevention or restoration of the decline in memory associated with aging is one of the most important issues for healthy aging. In the brain, which controls memory, neurons are responsible for processing information, and the function, or potential, of neurons should reduce in the aging process. Therefore, revealing the mechanism of the decline in neuronal potential and developing methods to prevent or restore it contribute to healthy aging.

The potential of a cell is determined by the gene sets that can express. The potential of gene transcription, the trigger for gene expression, is controlled by chromatin structure. Therefore, changes in chromatin structure should be involved in the decline of the potential of aged neurons. However, the mechanism for the changes in chromatin structure in aged neurons and the resulting decline in neuronal function has not been elucidated. In this study, I will comprehensively analyze the changes in chromatin structure during neuronal aging and reveal the mechanism of the decline in neuronal potential. I would also like to contribute to the development of methods to prevent or restore the decline in neuronal potential.

In this study, I will 1) isolate nuclei of neurons from the brains of young and old mice and monkeys, 2) perform comprehensive chromatin structural analysis, and 3) identify functional chromatin regions involved in aging and analyze their functions in order to clarify the mechanism of the decline of neuronal potential in aging.

The mechanism of developing dementia through age-related metabolic change

Principal Investigator	TONOKI Ayako
PI Primary Affiliation	Graduate School of Pharmaceutical Sciences, Chiba University
Co-Investigator(s)	none

PROJECT DESCRIPTION

In a super-aging society, an increase in dementia patients is one of the social problems to be solved. Dysregulation of insulin signal associated with diabetes and obesity is known as one of the causes of increasing the risk of developing dementia, which lowers the QOL of the elderly. However, there are many unclear points about how dysregulation of insulin signals reduces the ability of learning and memory. We genetically indicate that the multi-organ association of insulin-producing tissue, which has a blood glucose-regulating function, adipose tissue, and the brain are required for memory, and that the breakdown of these systems due to aging contributes to memory impairment, using *Drosophila* as a model animal (Tanabe et al., *Cell Reports.*, 2017; Tonoki et al., *J Neurosci.*, 2020). Based on these findings, we will elucidate a new molecular mechanism in which multi-organ association are linked to regulate memory, by analyzing gene expression patterns between tissues and performing candidate gene screening using olfactory associative learning in *Drosophila*. In addition, we will identify dietary habits and nutrients that prevent dementia and elucidate the mechanism by which such a memory regulating system breaks down due to aging and dietary habits that cause diabetes and obesity. The research results are expected to lead not only to the presentation of new drug discovery targets for the onset of

dementia through metabolism, but also to the development of supplements and foods containing nutrients that prevent dementia.

The mechanisms of lifespan regulation by dietary manipulation

Principal Investigator	OBATA Fumiaki
PI Primary Affiliation	RIKEN Center for Biosystems Dynamics Research
Co-Investigator(s)	none

PROJECT DESCRIPTION

Almost all animals, including humans, develop into adults and eventually age over time. As an environmental factor, dietary intake is essential throughout life. Diet contributes to metabolic and physiological homeostasis via nutrition, the composition of which is modulated by gut microbiota. Although the significance of dietary quality is well recognised, how each nutrient in diet and each bacterium in the gut influence the healthspan are poorly understood at a molecular level. This study utilises the short lifecycle and abundant genetic tools of fruit fly *Drosophila melanogaster* to understand how diet impacts organismal homeostasis and lifespan. We take advantage of an established chemically-defined diet. This synthetic medium is composed of approximately 40 compounds, which allows us to restrict a single nutrient to perform "loss of function" experiment. In particular, we focus on how each amino acid or mineral plays a role in lifespan determination. At the same time, the gnotobiotic experiment, by which we can produce flies with defined gut microbial composition, is readily accessible. We will elucidate the molecular mechanism of nutritional and microbial regulation of the host healthspan by multi-omics analysis and genetic dissection of responsible molecular pathways. We also aim to elucidate the mechanism underlying the prolonged effect of early-life diet on adult physiology. Our study would provide basic knowledge of evolutionally-conserved molecular mechanisms of dietary regulation of organismal longevity. This should help create an effective dietary intervention to extend the human healthspan by targeting people in all life stages.