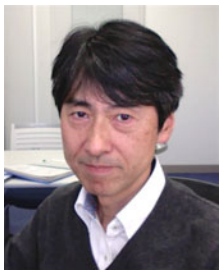

Title of Project：Deciphering of the epigenetic machinery that determines the hallmarks of hematopoietic stem cell aging

IWAMA Atsushi
(The University of Tokyo, The Institute of Medical Science, Professor)

Research Project Number：19H05653　Researcher Number：70244126
Keyword：Hematopoietic stem cell, aging, epigenetics

【Purpose and Background of the Research】

Hematopoietic cells represent the cell type with the greatest numbers in the body. They circulate throughout the body or stay in the organs and exert various functions such as supply of oxygen, hemostasis, and immune reactions. Hematopoietic stem cells (HSCs) are exposed to various stresses and show functional decline during aging.

Dysfunction of hematopoietic stem cells results in disorganized hematopoietic system, including anemia and immune dysfunction, thereby causing functional decline in various organs, eventually leading to the individual's functional impairment. Aged HSCs are also predisposed to transformation (Figure 1). Therefore, functional impairment of HSCs is tightly associated with individual's functional impairment. Understanding of the mechanisms regulating HSC function over the entire life course thus promotes understanding of the mechanisms underlying individual's functional impairment.

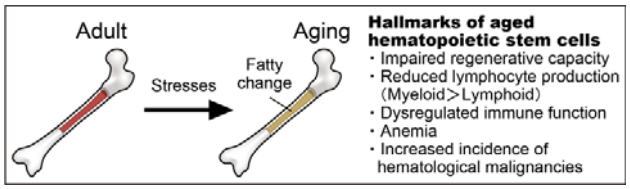

Figure 1. Hallmarks of aged hematopoietic stem cells

【Research Methods】

We will take bioinformatic comprehensive approaches to decipher the changes in epigenetic properties of HSCs over the entire life course: (1) maintenance phase in adult bone marrow and (2) functional impairment phase during aging. We will clarify how various stresses to which mice are exposed alter epigenetic patterns in HSCs and impair individual's function over the entire life course. We have already obtained a part of the epigenetic data of HSCs and identified unique chromatin properties that may account for functional impairment of HSCs with aging. We also take an advantage of single HSC profiling by RNA-seq and ATAC-seq analysis to decipher the alterations in heterogeneities in HSC populations with age (Figure 2).

Because the quality of bone marrow niche holds key to the functional maintenance of HSCs, we will also analyze the alterations in the quality of niche over the entire life course. Furthermore, we will clarify the epigenetic abnormalities responsible for the impaired differentiation of HSCs, which leads to abnormal production of differentiated progenies and transformation into age-associated hematological malignancies.

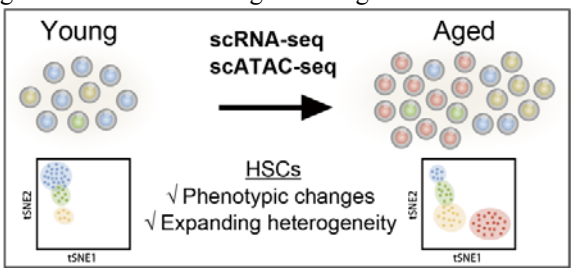

Figure 2. Single cell assays of HSCs

【Expected Research Achievements and Scientific Significance】

Through a series of analyses, we hope to identify factors responsible for the functional impairment of HSCs and their niche, which eventually affect individual's function over the entire life course. By manipulating those factors, we will develop the modalities to control the individual's functional impairment through reactivating HSCs

【Publications Relevant to the Project】

・Tara S, Isshiki Y, Nakajima-Takagi Y, Oshima M, Aoyama K, Tanaka T, Shinoda D, Koide S, Saraya A, Miyagi S, Manabe I, Matsui H, Koseki H, Bardwell VJ, Iwama A. *Bcor* insufficiency promotes initiation and progression of myelodysplastic syndrome. ***Blood*** 132(23):2470-2483, 2018.
・Sashida G, Harada H, Matsui H, Oshima M, Yui M, Harada Y, Tanaka S, Mochizuki-Kashio M, Wang C, Saraya A, Muto T, Inaba T, Koseki H, Huang G, Kitamura T, and Iwama A. Ezh2 loss promotes development of myelodysplastic syndrome but attenuates its predisposition to leukemic transformation. ***Nat Commun*** 5:4177, 2014.

【Term of Project】

FY2019-2023

【Budget Allocation】

153,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.ims.u-tokyo.ac.jp/molmed/
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Title of Project：Establishment of an integrated locomotive science including dynamics of bone-articular cells and regulation by immune system

TANAKA Sakae
(The University of Tokyo, The University of Tokyo Hospital, Professor)

Research Project Number：19H05654　Researcher Number：50282661
Keyword：Integrated Locomotive Science, Locomotive Disease, Single-cell Analysis

【Purpose and Background of the Research】

Locomotion is an essential activity to maintain homeostasis of the body. Representative locomotive diseases such as osteoarthritis (OA), osteoporosis and rheumatoid arthritis severely restrict patient's activities of daily living and thus lead to social problems. The difficulty in overcoming those diseases is caused by the diversity and the heterogeneity of the cellular complex associated with bone and articular cartilage. The purpose of this project is to analyze the underlying mechanism of bone and cartilage homeostasis. Using technology for molecular and cellular analysis, transgenic mouse analysis, we elucidate the mechanisms for maintaining the locomotive homeostasis, especially focusing on the regulation by immune system such as innate lymphoid cells (ILC).

【Research Methods】

We collect synovium, bone marrow, and articular cartilage from naïve mouse and OA model mouse, and we analyze comprehensively the proportion and the transition of synovial fibroblast, macrophage, other immune cells such as ILC, osteoblast, osteoclast, osteocyte, and chondrocyte, by using immunohistochemistry assay and mouse genetic modification technology. Furthermore, we perform single-cell RNA sequences (scRNAseq) to analyze the heterogeneity of those cells and the subsets related to each cell type.

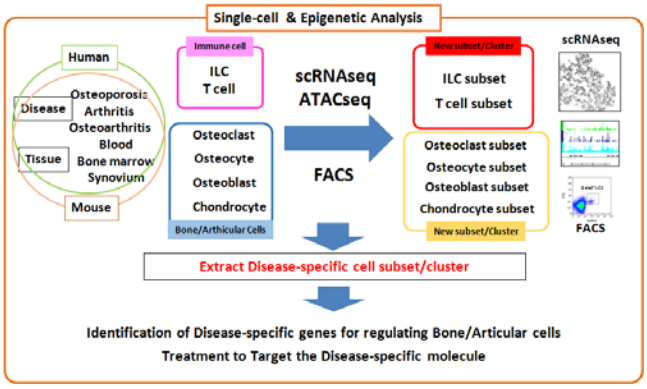

Figure. Integrated Locomotive Science.

We investigate the difference and the overlap between mouse and human about the phenomenon which were observed in those specific mice with OA.

【Expected Research Achievements and Scientific Significance】

This project focuses on the elucidation of heterogeneity of the cells associated with bone and cartilage by understanding the molecular changing in a single cell.From these insights, we could unveil the mechanisms for the locomotive system. Understanding of the mechanism by investigating the effect of immune cells such as ILC, it will be expected that those insights would discover the regulation of the diseases and lead to establishing treatment by targeting molecules associated.

【Publications Relevant to the Project】

- Komatsu N, Okamoto K, Sawa S, Nakashima T, Oh-hora M, Kodama T, Tanaka S, Bluestone JA, Takayanagi H., Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis, ***Nat Med***. 2014 Jan;20(1):62-8.
- Kobayashi H, Chang SH, Mori D, Itoh S, Hirata M, Hosaka Y, Taniguchi Y, Okada K, Mori Y, Yano F, Chung UI, Akiyama H, Kawaguchi H, Tanaka S, Saito T., Biphasic regulation of chondrocytes by Rela through induction of anti-apoptotic and catabolic target genes, ***Nat Commun***. 2016 Nov 10;7:13336.
- Omata Y, Frech M, Primbs T, Lucas S, Andreev D, Scholtysek C, Sarter K, Kindermann M, Yeremenko N, Baeten DL, Andreas N, Kamradt T, Bozec A, Ramming A, Krönke G, Wirtz S, Schett G, Zaiss MM.,Group 2 Innate Lymphoid Cells Attenuate Inflammatory Arthritis and Protect from Bone Destruction in Mice, ***Cell Rep***. 2018 Jul 3;24(1):169-180.

【Term of Project】

FY2019-2023

【Budget Allocation】

154,300 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.u-tokyo-ortho.jp/

Title of Project：Anti-cancer therapies aiming for cure through inhibiting tumor-specific responses to environmental fluctuation

ISHIKAWA Fuyuki
(Kyoto University, Graduate School of Biostudies, Professor)

Research Project Number：19H05655　Researcher Number：30184493
Keyword：Stress Response, Cancer Progression

【Purpose and Background of the Research】

Precision medicine based on molecularly targeting anti-cancer drugs has been revolutionized the strategy in cancer therapeutics. One drawback is, however, relapse of cancer cells resistant to the cognate medicine is not infrequent. Molecular targeting inhibits specific signaling molecules, activated by mutations (driver mutations, Fig 1a and b), thereby halt the autonomous cell proliferation (Fig 1c). However, cancer cells show genetic instability, which generates numerous random mutations in the genome. Accordingly, cells possessing a second driver mutation that bypasses the molecular targeting drug arises in time, leading to relapse (Fig 1d).

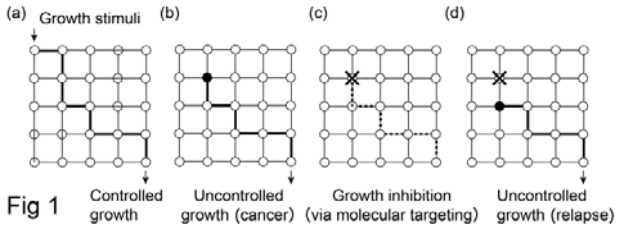

Fig 1

This argument led to a conclusion that to achieve cure without relapse in treating cancer patients, we need to target the system that uniquely enables cancer cells to undergo malignant progression, in addition to the molecular targeting towards driver genes.

【Research Methods】

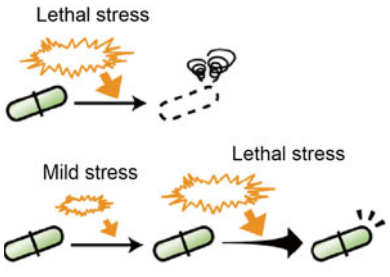

Fig 2　Acquired Tolerance

We have previously investigated how cells respond to non-lethal mild stress. Specifically, we have revealed molecular mechanisms of acquired tolerance (Fig 2), a widely observed phenomenon where a preceding mild stress equips cells with resistance to a following lethal stress, using genetic screening in fission yeast. We will investigate to test whether tumors depend on acquired tolerance for their progression. If yes, we will exploit the dependence to develop novel cancer therapeutics.

【Expected Research Achievements and Scientific Significance】

Normal tissues in organisms are maintained under constant environment thanks to homeostasis. However, tumors are not benefitted by it, and accordingly are exposed to constant environmental fluctuation, such as those of oxygen and nutrient concentrations. As such, responses to non-lethal stresses are vital to tumors in maintaining their viability. This research will give a basic framework in targeting acquired tolerance as a novel strategy to develop cancer treatment aiming at cure.

【Publications Relevant to the Project】

・Chujo, M., Tarumoto, Y., Miyatake, K., Nishida, E., and Ishikawa, F. (2012). ***J Biol Chem*** 287(28), 23440-23450.

【Term of Project】

FY2019-2022

【Budget Allocation】

128,100 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.fish.lif.kyoto-u.ac.jp/en/home_en.html

Title of Projec： Comprehensive studies on the molecular basis of early development and clonal evolution in cancer using advanced genomics.

OGAWA Seishi
(Kyoto University, Graduate School of Medicine, Professor)

Research Project Number：19H05656　Researcher Number：60292900
Keywords: Genome biology, Oncology

【Purpose and Background of the Research】

Whole spectrum of genetic mutations has been clarified in most of the common cancers. However, it remains unclear how heterogeneity in cancer is acquired during initial development and clonal selection in the clinical course. To address these issues, combinations of multiple mutations, cryptic noncoding genomic lesions, and their functional implication should be elucidated. By single-cell sequencing, micro-scale sampling, whole genome sequencing, long-read sequencing, we will elucidate the molecular basis of early development and clonal evolution in cancer. We will also analyze large cohort of cancer patients to establish novel disease classification, to stratify patients according to prognosis, and to identify therapeutic molecular targets, which will be validated for functional implication in mouse models.

【Research Methods (Figure 1)】

1) Sequencing of high-density micro-scale samples, organoid, and laser microdissection tissues will demonstrate the process of clonal expansion in pre-cancer lesions of pancreatic, colon, and breast cancers. Serial sampling of multiple lesions will allow for clonal evolution from primary to aggressive, metastatic, and recurrent cancers.
2) The in-house single-cell sequencing method will simultaneously provide both information of genetic mutations and gene expression levels from a single cell, which will make it possible to display expression profile in each mutated cell from heterogenous fractions.
3) Whole genome and long-read sequencing will identify structural variants in noncoding regions whose significance will be validated by mouse model.
4) Large scale study of cancer patients will reveal association of genetic lesions with disease phenotype, therapeutic response, and prognosis. Coincidence and mutual exclusiveness of genetic mutations will be investigated to elucidate mechanism of stepwise acquisition of heterogeneity in cancer.

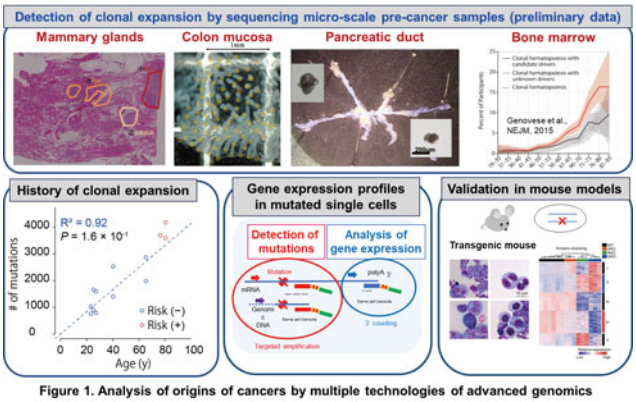

Figure 1. Analysis of origins of cancers by multiple technologies of advanced genomics

【Expected Research Achievements and Scientific Significance】

We will comprehensively demonstrate the process of age-related clonal expansion and remodeling in pre-cancer tissues using single-cell sequencing and micro-scale sampling and clarify their adaptive response to environmental stress and association with initial process in cancer development. We will also identify implication of noncoding lesions by whole genome / long-read sequencing (Figure 2). Moreover, we will conduct such studies in large cohort of patients for achievement of precision medicine on the basis of 'personality of each case' which will be defined by establishment of novel disease classification, prognostic stratification of patients, and identification of therapeutic molecular targets.

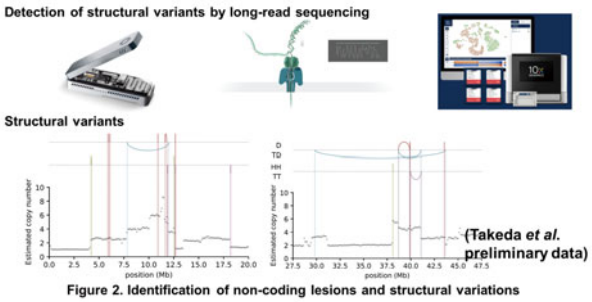

Figure 2. Identification of non-coding lesions and structural variations

【Publication Relevant to the Project】

・Yokoyama A, Ogawa S, *et al*., Age-related remodelling of oesophageal epithelia by mutated cancer drivers. ***Nature.*** 565:312-317, 2019
・Yoshizato T, Ogawa S, *et al*., Somatic mutations and clonal hematopoiesis in aplastic anemia. ***N Engl J Med.*** 373:35-47, 2015

【Term of Project】

FY2019-2023

【Budget Allocation】

153,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://plaza.umin.ac.jp/kyoto_tumorpatho/
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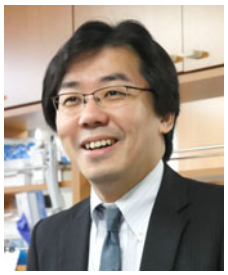

Title of Project： Identification and control of pathogenic osteoclasts

ISHII Masaru
(Osaka University, Graduate School of Frontier Biosciences, Professor)

Research Project Number：19H05657　Researcher Number：10324758
Keyword：medicine and welfare, immunology

【Purpose and Background of the Research】

Osteoclasts play a key role in maintaining skeletal homeostasis by supporting steady-state bone remodelling in the bone marrow (BM). However, in contrast to this physiological role, osteoclasts are also involved in pathological arthritic bone erosion in patients with rheumatoid arthritis (RA), which occurs where the hypertrophied synovium (called “pannus”) invades the outer surface of the articular bone. Extensive studies have been performed to identify the osteoclast precursor (OP) population in BM. Nevertheless, precise analysis of OPs in inflammatory conditions has not yet been performed, especially in “inflamed synovium”, the actual site of bone erosion in arthritis, mainly due to technical difficulties associated with approaching and isolating tiny synovial tissues. Thus, whether the two osteoclast populations in the BM and synovial tissue settings have a similar pathway of differentiation and arise from similar precursor states remains unknown (Figure 1). The objectives of the current study are, (1) to identify the osteoclast precursor (OP) population in the inflamed synovium and elucidate the molecular mechanisms responsible for regulating this population, and (2) to elucidate the functional characteristics of osteoclasts in the pannus-bone interface compared with conventional osteoclasts in BM.

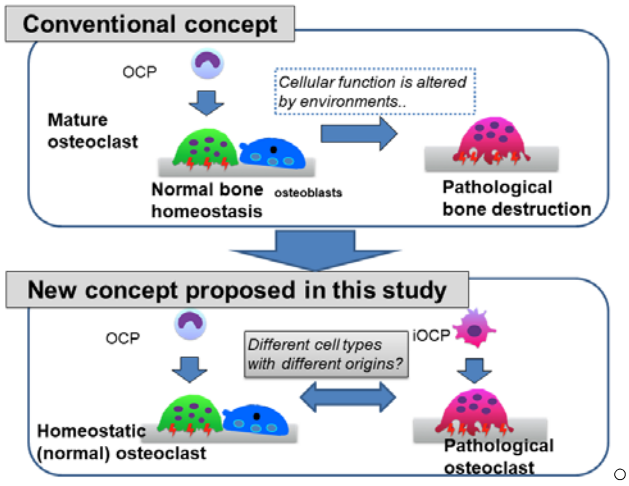

Figure 1. Conventional and novel concepts on osteoclasts in inflammatory conditions. Are they just different in terms of their activation status, or they are essentially different cell types from respectively distinct precursors?

【Research Methods】

Using the original protocol to isolate the inflamed synovium, we identify the OP population in the synovium and then characterize the molecular mechanisms as well as the predicted critical regulator for differentiation of these cell types, by using exhaustive expression analyses, such as RNA sequencing. For analyzing their origins, cellular tracing and intravital imaging with photo-convertible fluorophore will be conducted. Furthermore, by targeting the molecule(s) which we identify specifically expressed in inflammatory OP fractions, we plan to develop novel therapeutics against inflammatory bone destructions. We also further analyze the possible involvement of this novel line of osteoclasts in another pathological conditions such as bone-metastatic tumors. For such analyses, direct visualization of osteoclasts will clarify the differences and characteristics, which may lead to the development of optimized treatment for bone diseases.

【Expected Research Achievements and Scientific Significance】

This work, with its identification and characterization of a novel OPs specifically involved in arthritic bone destruction, and with elucidation of the functional differences between osteoclasts in the BM and pannus-bone interface, will lead to pathogenic-osteoclast specific treatment in patients with rheumatoid arthritis (RA).

【Publications Relevant to the Project】

Furuya, et al., Direct cell-cell contact between mature osteoblasts and osteoclasts dynamically controls their functions in vivo. Nat. Commun., 9: 300, 2018.
Matsuura et al. In vivo visualization of different modes of action of biologic DMARDs inhibiting osteoclastic bone resorption. Ann. Rheum. Dis., 77 :1219-1225, 2018.

【Term of Project】

FY2019-2023

【Budget Allocation】

153,700 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.icb.med.osaka-u.ac.jp/
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Title of Project：Elucidation of abnormal functioning of neuronal circuits underlying neuropathic pain and its application for drug discovery

TSUDA Makoto
(Kyushu University, Graduate School of Pharmaceutical Sciences, Professor)

Research Project Number：19H05658　Researcher Number：40373394
Keyword：Neuropathic allodynia, primary afferent Aβ fiber, optogenetics, spinal dorsal horn neuronal circuit

【Purpose and Background of the Research】

Damage to the nervous system causes neuropathic pain, a highly debilitating chronic pain condition that is frequently resistant to morphine. A cardinal symptom of neuropathic pain is mechanical allodynia, pain that is produced by innocuous mechanical stimulus, such as light touch. A major question is where and how touch information is pathologically converted to pain in the context of nerve damage.

We have previously discovered an essential role of glial cells in the spinal cord in the pathogenesis of neuropathic pain (Nature 2003) and indicated a strong ability of glial cells to alter the function of neuronal cells in the nervous system (Nat Rev Neurosci 2018). In this proposed research program, by using our developed research skills and scientific knowledge in the field of pain and glia combined with a new approach for investigating neuropathic allodynia and a technique for visualization and functional operation of neuronal subsets, we will identify neuronal circuits that are required for neuropathic allodynia. Furthermore, we will determine a cause of functional abnormality of the circuits after nerve injury by focusing on the role of glial cells and top-down signaling from the brain to the spinal dorsal horn. In addition, we will explore drugs that act on neurons and glia implicated in neuropathic allodynia by performing a screening of small-molecule chemical libraries (mainly clinically approved drugs).

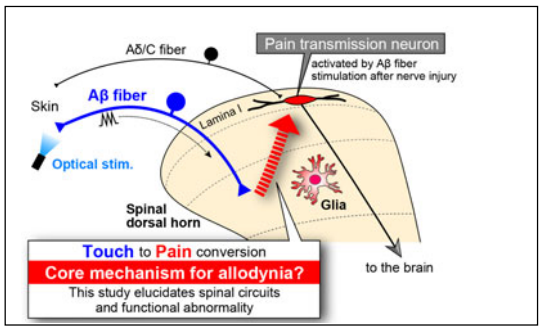

Figure 1　Proposal

【Research Methods】

In our proposed research program, experiments will be performed by using a new approach for investigating morphine-resistant neuropathic allodynia by optogenetics that enable a selective stimulation of touch-sensing primary afferent Aβ fibers and a technique for functional operation of neuronal subsets combined with histology, electrophysiology and imaging. First, we will examine the role of neuronal subsets in the spinal dorsal horn in Aβ fiber-derived neuropathic allodynia. Furthermore, the role of these subsets in Aβ fiber-derived information signal to lamina I SDH neurons after nerve injury will be analyzed. Abnormal functioning of identified neuronal subsets will be examined by focusing on the role of glia. Because recent studies have shown that top-down signaling from the brain directly affects pain processing in the spinal dorsal horn, we will examine the role of identified subsets of dorsal horn neurons as receiving cells of top-down signaling from the brain. Lastly, we will explore drugs that act on neurons and glia implicated in neuropathic allodynia by screening clinically approved drugs.

【Expected Research Achievements and Scientific Significance】

These findings from our proposed research program will identify neuronal circuits that are required for neuropathic allodynia and determine the role of glia and top-down signaling from the brain, which in turn advances our knowledge of 'how touch-sensing Aβ fiber signals are pathologically converted to pain in the context of nerve damage'. Our findings will not only advance in understanding of the mechanisms that underlie neuropathic pain but also provide new targets for treating this chronic pain.

【Publications Relevant to the Project】

・Tsuda M: New approach for investigating neuropathic allodynia by optogenetics. Pain 160 (Suppl 1): S53-S58 (2019)
・Inoue K, Tsuda M: Microglia in neuropathic pain: cellular and molecular mechanisms and therapeutic potential. Nat Rev Neurosci 19: 138-152 (2018)

【Term of Project】FY2019-2023

【Budget Allocation】153,700 Thousand Yen

【Homepage Address and Other Contact Information】

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Title of Project：Integrative study of brain mechanisms to induce hypertension

NODA Masaharu
(Tokyo Institute of Technology, Institute of Innovative Research, Cell Biology Center, Professor)

Research Project Number：19H05659　Researcher Number：60172798
Keyword：hypertension, leptin, angiotensin II, aldosterone, Na_x channel

【Purpose and Background of the Research】

It is well-known that excess salt intake (HS) induces hypertension; however, the mechanism has not been elucidated until recently. We, for the first time, revealed brain mechanisms for salt-induced elevations of blood pressure (BP) (Fig. 1). Briefly, increases of [Na^+] in body fluids activate Na_x channels in the organum vasculosum lamina terminalis (OVLT). The Na_x signal in glial cells in transferred to the central nuclei [paraventricular nucleus (PVN) and rostral ventrolateral medulla (RVLM)] controlling sympathetic nerve activity (SNA). The increase in SNA leads to BP elevations.

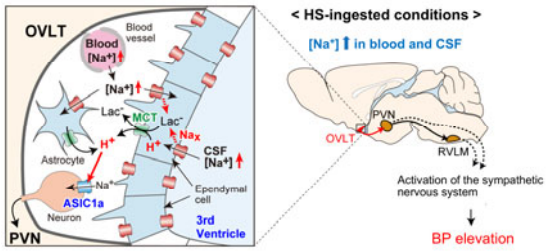

Figure 1　Central mechanisms of salt-induced BP elevations

On the other hand, it is known the obesity and psychological stress elevate BP through activation of SNA. The present study is aimed to elucidate the overall picture of brain mechanisms controlling BP.

【Research Methods】

Under obesity or stress conditions, the level of leptin, angiotensin II, or aldosterone increase in blood. After these factors are received by specific brain loci, the signals are eventually transferred to the central nuclei controlling SNA. We hypothesized that sensing receptive loci of these factors are circumventricular organs (CVOs) that lack blood-brain barrier in the brain.

In the present study, we will promote the following projects: Elucidation of; 1) receptive loci for respective factors, 2) signaling pathways to the PVN or RVLM and, 3) integration mechanism of these signals in some central nuclei. For this purpose, we employ modern research techniques, such as tracing methods by using multiple viral vectors, optogenetics to reveal the function of a specific neural pathway, and Ca^{++} imaging of a nucleus at the single neuronal level.

【Expected Research Achievements and Scientific Significance】

Although the mechanism of salt-induced hypertension has long been studied, the details have not been clarified until recently. The main reasons were that the sensor to detect increases in [Na^+] of body fluids was an enigma, and the mechanisms for sensing and transmitting signal to the center controlling SNA were unknown.

We do not know the sensing loci of leptin, angiotensin II, or aldosterone in the brain, nor the cellular mechanisms of signal transmission. The present study will integratively elucidate brain mechanisms of BP elevations caused by these endogenous pressor factors. This study is of marked academic value and will contribute to the development of a novel strategy to treat hypertension.

【Publications Relevant to the Project】

・Nomura K, Hiyama TY, et al. and Noda M. [Na^+] increases in body fluids sensed by central Na_x induce sympathetically mediated blood pressure elevations via H^+-dependent activation of ASISC1a. **Neuron** *101*, 60-75 (2019).
・Matsuda T, Hiyama TY, et al. and Noda M. Distinct neural mechanisms for the control of thirst and salt appetite in the subfornical organ. **Nature Neurosci.** *20*, 230-241 (2017).
・Noda M, and Sakuta H. Central regulation of body-fluid homeostasis. **Trends Neurosci.** *36*, 661-673 (2013).

【Term of Project】

FY2019-2023

【Budget Allocation】

140,500 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.rcb.iir.titech.ac.jp/index.html