Title of Project : Multi Regulatory System for Gut Homeostasis and Inflammation

Hiroshi Kiyono
(The University of Tokyo, The Institute of Medical Science, Distinguished Professor)

Research Project Number : 18H05280 Researcher Number : 10271032
Keyword : Mucosal Immunology, Organ association, Inflammatory bowel diseases

【Purpose and Background of the Research】

Intestinal mucosa senses the alteration of nutrients and commensal bacteria and modulates both maintenance of homeostasis/ induction of pathogenesis. The important effects of the intestine in the regulation of physiological function beyond the organ has been well recognized, so that it is called the "Super organ."

Crohn's disease is a chronic inflammatory disease occurring in the intestinal mucosa. It has been reported that patients of Crohn's disease show inflammation and dysfunction in not only mucosa but surrounding tissues, such as pancreas and *Muscularis externa*. In addition, complications associated with Crohn's disease develop in the remote organs such as eyes and joints. However, the detailed mechanism of the crosstalk between surrounding tissues, or mucosa-supportive tissues, and intestinal mucosa has not been revealed yet.

With our past researches of mucosal immune system together with pioneering and accumulated profound knowledge/skills, we aim to elucidate mucosal defense mechanisms which is mediated by the gut hierarchically-organized mucosal supportive system.

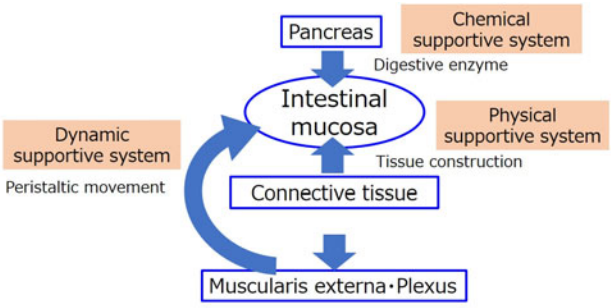


Figure 1. The gut hierarchically-organized mucosal supportive system

【Research Methods】

In this study, we focus on following organ or tissue association/crosstalk.

1. pancreas-intestinal association
2. mucosal-connective tissue association
3. mucosa-muscular association

It is thought that the breakdown of association/crosstalk leads to the intestinal pathogenesis, especially inflammatory bowel disease. By the creation of novel experimental disease models, we aim to reveal the multiple organ/tissue crosstalk systems. We also try to orchestrate these three research pieces for further understanding of gut hierarchically-organized mucosal supportive system.

【Expected Research Achievements and Scientific Significance】

The accomplishment of this study will provide further understanding of the contribution of mucosal system for systemic homeostasis and pathogenesis. In addition, we aim to establish the basis for therapeutic strategy of mucosal inflammation and its complications.

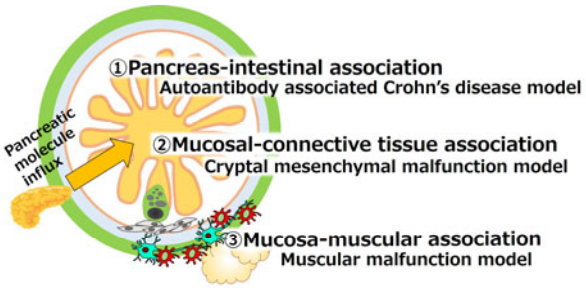


Figure 2. Understanding of mucosal supportive system and establishment of the therapeutic strategy

【Publications Relevant to the Project】

1. Kurashima Y and Kiyono H. Mucosal ecological network of epithelium and immune cells for gut homeostasis and tissue healing. *Ann Rev Immunol.* 35:119-147. 2017.
2. Goto Y, Uematsu S and Kiyono H. Epithelial glycosylation is a key immunological event for gut homeostasis and inflammation. *Nature Immunol.* 17(11):1244-1251. 2016.

【Term of Project】 FY2018-2022

【Budget Allocation】 147,200 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.ims.u-tokyo.ac.jp/enmen/index_j.htm
http://www.m.chiba-u.jp/class/innovativemed/index.html

Title of Project : Molecular Analysis of Spermatogonial Stem Cell Aging

Takashi Shinohara
(Kyoto University, Graduate School of Medicine, Professor)

Research Project Number : 18H05281 Researcher Number : 30322770
Keyword : spermatogenesis, stem cells, aging

【Purpose and Background of the Research】

Germline stem (GS) cells proliferate in vitro as clumps of spermatogonia, but they can reinitiate spermatogenesis following spermatogonial transplantation. Unlike embryonic stem (ES) cells, GS cells are resistant to reactive oxygen species (ROS) and have a lower mutation frequency. Moreover, although GS cells express telomerase, their telomeres become shorter during two years of consecutive culture. These results suggest that GS cells have anti-aging machinery, which is different from those found in ES cells or somatic cells. We will analyze 1) the mechanism underlying telomere regulation in GS cells, 2) DNA repair or ROS resistance and 3) identify aging-inducing signals from somatic cells.

【Research Methods】

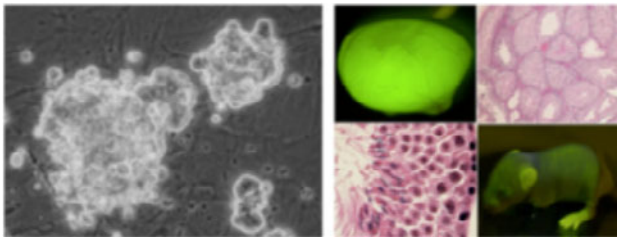


Figure 1 GS cells can differentiate into sperm

1) Analysis of telomere maintenance in GS cells
We will visualize the telomeres in GS cells and analyze the spatiotemporal distribution of telomeres. We will identify molecules involved in telomere maintenance in GS cells.
2) Analysis of DNA repair machinery and ROS level regulation in GS cells.
Cells in the germline lineage have a lower mutation frequency compared with somatic cells. However molecules involved in genome quality control have not been identified. We will analyze the impact of aging on mutation frequency and identify molecules involved in DNA repair.
3) Identification of aging inducing signals from somatic cells
Because germ cells do not show circadian rhythms. it is possible that testicular somatic cells induces germ cell aging. To identify these molecules, we will analyze gene expression patterns in aged animals from several species and identify genes responsible for testis aging.

【Expected Research Achievements and Scientific Significance】

Identification of telomere regulatory factors will bring new insight into telomere biology. Moreover, understanding the mechanism underlying the low mutation frequency in GS cells will open up a new field of germline genome quality control. The identification of aging-inducing signals from somatic cells will promote development of new strategies of male infertility treatment.

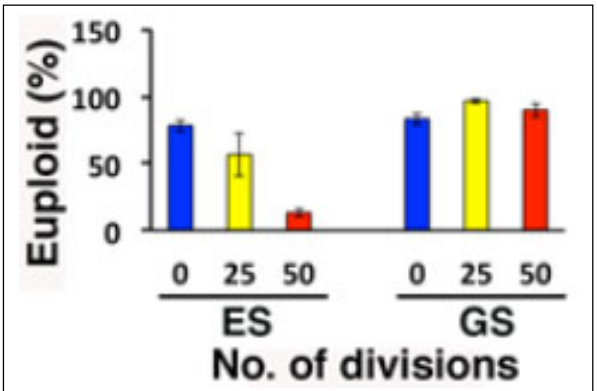


Figure 2 Karyotype stability of GS cells

【Publications Relevant to the Project】

・ Kanatsu-Shinohara, M.. et al. Nonrandom germline transmission of mouse spermatogonial stem cells. Dev. Cell 2016;38: 248-261.

【Term of Project】

FY2018-2022

【Budget Allocation】

148,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www2.mfour.med.kyoto-u.ac.jp/~molgen/research_summary.html

Title of Project : Investigation on pathological implications of guidance molecules in neuro-immune-metabolism

Atsushi Kumanogoh
(Osaka University, Graduate School of Medicine, Professor)

Research Project Number : 18H05282 Researcher Number : 10294125
Keyword : Immunometabolism, axon guidance molecule, chronic inflammation

【Purpose and Background of the Research】

The immune, nervous, and metabolic systems are indispensable for body homeostasis. Previous studies showed that these systems interact closely with each other. However, the mechanisms underlying these interactions remain unknown. We have unveiled the existence of the semaphorins, a group of molecules that works in both the nervous and immune systems. In addition, we have obtained insights suggesting that the expression of semaphorins and their related molecules is regulated by metabolic signaling, and that breakdown in the regulatory system can result in development of lesions of chronic inflammatory diseases, including angiitis, multiple sclerosis, metabolic diseases and malignancies. By establishing the novel concept of "Neuro-Immune-Metabolism," this research aims to elucidate the mechanism underlying the interactions among the immune, nervous, and metabolic systems (Fig. 1).

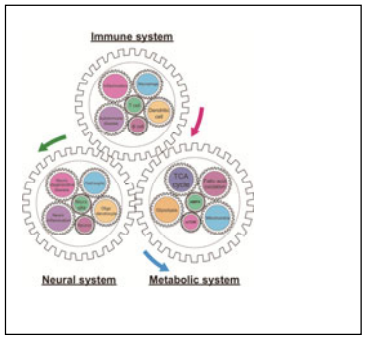


Figure 1

【Research Methods】

Towards elucidation of Neuro-Immune-Metabolism regulatory mechanism and control of the mechanism, this study employs the following viewpoints to achieve the study objective:

1) Elucidation of the mechanism by which immune and inflammatory cells are activated or differentiated by molecules that regulate Neuro-Immune-Metabolism

2) Elucidation of the involvement of abnormal expression of semaphorin-related molecules in disease pathology and the control of semaphorin expression

Additionally, this study proactively integrates basic and clinical approaches ("Bench to Bed" and "Bed to Bench") to pursue the study objectives.

【Expected Research Achievements and Scientific Significance】

Studies that focus on the pairwise relationships between the immune, nervous, and metabolic systems have started to receive a great deal of attention. However, studies that examine the relationships among these three systems from a single perspective have just begun. This research uses the "window" of semaphorins and their related molecules to investigate the relationships among these three systems, with the aim of elucidating the mechanisms underlying their interactions. Based on the novel concept of Neuro-Immune-Metabolism, the resultant knowledge should contribute to development of diagnostic and therapeutic mechanisms for diseases that are caused by any breakdown of the control system (Fig. 2).

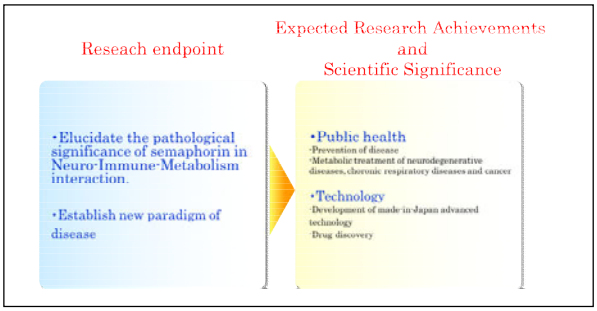


Figure 2

【Publications Relevant to the Project】

・Kang S, Nakanishi Y, Kumanogoh A et al. (2018) Semaphorin 6D reverse signaling controls macrophage lipid metabolism and anti-inflammatory polarization. ***Nature Immunology***, 19, 561-570.

・Hosen N, Kumanogoh A et al. (2017) The activated conformation of integrin β7 is a novel multiple myeloma-specific target for CAR T cell therapy. ***Nature Medicine***, 12, 1436-1443.

【Term of Project】 FY2018-2022

【Budget Allocation】 147,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.imed3.med.osaka-u.ac.jp/

【Grant-in-Aid for Scientific Research (S)】

Broad Section I

Title of Project : Elucidation of the mechanism in the regulation of chondrocyte-specific Runx2 enhancer and development of the drug for osteoarthritis

Toshihisa Komori
(Nagasaki University, Graduate School of Biomedical Sciences, Professor)

Research Project Number : 18H05283 Researcher Number : 00252677
Keyword : chondrocyte, osteoarthritis, enhancer, Runx2

【Purpose and Background of the Research】

We are pursuing to elucidate the mechanism of the formation and maintenance of bone and cartilage focusing on Runx2. We clarified that Runx2 is an essential transcription factor for osteoblast differentiation and chondrocyte maturation, and is responsible for osteoarthritis (Fig. 1, 2). Therefore, Runx2 positively works in adult bone by increasing bone formation and negatively works in articular cartilage by destructing it. The elucidation of the transcriptional regulation of Runx2 in osteoblasts and chondrocytes makes a great advance in the understanding of the molecular mechanism of skeletal development and maintenance. Further, it makes possible to regulate Runx2 in osteoblasts and chondrocytes separately, which allows us to develop the drugs for osteoporosis and osteoarthritis. In this study, we elucidate the

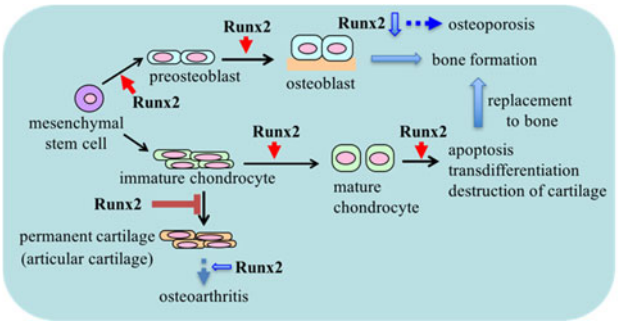


Fig. 1 The functions of Runx2

mechanism of the activation of chondrocyte-specific enhancers and develop the drugs for osteoarthritis using the enhancers.

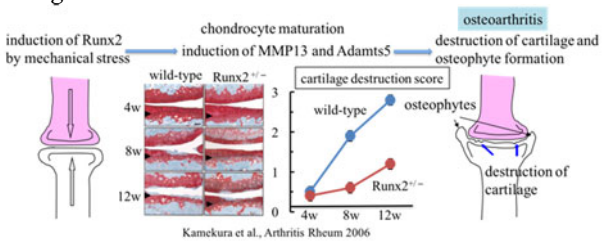


Fig. 2 The development of osteoarthritis by Runx2

【Research Methods】

We clarify the transcription factors and cofactors, which activate chondrocyte-specific Runx2 enhancers, the structure of the enhanceosome, and the interaction of enhancers and promoters. By high throughput screening, we identify the chemical compounds, which inhibit the activity of chondrocyte-specific enhancers and suppress Runx2 expression only in chondrocytes. We evaluate the effect of the selected chemical compounds using osteoarthritis mouse models, identify the molecules interacting with them, and elucidate the mechanisms of action of the selected compounds. From these data, we determine the candidates for the drug for osteoarthritis.

【Expected Research Achievements and Scientific Significance】

More than 25 million people suffer osteoarthritis of knee joints in Japan. Osteoarthritis is caused by the destruction of articular cartilage through the repetitive mechanical stress and its accumulation. It causes disability of walking due to the severe pain. The prosthetic replacement arthroplasty is the only therapy. This is a unique study to develop the drugs for osteoarthritis by using chondrocyte-specific Runx2 enhancers. From this study, we will have the drugs to inhibit the development and progress of osteoarthritis.

【Publications Relevant to the Project】

Komori T: Runx2, an inducer of osteoblast and chondrocyte differentiation. Histochem Cell Biol. 149(4):313-323, 2018.
Kawane T, et. al.: Dlx5 and Mef2 regulate a novel Runx2 enhancer for osteoblast-specific expression. J Bone Miner Res. 29(9):1960-1969, 2014.

【Term of Project】 FY2018-2022

【Budget Allocation】 148,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.de.nagasaki-u.ac.jp/dokuji/kaibou-2/index.html

Title of Project : Self-Renewal Capacity of Hematopoietic Stem Cells through the Regulation of Mitochondrial Metabolism

Toshio Suda
(Kumamoto University, International Research Center for Medical Sciences, Distinguished Professor)

Research Project Number : 18H05284 Researcher Number : 60118453
Keyword : Hematopoietic Stem Cells (HSCs), Stem Cell Niche, Mitochondrial Metabolism

【Purpose and Background of the Research】

Ex vivo expansion of HSCs is a long-standing subject in the research field of hematopoiesis, but it has not been realized yet. We hypothesize that HSCs show two types of cell division patterns; self-renewal cell division, which reproduces stem cells, and differentiation division, which produces functioning mature cells.

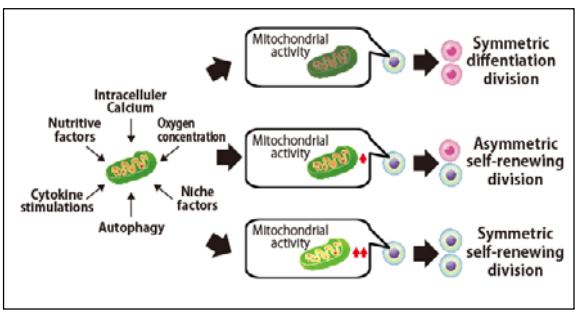
In this project, we will validate how mitochondrial function affects the cell division of HSCs. We have previously shown that oxidative metabolism is critical for the regulation of quiescence and maintenance of HSCs, as well as glycolysis.

Here we will clarify the underlying molecular mechanisms how mitochondria metabolism regulates the stem cell division. We will approach the self-renewal division in HSCs. This project is aiming for the *ex vivo* expansion of HSCs by increasing the self-renewal division by the mitochondrial regulation (see Figure).

【Research Methods】

At first, we will clarify how mitochondrial activation induces the stem cell division and differentiation. We will monitor the mitochondrial mass, membrane potential (MMP) and ROS.

Next, we will clarify how mitochondrial activation is regulated by the HSCs niche. We have previously suggest the following signaling; exogenous adenosine— intracellular Ca^{2+} increase—MMP upregulation—cell division. We will try to connect the missing link in this axis. Especially, to clarify how Ca^{2+} is regulated in HSCs, we will analyze the Ca^{2+} efflux and influx from extracellular compartment and endoplasmic reticulum (ER).



We will analyze the HSC division in MITOL-deficient HSCs, which has abnormalities in mitochondria-ER interaction.

Then, we will examine the quality of mitochondria from the aspect of autophagy/mitophagy. It is interesting to see the regulation of mitochondrial biogenesis and exclusion of damaged mitochondrial in HSCs. We will dissect HSCs of autophagy-defective mice such as ATG7 cKO mice and folliculin (FLCN) cKO mice, in which HSCs are defective. We will analyze the effect of FLCN and downstream signal TFE3 on mitochondria and lysosomal function.

Finally, we challenge to modulate mitochondrial function to increase the self-renewal activity in HSCs and realize the *ex vivo* expansion of HSCs.

【Expected Research Achievements and Scientific Significance】

We will focus on the understanding the mitochondrial function in HSCs. On the basis of these basic data, we will realize the *ex vivo* expansion of HSCs through the mitochondrial modulation.

【Publications Relevant to the Project】

・Ito K, Suda T: Metabolic requirements for the maintenance of self-renewing stem cells. ***Nat Rev Mol Cell Biol*** 141: 243-256, 2014
・Umemoto T, Hashimoto M, Matsumura T, Nakamura-Ishizu A, SudaT: Ca^{2+}-Mitochondrial axis drives cell division in hematopoietic stem cells. ***J Exp Med***, in press. 2018 doi: 10.1084/jem.20180421.

【Term of Project】 FY2018-2022

【Budget Allocation】 140,000 Thousand Yen

【Homepage Address and Other Contact Information】

http:// ircms.kumamoto-u.ac.jp/
sudato@keio.jp

Title of Project : Establishment of a novel strategy for pathological analysis of multifactorial diseases using genetic risk variants

Kazuhiko Yamamoto
(RIKEN, Center for Integrative Medical Sciences, Deputy Director)

Research Project Number : 18H05285 Researcher Number : 80191394
Keyword : multifactorial disease, genome-wide association study, risk variants

【Purpose and Background of the Research】

The purpose of this study is to establish a method for identifying causal intermediate phenotypes in the research of human multifactorial diseases based on the principle that genomic factors clearly indicate causality to disease. Recent findings have indicated that the majority of disease risk variants in multifactorial diseases affect gene expression or splicing. In our study, we will focus on immune diseases. Using genetic information, factors having a causal relationship with diseases will be identified from intermediate phenotypes, such as gene expression, epigenetic changes, protein expression, and cellular alterations, among others. These data will enable the pathogenesis of diseases to be more clearly understood, and facilitate the development of new therapies. Once our method has been established, it could also be applied to multifactorial diseases other than immune diseases.

【Research Methods】

The effects of risk variants, such as single nucleotide polymorphisms (SNP), which are identified in genome-wide association study (GWAS), can be elucidated by combining and analyzing various cellular phenotypes and risk variants. Therefore, we will construct datasets of the relationships between gene expression and genetic variants in subsets of immunocompetent cells. To avoid the influence of diseases or treatments, and to obtain clear causal relationships between the genetic variants and intermediate phenotypes, peripheral blood of healthy individuals will be mainly analyzed. After separating cells into approximately 20 different cell subsets with a cell sorter, gene expression analysis and epigenetic analyses will be carried out. We will then combine and analyze the disease risk variants in each subset according to gene expression, splicing, and epigenetic alternations

【Expected Research Achievements and Scientific Significance】

Many diseases have been studied using animal models, but the differences between humans and these animal models remain a major obstacle for clinical application. With human studies, data can be obtained for some intermediate phenotypes, but the identification of causal factors remains difficult. Data without a demonstrated causal relationship is less useful for subsequent research. Therefore, our system for identifying causal factors of diseases will enable us to gain more information to better understand the exact pathogenesis of diseases for developing new therapies.

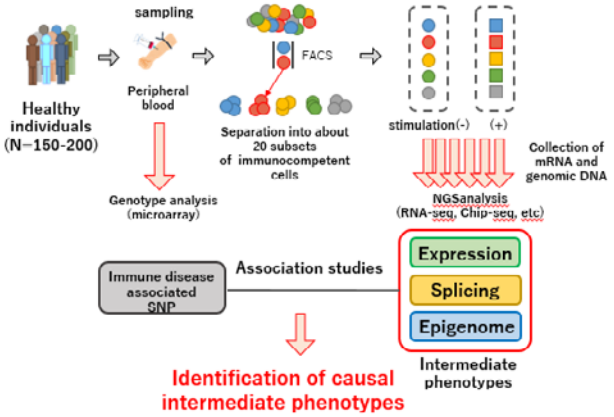


Figure: Novel analysis of multifactorial diseases with risk variants

【Publications Relevant to the Project】

・Ishigaki K, Kochi Y, Suzuki A, et al. and Yamamoto K. Polygenic burdens on cell-specific pathways underlie the risk of rheumatoid arthritis. **Nat Genet**, 2017;49;1120-1125
・Okada Y, Wu D, Trynka G, (+94), Matsuda F, Yamamoto K, and Plenge RM. Genetics of rheumatoid arthritis contributes to biology and drug discovery, et al. **Nature**. 2014; 506:376-81

【Term of Project】 FY2018-2022

【Budget Allocation】 148,800 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.riken.jp/research/labs/ims/autoimmun_dis/

Title of Project : Role of ILC2 in idiopathic interstitial pneumonia

Kazuyo Moro
(RIKEN, Center for Integrative Medical Sciences, Team leader)

Research Project Number : 18H05286　Researcher Number : 90468489
Keyword : Respiratory medicine

【Purpose and Background of the Research】

Idiopathic interstitial pneumonias (IIPs) are a set of diseases that are characterized by progressive deposition of collagen in the pulmonary alveolar interstitium. It has been reported that type 2 immune responses are inappropriately upregulated in the lungs of IIPs patients. However, the etiology of the disease is not fully understood. Group2 innate lymphoid cells (ILC2), which we discovered in 2010 produce large amounts of type 2 cytokines in response to IL-33. IL-33-activated ILC2 have been reported to exacerbate IIPs in the bleomycin-induced mouse model of pulmonary fibrosis. In this project, we will investigate the role of ILC2 in pulmonary fibrosis and verify the pathology of IIPs.

【Research Methods】

The mouse model of bleomycin-induced fibrosis is the most common model used to study IIPs. However, this is a model of acute fibrosis that occurs in 2 weeks and is resolved spontaneously after several weeks. To understand the chronic fibrosis that characterizes idiopathic pulmonary fibrosis (IPF), we have established a new mouse strain which lacks several systems for inhibition of ILC2 and develops pulmonary fibrosis. Unlike conventional models, the fibrosis in this strain occurs spontaneously and worsens in an age-dependent manner. In this project, we will investigate the pathogenic mechanism of IPF by single cell RNA-Sequence analysis, thorough the use of samples from this mouse model of fibrosis and from IPF patients.

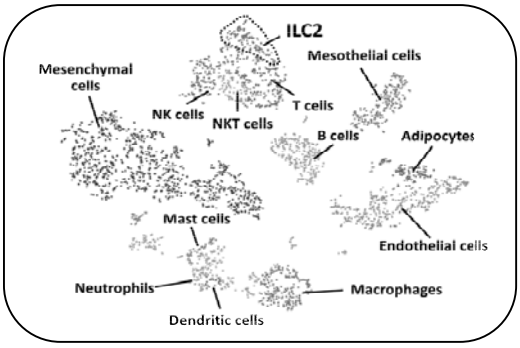


Fig. 1 Single cell RNA-Sequence

【Expected Research Achievements and Scientific Significance】

Find a candidate factors that could be a target for new therapy for IIPs.

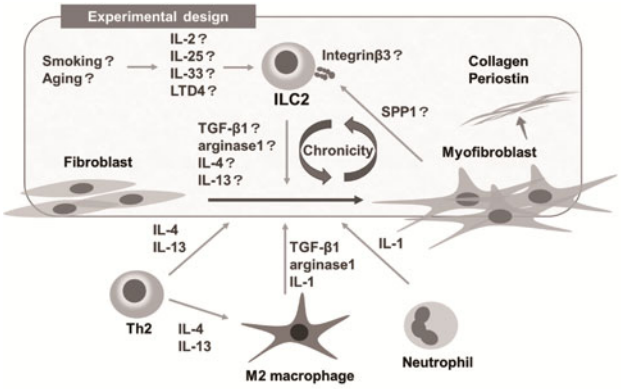


Fig. 2 ILC2 and pulmonary fibrosis

【Publications Relevant to the Project】

・Koga S, Hozumi K, Hirano KI, Yazawa M, Terooatea T. Peripheral PDGFRalpha(+)gp38(+) mesenchymal cells support the differentiation of fetal liver-derived ILC2. (2018)
・Moro K, Kabata H, Tanabe M, Koga S, Takeno N, Mochizuki M, Fukunaga K, Asano K, Betsuyaku T, Koyasu S. Interferon and IL-27 antagonize the function of group 2 innate lymphoid cells and type 2 innate immune responses. Nat Immunol, 17(1): 76-86 (2016)
・Moro K, Yamada T, Tanabe M, Takeuchi T, Ikawa T, Kawamoto H, Furusawa J, Ohtani M, Fujii H, Koyasu S. Innate production of T(H)2 cytokines by adipose tissue-associated c-Kit(+)Sca-1(+) lymphoid cells. Nature, 463(7280): 540-544 (2010)

【Term of Project】 FY2018-2022

【Budget Allocation】 148,200 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.ims.riken.jp/labo/56/index.html

Title of Project : Neural Mechanisms of Functional Recovery via Artificial Neural Connection

Yukio Nishimura
(Tokyo Metropolitan Institute of Medical Science, Department of Dementia and Higher Brain Function, Neural Prosthesis Project, Project Leader)

Research Project Number : 18H05287 Researcher Number : 20390693
Keyword : Artificial Neural Connection, Spinal cord Injury, Functional Recovery

【Purpose and Background of the Research】

Motor impairment in individuals with spinal cord lesion is attributed to the interruption of descending pathways to the spinal circuit, whereas neural circuits below and above the lesion maintain their functional capability. An artificial neural connection (ANC), which bridges supraspinal centers and spinal networks beyond the lesion, may restore the functional impairment. We have shown that ANC enable to compensate for the dysfunction of descending pathways by sending commands to the preserved spinal circuits and enable individuals with paraplegia to regain volitionally controlled paralyzed limb. Individuals may be required to learn a novel causal input-output relationship to control the paralyzed limb. Although, how the brain incorporates a novel "artificial" neural pathway into volitional limb control within the surviving cortical areas remains largely unclear. Using animal model of spinal cord injury (SCI) and SCI patients, the aim of study is elucidate neural mechanisms of adaptation and plasticity in central nervous systems induced by ANC (Fig. 1).

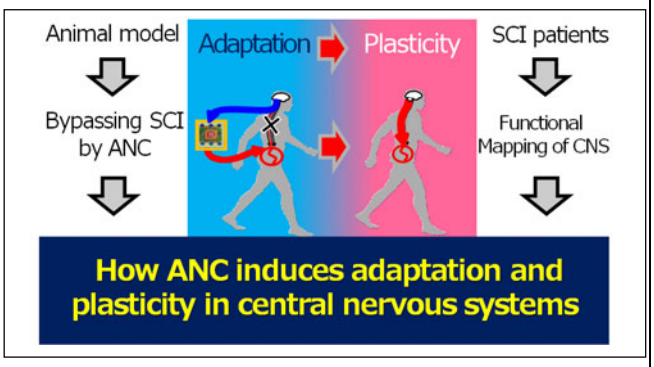


Fig. 1 Research aim

【Research Methods】

We implants multi-channel electrode array in cortical motor area and spinal cord in monkeys. To regain volitional control of the paralyzed hand, the monkeys SCI models are connected to the ANC which bridge cortical motor area and preserved spinal circuits. We investigates neural firing of population cortical cells throughout adaptation process to ANC.

We apply non-invasive ANC in paraplegic humans with chronic SCI to induce functional recovery of voluntary limb control. We investigates cortical and spinal reorganization by MRI and electrophysiological methods.

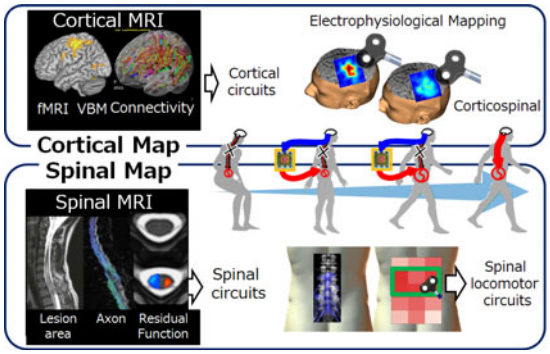


Fig. 2 Functional Mapping

【Expected Research Achievements and Scientific Significance】

The results will show neural mechanisms of functional recovery induced by ANC and propose an innovative neruorehabiliation for SCI

【Publications Relevant to the Project】

・Nishimura Y, Perlmutter SI, Eaton RW, Fetz EE. Spike-timing-dependent plasticity in primate corticospinal connections induced during free behavior. Neuron. 2013;80(5):1301-9.
・Sasada S, Kato K, Kadowaki S, Groiss SJ, Ugawa Y, Komiyama T, Nishimura Y. Volitional walking via upper limb muscle-controlled stimulation of the lumbar locomotor center in man. J Neurosci. 2014 Aug 13;34(33):11131-42.

【Term of Project】 FY2018-2022

【Budget Allocation】 113,200 Thousand Yen

【Homepage Address and Other Contact Information】

http://www.igakuken.or.jp/project/detail/neuroprosth.html