Spatiotemporal programs regulating hematopoietic stem cell maturation

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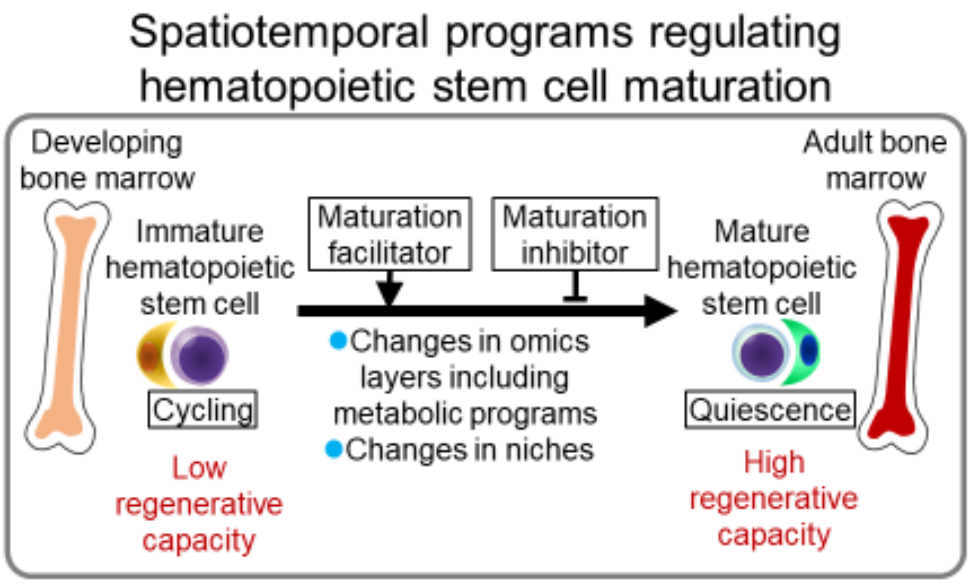
In blood cells, maturation is associated with acquisition of cell cycle quiescence in hematopoietic stem cells (HSCs) with high regeneration capacity of entire blood system. Maturation of HSCs and their niches likely contributes to high engraftment capacity after HSC transplant. However, expanded HSCs or induced HSCs from pluripotent cells show immature phenotypes including loss of quiescence with low stem cell capacity. In this project, we will define mechanisms that underlie hematopoietic maturation and resulting technologies that improve transplantation. We will evaluate transcriptional/epigenetic/metabolic programs associated with maturation-related changes in HSC metabolism using single cell analysis.

We will also model HSC maturation using a novel HSC culture mimicking the physiological niche by testing candidate factors that induce HSC maturation. Furthermore, we will evaluate the effect of microenvironment changes on HSC maturation. We will assess dynamics of HSCs and niche cells in maturating BM using intravital multiphoton imaging technique. Our studies should reveal mechanisms underlying HSC maturation at cellular and molecular levels, suggest how to enhance HSC maturation and improve expansion of HSCs and generation of transplantable HSCs from pluripotent cells in vitro.



■ URL http://www.ri.ncgm.go.jp/department/pro/04/abstract.html

Generation of T cells with diverse reactivity by regenerating thymic function

HAMAZAKI Yoko

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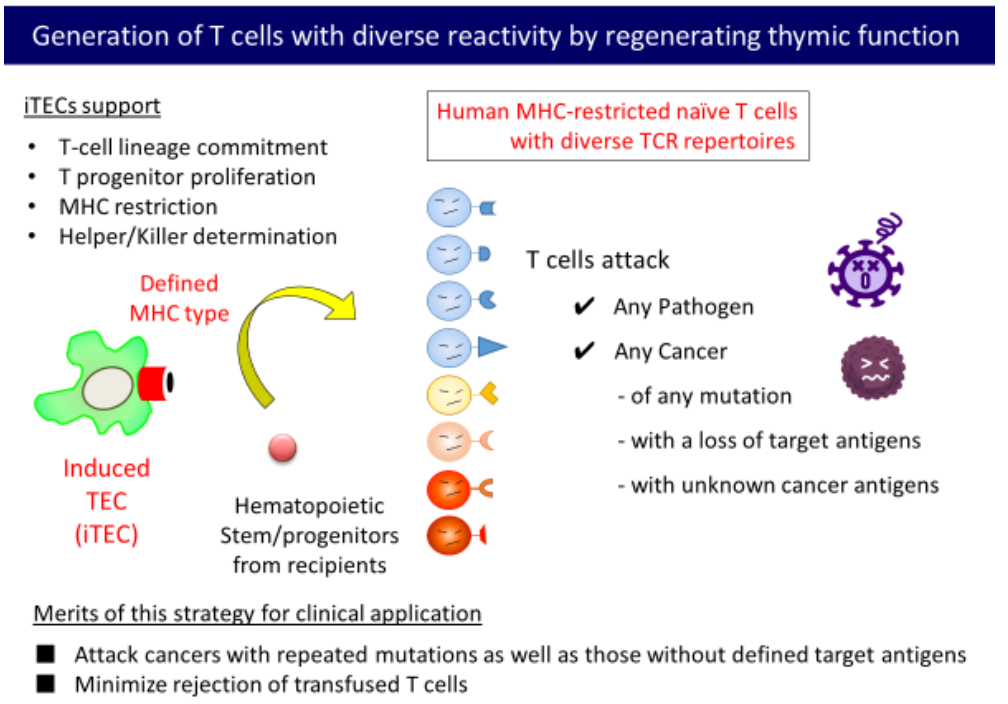
The regeneration of T cells that recognize antigens specifically expressed on cancers is an emerging form of immunotherapy. However, a frequent recurrence of tumors losing the expression of the target antigens by further mutations has compromised this strategy. T cells can theoretically react to any antigen by generating a highly diverse T-cell receptor (TCR) repertoire that can be only achieved during their differentiation in the thymus. The aim of this project is to regenerate thymic function by inducing thymic epithelial cells (TECs), which are indispensable stromal components for developing T cells and ensuring MHC restriction. As cellular sources, we will use human iPS cells, which are available from desired MHC haplotype donors, or human keratinocytes, which are easily available self-somatic cells and share similar biological properties as TECs. Using induced TECs (iTECs), we will establish a method to induce human T cells with a diverse TCR repertoire from primary hematopoietic stem/progenitor cells. This strategy could lead to significant advances in T-cell immunotherapies that reduce relapse and increase the number of patients who benefit from the immunotherapy. Furthermore, this strategy avoids the risks related to gene transduction in T cells and minimizes the rejection of transfused T cells in patients.



■ URL : https://www.cira.kyoto-u.ac.jp/j/research/hamazaki_summary.html

Realization of human cardiomyocyte proliferation – A key for cardiac regeneration -

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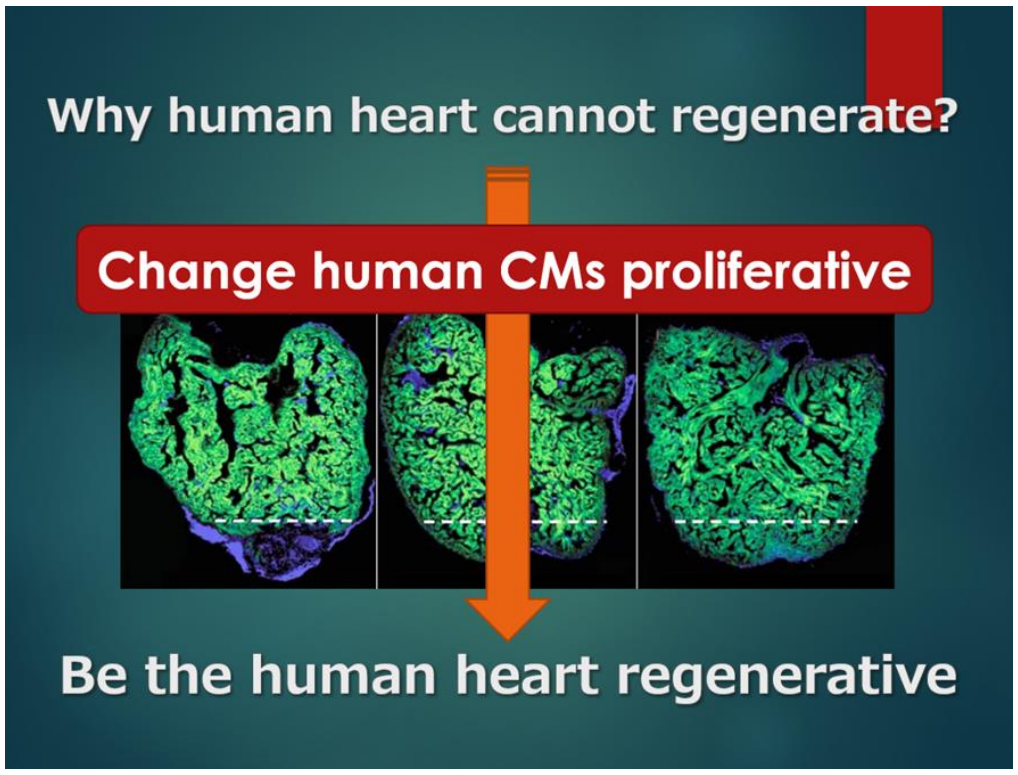
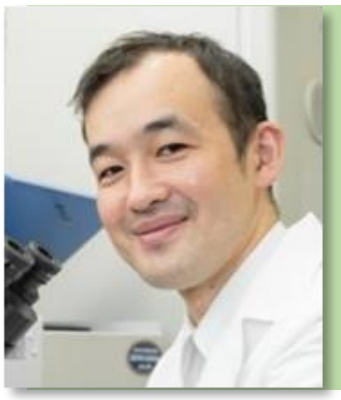
The heart of human is postulated to be a non-regenerative organ, although hearts of zebrafish or neonatal mouse within 1 week after birth can regenerate after injury. Due to the above facts, proliferative ability of cardiomyocytes is now considered to be a determinant factor for cardiac regeneration, and elucidating and manipulating the machinery that regulates human cardiomyocytes would be an important key to realizing human cardiomyocyte proliferation. We have already succeeded in inducing cardiomyocytes from human pluripotent stem cells and inducing their proliferation to some degree with small molecules. In this project, we will analyze the human cardiomyocyte proliferation process with single cell analysis comparing global gene expression between proliferating and non-proliferating cardiomyocytes. Through this analysis, we hope to elucidate the regulatory mechanisms for human cardiomyocyte proliferation and realize a future that views the human heart as a regenerative organ.



■ URL https://www.cira.kyoto-u.ac.jp/j/research/yamashita_summary.html



Modeling Skeletal Muscle Tissue Regeneration with Stem Cell-Macrophage Interaction

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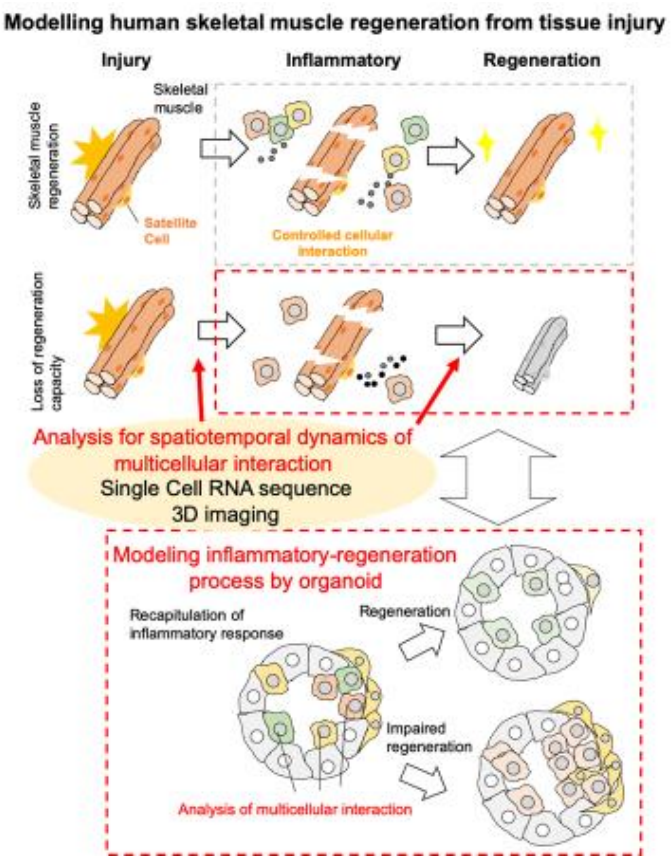
Nippon Medical School

The loss of muscle mass due to aging is called sarcopenia, and the elucidation of its pathogenesis and the development of treatment and prevention methods are important issues for our country. Sarcopenia is a pathological condition that mainly involves post-injury muscle regenerative failure, and a full understanding of the molecular mechanisms of the regenerative process is expected to provide important insight into the development of prevention methods.
However, most of the previous studies have been limited to analysis of genetically modified animals, and it has been difficult to elucidate the complex interactions that vary with the progress of muscle regeneration. To solve these issues, the establishment of an *in vitro* evaluation system that can easily analyze complex molecular mechanisms in human skeletal muscle tissue has been desired. In this study, I focus on secondary sarcopenia, a pathological condition mainly caused by post-injury muscle regeneration failure, and aim to establish a system to mimic *in vivo* to evaluate this condition by applying the method for constructing functional miniature tissues called organoids from human iPS cells.



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Development of novel safe medical device to promote regeneration of injured peripheral nerve by human iPSC-derived neuron and bioabsorbable material

SHIBATA Shinsuke

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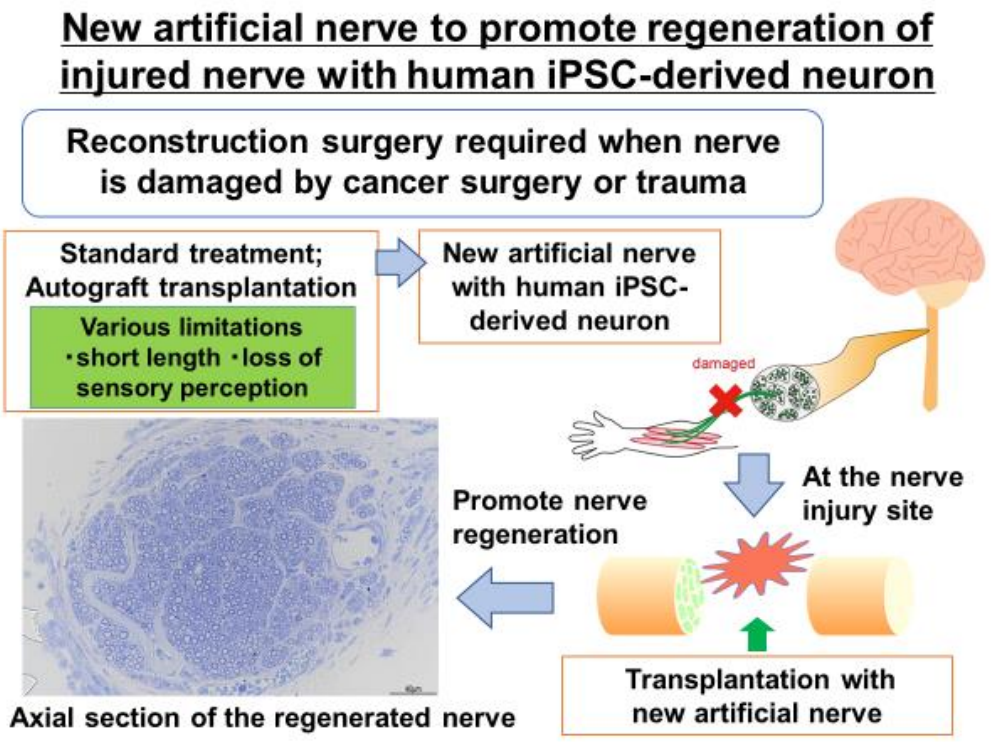
When a nerve is damaged due to a cancer operation or an injury, it must be treated by surgery. Currently, the most recommended treatment is to transplant at the expense of their own peripheral nerves, but there are some demerits that include the feeling of touch being diminished due to the removal of the sensory nerves and that treatment cannot be performed depending on the size of the nerve gap. In recent years, artificial nerves for transplantation made of artificial materials have been developed, but the therapeutic effect is still not sufficient. Therefore, we decided to generate new optimal artificial nerves for transplantation by using neurons generated from human-derived iPS cells. From this project, we hope to achieve a ready-made artificial nerve with close to parallel abilities to that of natural nerves which can be prepared in advance for transplantation surgeries required in patients with serious nerve damage due to injury. This project has the potential to develop safe new medical device that can restore the injured nerve function.



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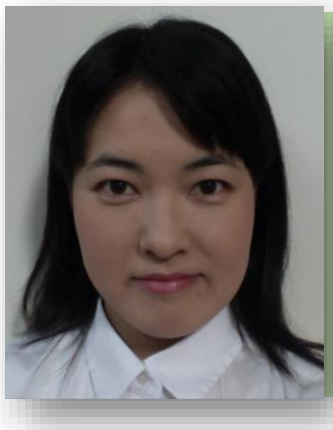

3D tissue reconstruction with hierarchical vascular networks

TADOKORO Tomomi

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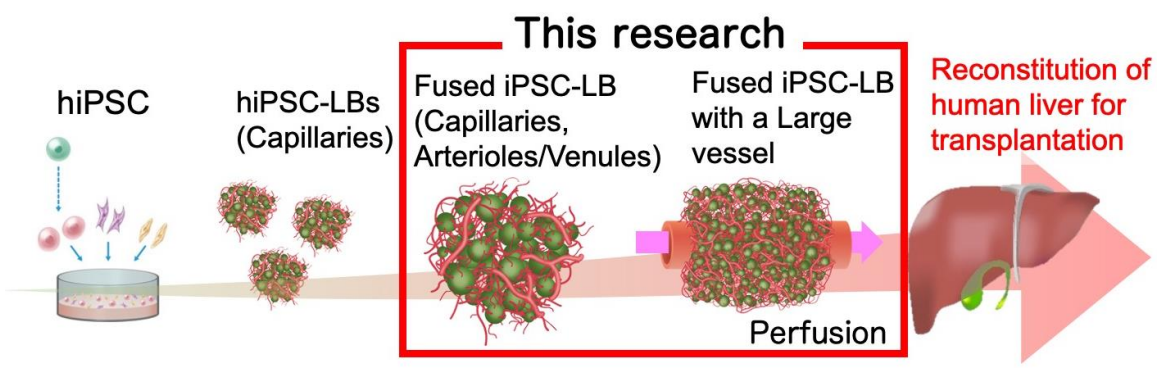
Organoid culture technology, which allows us to create complex tissue structures by induction of self-organization through cell–cell interactions, has rapidly developed by using somatic stem cells and pluripotent stem cells. Organoids have been utilized for drug discovery, generation of disease models, and reconstruction of various organs over the last decade. Compared to tissue transplants, cell/organoid transplants take more time to connect to blood vessels and exert their function. Vascularized organoid technology shortens this process; however, a certain time lag exists until a connection is made with the blood stream. Another issue in regenerative medicine is the procurement of a large mass of tissue. To solve this problem, we have developed a cell culture method to generate 3D organoids with hierarchical vascular networks that seamlessly connect capillaries to large vessels. With such vascular networks, large organs can be generated by perfusion culture. In the future, this technology may be applied to pharmaceutical research and contribute to enhance transplant engraftment and improve tissue functions.

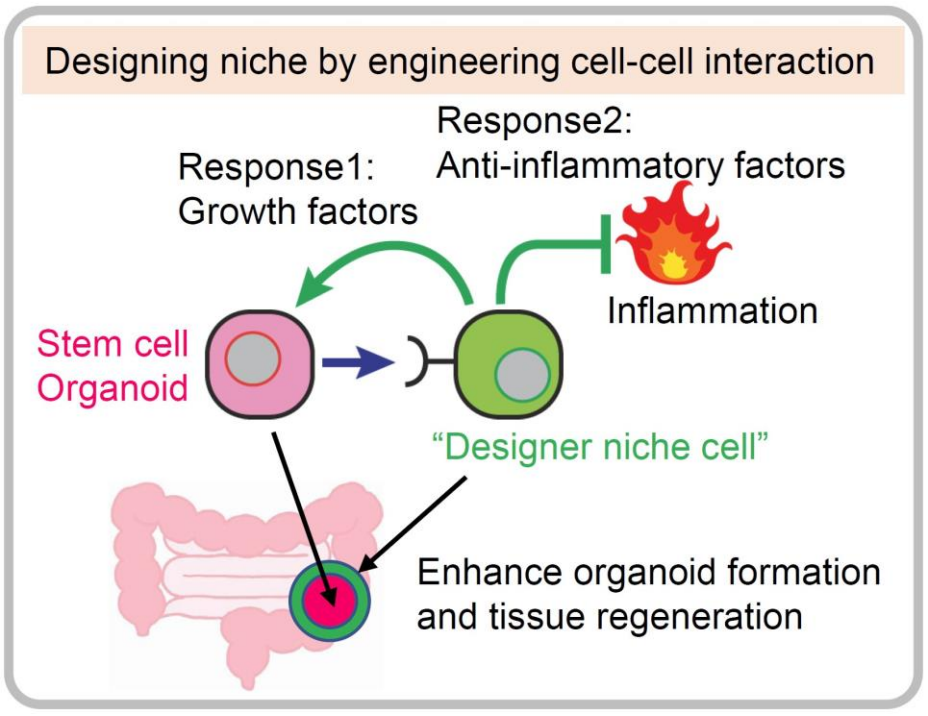


Development of designer niche cell that governs tissue microenvironment

TODA Satoshi

Assistant Professor, Nano Life Science Institute, Kanazawa University

Recent advances in stem cell biology have enabled researchers to culture *in vitro* 3D tissues called organoids, which capture some of key features of organs. Organoid systems allow us to analyze organogenesis processes and have enormous potentials in medical applications such as an alternative therapy for organ transplantation. However, how transplanted organoids can regenerate patient organs where tissue microenvironment or niche is destroyed by disease and inflammation remains unresolved. In this study, using synthetic receptor technologies, we aim to develop new therapeutic cell called "designer niche cell", which can recognize transplanted organoids and provide user-defined growth factors that can form a niche. By transplanting both organoids and designer niche cells, the designer niche cells will form a local niche around transplanted organoids to specifically enhance tissue regeneration by transplanted cells. We will also program the secretion of both growth factors and anti-inflammatory factors in the designer niche cells to induce tissue regeneration and reduce inflammation at the same time to achieve more effective cell-based regenerative therapy.



■ URL: https://sites.google.com/view/satoshitodalab/home

Development of human kidney regeneration technology from iPS cell-derived progenitor cells using mouse nephrogenic niche

YAMANAKA Shuichiro

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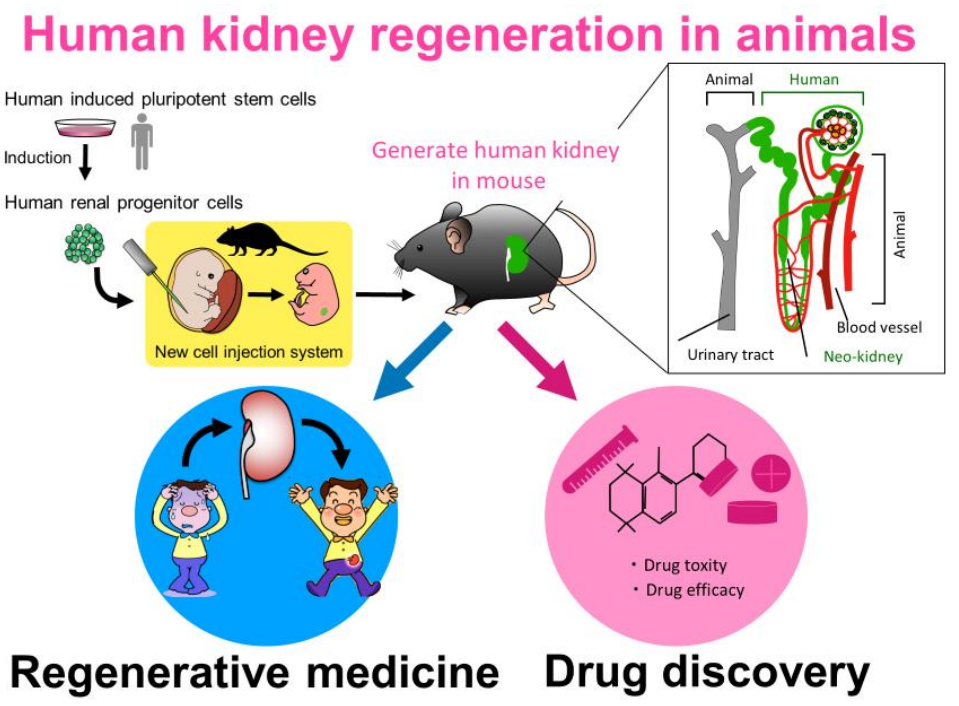
One in eight people in Japan suffer from chronic kidney disease (CKD), which is considered to be the new national disease. 330,000 patients with CKD need to undergo hemodialysis three to five hours a day for three times a week. The development of regenerative medicine as a new treatment method is desired because of the great physical and mental burden associated with usual treatment. However, the kidney not only removes waste from the body but also plays many roles in bone health and in regulation of anemia. Thus, the kidney is a complex organ that is difficult to regenerate. We focused on kidney progenitor cells, which are unique to the kidney, and have developed a technology to regenerate a functioning kidney based on a completely novel concept. This method involves transplanting progenitors from the kidneys of fetal animals and regenerating functional nephrons from the transplanted cells. By using human induced pluripotent stem cells, we are promoting the application of this technology for renal failure treatment as well as drug discovery. With this development, we aim to accomplish a method of kidney regeneration at the earliest, so that it can be used as a treatment modality in patients with kidney failure.



■ URL: https://jikei-kidneyht.jp/movie

3D Human Axial Development In Vitro: using novel human in vitro somitogenesis models to study birth defects with patient-relevant iPS cell lines

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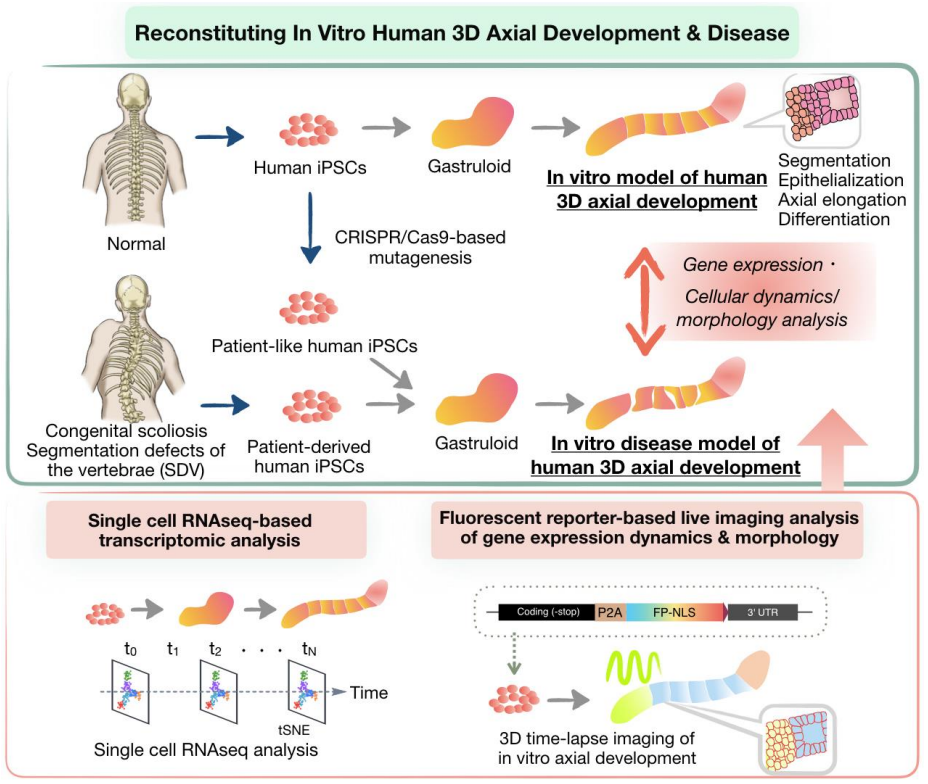
Institute for the Advanced Study of Human Biology (ASHBi), Kyoto University

Congenital scoliosis and segmentation defects of the vertebrae (SDV) are medical conditions characterized by abnormal formation and patterning of the spine and axial skeleton, thought to be caused by defects during somitogenesis and axial development of the early embryo. Although it is highly warranted to study human somitogenesis for proper understanding of normal as well as abnormal human development, analyzing human embryos is difficult due to ethical and technical restrictions. Here we propose the establishment of an induced pluripotent stem cell (iPSC)-based in vitro model of human 3D axial development & disease, aiming to characterize the functional and molecular features of human somitogenesis and to study the pathogenesis of congenital defects of the spine and axial skeleton. To this end we will utilize our newly established in vitro 3D axial development model in combination with patient-derived and patient-like iPSCs containing pathogenic mutations. We will analyze and compare in vitro human 3D axial development and somitogenesis under normal and disease conditions by utilizing single cell RNAseq-based transcriptomic analysis and fluorescent live imaging-based assessment of gene expression dynamics and morphological changes. Successful implementation of our joint research project will increase our still limited understanding of human development and disease.



■URL: https://ashbi.kyoto-u.ac.jp/ja/groups/alev/

Generating platelets in vitro for the clinic: optimisation and added clinical efficacy

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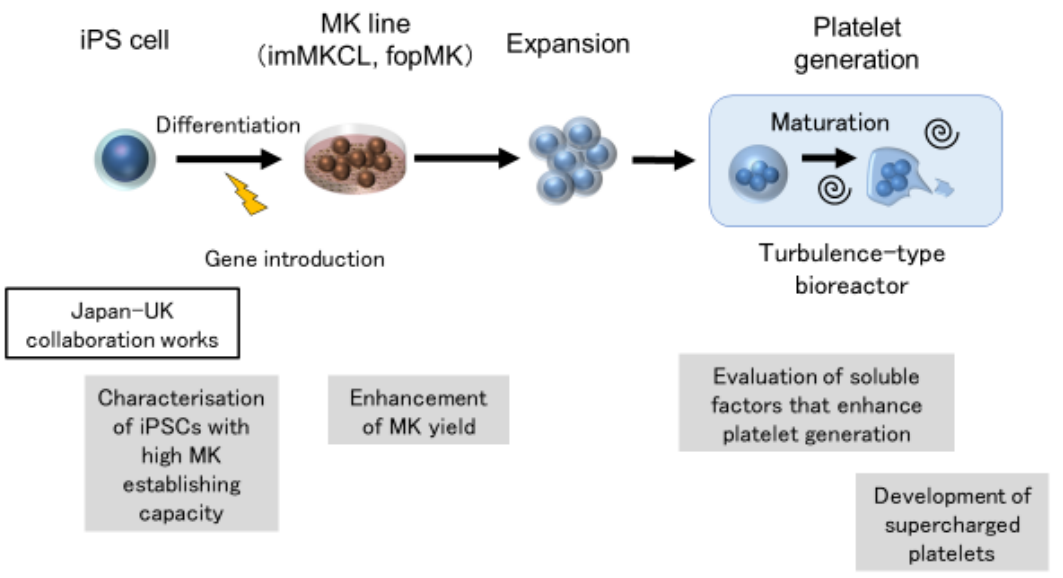
A large amount of platelet preparations is transfused every year to prevent or treat bleeding associated with thrombocytopenia. However, the current system relies solely on blood donation and thus bears the risk of unstable supply, infection and immunological incompatibility. Therefore, we and Ghevaert of the United Kingdom established expandable cell lines of megakaryocytes, which are mother cells of platelets, from iPS cells named imMKCLs and fopMKs, respectively. Nevertheless, there are still common problems. To overcome these problems, both groups will exchange reagents and cells, and first establish a method for selecting iPS cell lines suitable for mass production of platelets. Next, we will elucidate the mechanism of induction of megakaryocyte progenitor cells, identify useful cell markers, and improve the culture method. Furthermore, a small molecule substance that promotes platelet production identified by the UK side will be used in the "turbulent" bioreactor we have developed to improve platelet production efficiency. We will also create platelets with enhanced specific functions and pursue their effectiveness in treating bleeding, fractures, and infections.



■ URL: http://www.cira.kyoto-u.ac.jp/eto/

Improving haematopoietic reconstitution in blood stem cell transplantation procedures through the regulation of stem cells and their niches

TAKIZAWA Hitoshi

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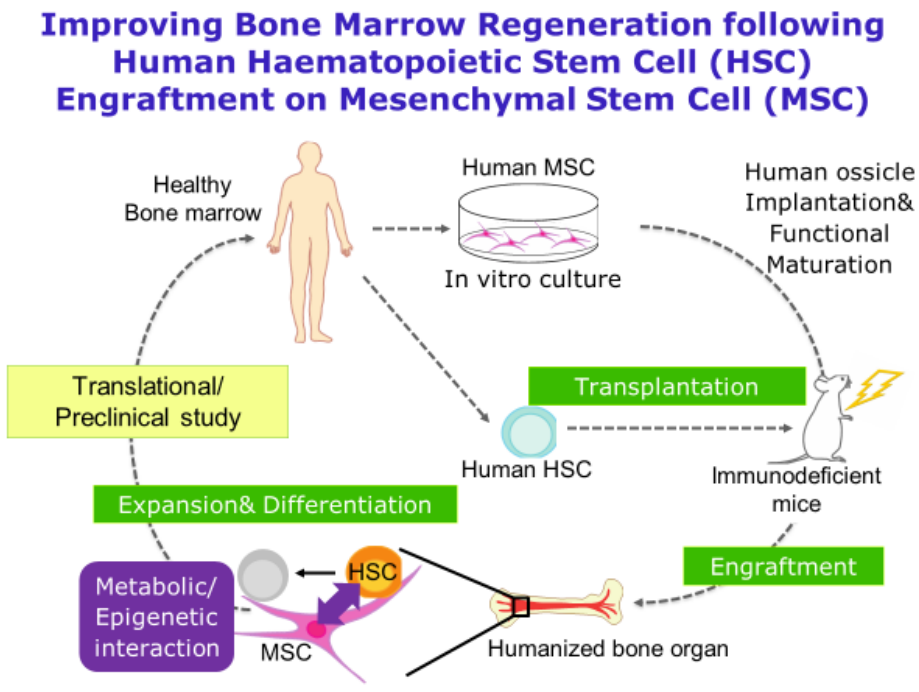
Haematopoietic stem cell (HSC) transplantation (HSCT) is now routinely performed to save the life of patients with haematological, genetic, and metabolic disorders. Regenerating normal blood cell production after chemotherapy/irradiation relies upon 3 key steps: 1) harvesting sufficient donor HSCs, 2) optimal HSC homing to the bone marrow, and 3) expansion and differentiation of donor HSCs in the recipient. However, efficacy of HSC engraftment significantly decreases during ageing and in certain haematological diseases, hampering the use of HSC transplantation as a therapeutic option. Increasing the success of HSCT requires a more detailed understanding of the factors which affect HSC engraftment, maintenance, proliferation and differentiation. Thus, this proposal aims at understanding and modelling the regeneration process following HSCT. The main goal is to identify cell-intrinsic and -extrinsic mechanisms as well as the cellular interactions with the surrounding microenvironment that regulate HSC proliferation and lineage commitment during bone marrow regeneration. Our translational aim is instructing HSC to more efficiently/rapidly/long-lastingly regenerate the haematopoietic and immune systems. The complementary expertise of the Japan-UK teams raise confidence that these current limitations in HSCT can be overcome.



■ URL: https://ircms.kumamoto-u.ac.jp/research/hitoshi_takizawa/

Elucidating and modulating macrophage and stem cell responses to bioactive nanoclays for bone regeneration

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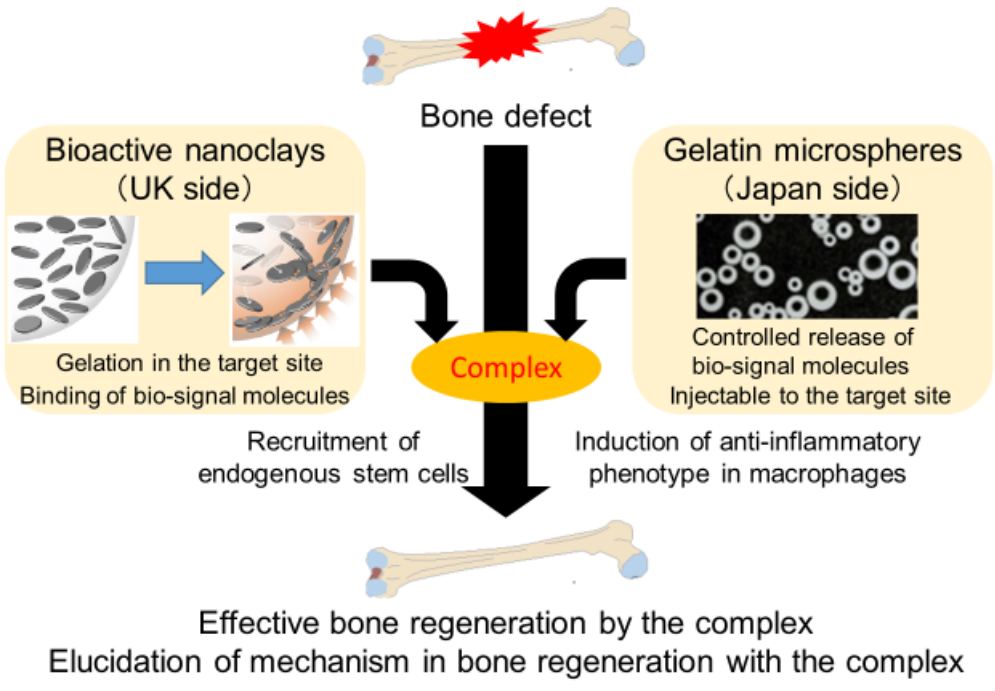
Recently, it has been well recognized that the inflammation process is tightly related to the regeneration and repair of biological tissue. Although inflammation is initially required for tissue regeneration and repair, continuous and chronic inflammation often impairs tissue regeneration and repair. Therefore, it is indispensable to recruit the endogenous stem cells to terminate the inflammation in the site to be regenerated toward the effective induction of tissue regeneration and repair. It is well known that macrophages are involved in the entire inflammation process and possess two contrary phenotypes of “pro-inflammatory” and “anti-inflammatory” to modulate the inflammation process. Through the tight Japan-UK collaboration, this project will create a novel biomaterial which enables the recruitment of endogenous stem cells and induce the anti-inflammatory phenotype of macrophages based on the fusion of each original biomaterial technology (gelatin hydrogel microspheres and bioactive nanoclays) and aim at the efficient induction and elucidation of bone regeneration.



■ URL: http://www2.infront.kyoto-u.ac.jp/te02/index-j.php3

Non-invasive monitoring of human pluripotent stem cell differentiation into midbrain dopaminergic neural cells

MORIZANE Asuka

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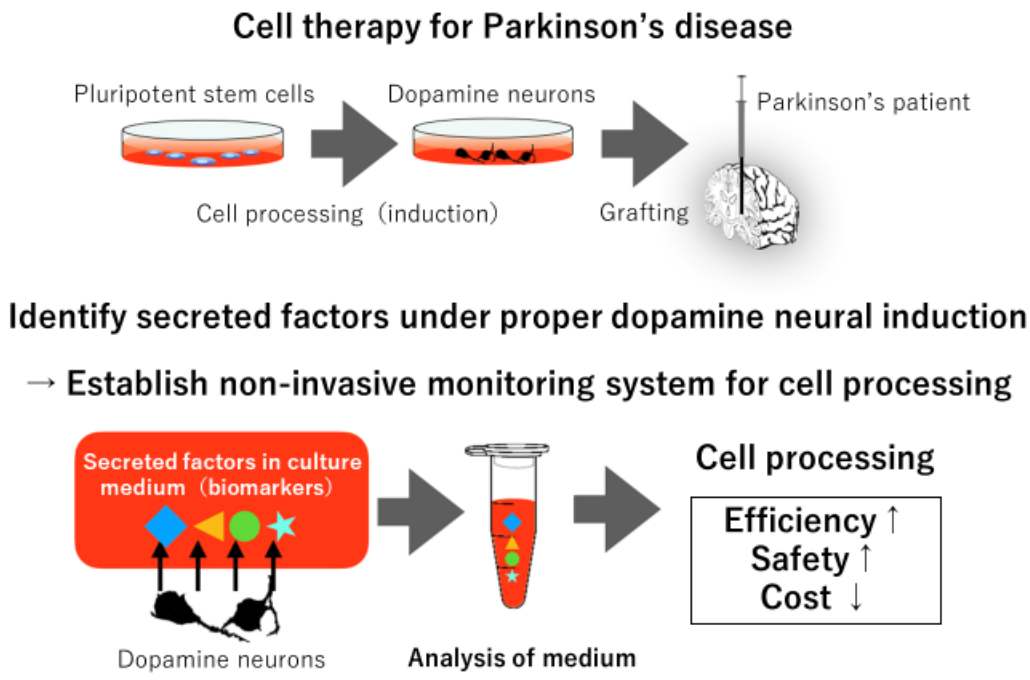
Cell therapy using iPS cells for Parkinson‘s disease began in 2018 as a doctor-led clinical trial in Japan. Dopaminergic neurons required for transplantation into patients must be carefully prepared from human iPS cells. The quality of these cells is the key to success of the treatment. As the cell production has many uncertainties, it requires delicate quality control with high cost. This research is a collaboration project with Dr. Tilo Kunath at the University of Edinburgh. In this research, we focus on biomarkers secreted from cells into culture medium during the differentiation process. We will identify molecules unique to developing dopaminergic cells and investigate those biomarkers in real time during their formation. The project aims at developing technology that makes the cell production safer and more effective at low cost. It is known that pluripotent stem cells, iPS cells and ES cells, have a unique differentiation character that varies between cell lines. Experiments with multiple cell lines in Japan and the United Kingdom are expected to yield more versatile and robust research results. Through this research, we aim to evolve and promote regenerative medicine for Parkinson's disease.



■ URL: http://www.cira.kyoto-u.ac.jp/jtakahashi/

Human blood stem cell expansion: Empowering new technology for stem cell medicine

YAMAZAKI Satoshi

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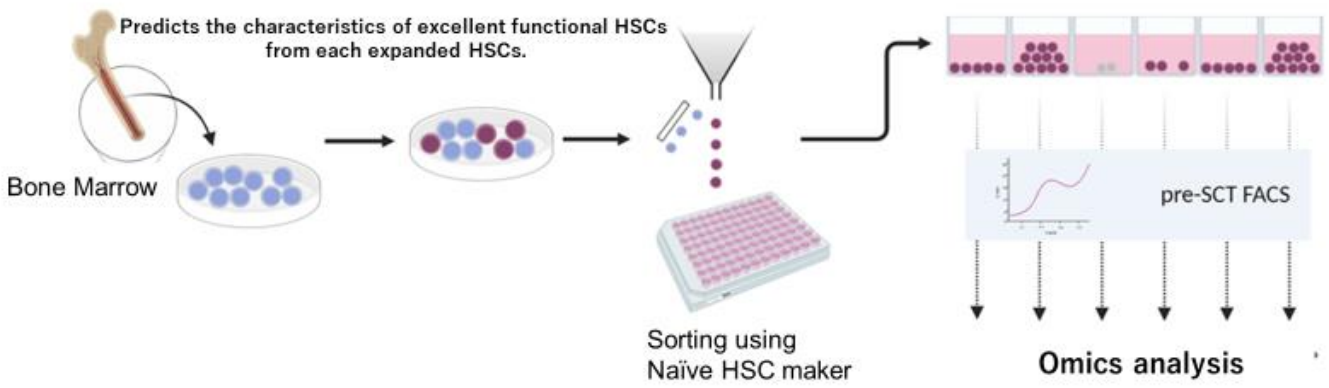
The University of Tokyo

Hematopoietic stem cells (HSCs) are one of the somatic stem cells that drive regenerative medicine because they can supply whole blood and cells of the immune system that exist in vivo. Also, HSCs are already clinically applied in hematopoietic stem cell transplantation and is known to many people. However, details regarding the characteristics and functions of HSC are still unclear, and some problems remain in its clinical application. The cause of the problem is that only a small number of HSCs can be extracted from the living body. This problem makes it difficult to develop gene therapy using HSC, establish HSC transplantation method, production of sufficiently mature cells in vitro, and analyze HSC in more detail biochemically. As a result, there are many limitations to the clinical application of HSC. Last year, we succeeded in developing a significant in vitro amplification technology for mouse HSCs with the support of AMED. We believe that this new cell culture system can be a “breakthrough” that solves the problems of HSCs. This project will clarify the molecular and functional characterization of mouse and human HSCs by utilizing the expertise of researchers in Japan and the UK to ensure that this technology will be applied clinically to humans.

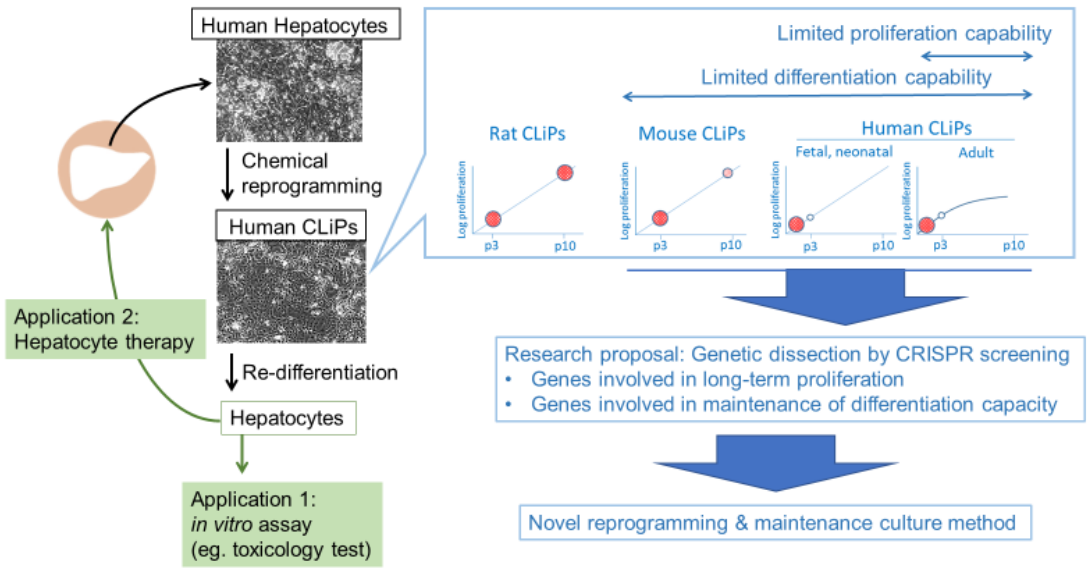


■ URL: http://www.ims.u-tokyo.ac.jp/saisei/

Reprogramming adult human hepatocytes into liver progenitors with unlimited self-renewal, efficient differentiation and transplantation capacities

YUSA Kosuke Professor, Stem Cell Genetics laboratory, Institute for Frontier Life and Medical Sciences, Kyoto University

Chronic or large acute liver diseases often require liver transplantation for cure; however, the shortage of liver donor is the major issue. As an alternative to whole liver transplantation, hepatocyte transplantation therapy has been proposed but the supply of large-scale high-quality hepatocytes has yet to be solved. Our research group aims to solve this issue by using chemically reprogrammed hepatocyte progenitors, which has been recently reported using rat hepatocytes. Progenitors derived from adult human hepatocytes were successfully generated and capable of proliferating and re-differentiating into hepatocyte; however, the progenitors rapidly lost both abilities. In this project, we employ a CRISPR-based forward genetic screening approach to genetically dissect gene/pathways involved in proliferation and differentiation of hepatocyte progenitors, and establish a culture method that supports long-term proliferation of human liver progenitors with a differentiation capability. Our research not only brings hepatocyte-based therapy closer to the clinic but also provides a more reliable hepatocyte source for in vitro use.



■ URL: https://www.infront.kyoto-u.ac.jp/research/lab40/

Harnessing spermatogonial stem cell flexibility to increase transplantation efficiency

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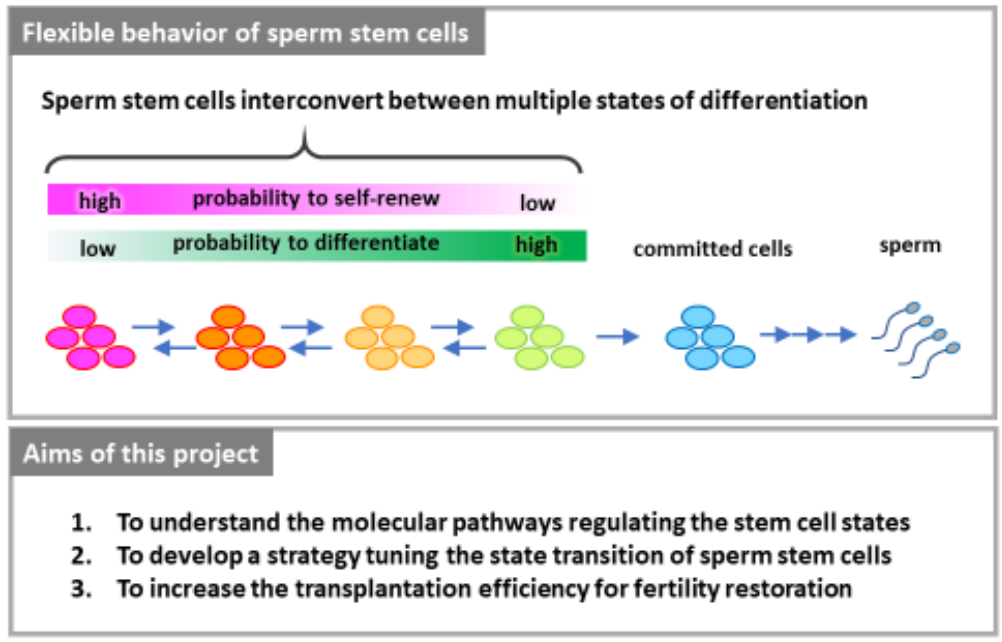
Professor, Division of Germ Cell Biology, National Institute for Basic Biology

In the testis, numerous sperm are produced based on the function of sperm stem cells, ensuring the success of reproduction. Transplantation of sperm stem cells reconstitutes spermatogenesis in the host testis whose germ cells have been depleted. However, current transplantation efficiency is not necessarily high enough for practical use. Through careful examinations of the behavior of sperm stem cells in the host testes, we have revealed that the self-renewing and differentiating states cannot be distinguished clearly. Rather, stem cells maintain persistent spermatogenesis through continual inclining toward differentiation and returning to undifferentiated states. This international research project involves researchers in Japan and in the United Kingdom and aims to conduct detailed analysis of such state changes of sperm stem cells, and to reveal the molecular mechanisms that control such state conversions. Based on these achievements, we will further tune the balance between self-renewal and differentiation of donor stem cells and augment the efficiency of spermatogenesis restoration. The results would develop a strategy to improve the infertility treatment and/or fertility restoration following disease treatment.



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