Center for Stem Cell Biology and Regenerative Medicine

Division of Regenerative Medicine
再生医学分野

Professor	Hideki Taniguchi, M.D., Ph.D.	教　授	博士(医学)	谷　口　英　樹
Associate Professor	Naoki Tanimizu, Ph.D	准教授	博士(農学)	谷　水　直　樹
Assistant Professor	Yun-Zhong Nie, Ph.D.	助　教	博士(医学)	聶　　運　中
Assistant Professor	Takayoshi Oba, M.D., Ph.D.	助　教	博士(医学)	大　場　敬　義
Project Assistant Professor	Yasuharu Ueno, Ph.D.	特任助教	博士(医学)	上　野　康　晴

Currently, organ transplantation is the only effective treatment for patients with end-stage organ failure. Unfortunately, the limited number of transplantable organs hinders the extensive application of this treatment. On the other hand, recent development of regenerative medicine that aims to generate transplantable organs on a dish has attracted much attention. Regenerative medicine is a challenging scientific field that attempts to convert knowledge from developmental biology and stem cell biology into clinical application. Our established novel organoid culture technologies reconstruct functional human organs derived from human induced pluripotent stem cells (hiPSCs) and finally aim to develop ex vivo human liver disease models and a substitute for organ transplantation therapy. Currently, we are trying to conduct the transplantation of human liver organoids (LOs) generated from hiPSCs to treat liver diseases, such as metabolic disorders and liver fibrosis. Moreover, we expand the application of our technologies to reconstruct artificial cancer tissue (cancer organoid) with a refractory tumor microenvironment for developing a new drug-screening platform to discover candidate compounds that could prevent cancer relapse and metastasis.

1. Development of treatment for metabolic liver disease by transplantation of human iPS cell derived 3D-organoids.

Yasuharu Ueno[1], Naoki Tanimizu[1], Yunzhong Nie[1], Satoshi Okamoto[1], Yu Kamishibahara[1], Takashi Okumura[1], Tomonori Tsuchida[1], Toshiharu Kasai[1], Tatsuya Kobayashi[1], Kerrigan Kilpatrick[1], Tomomi Tadokoro[2] and Hideki Taniguchi[1,2]:

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

The liver plays a crucial role in maintaining homeostasis in the living organism by performing various metabolic functions such as glucose metabolism, lipid metabolism, and ammonia metabolism. On the other hand, abnormalities in these metabolic functions can lead to a variety of liver diseases. Liver transplantation is the only curative therapy for end-stage liver disease, but the absolute shortage of donor organs is a serious challenge, and alternative treatments are clinically highly demanded. We established a technique to produce and evaluate human pluripotent stem cell (hiPSC) derived liver organoids (hiPSC-LO) by inducing differentiation of hepatic endodermal cells, vascular endothelial cells, and mesenchymal cells from hiPSCs and co-culturing them in a 3D manner (***Nature*** 2013, ***Nature*** 2017, ***Cell Reports*** 2017, ***Sci Rep*** 2020,

Stem Cell Rev Rep. 2022). Furthermore, utilizing Air-Liquid interface (ALI) cell culture technique, we newly developed a larger, more advanced fused liver organoid with enhanced vascular structures (***Sci Transl Med***. 2024, Patent No. 7228269, PCT/JP2019/13114). Transplantation of these fused liver organoids into a drug-induced liver fibrosis model ameliorated fibrosis and significantly increased survival rates.

Based on these findings, we are currently advancing research and development of a fusion-type liver organoid transplantation therapy for metabolic dysfunction-associated steatohepatitis (MASH) with support from AMED. Currently, there are approximately 300,000 MASH patients in Japan and about 50 million worldwide, making the establishment of effective treatments an urgent social issue. However, no established treatment exists globally for MASH with advanced fibrosis. To establish novel therapies for MASH, we are advancing efforts to enhance the anti-inflammatory effects of fused liver organoids, develop in vitro evaluation systems for their anti-inflammatory activity (***Biochem Biophys Res Commun***. 2024) and establish large-scale organoid production (JP Patent Application 2024-13672, PCT/JP2025/3033).

2. Liver repopulation with hiPSC derived proliferative progenitors

Yun-Zhong Nie[1], Yoshihito Hayashi[1], Qing-Lin Li[1], Xiao-Shan Deng[1], Luo Na[1], Xia Yang[1], Riana Plummer[1], Naoki Tanimizu[1], Yasuharu Ueno[1], Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

hiPSCs have shown immense potential for cell replacement therapy for disease treatment. However, hiPSC-derived cells that can effectively repopulate damaged tissues such as liver have not been reported. Here, we present the generation of expandable hiPSC-derived hepatoblast (hiPSC-HB) with robust repopulation capacity after transplantation to immunodeficient mice. These hiPSC-HB exhibited remarkable expansion capacity and displayed bipotential differentiation abilities both *in vitro* and *in vivo*. Notably, we found that hiPSC-HB transplantation could rescue mice from liver failure, demonstrating a repopulation capacity comparable to that of primary human hepatocytes (PHHs). Further, the engrafted hiPSC-HB matured into functional human hepatocytes with tissue-specific structural features in kinds of disease models. Remarkably, hiPSC-HB could also robustly reconstitute functional human tissue in livers with toxin induced chronic injury, resulting in significant attenuation of fibrosis. This study represents a major breakthrough, demonstrating the successful generation of cells from hiPSC capable of reconstituting tissue function in vivo and offering promising prospects for innovative therapeutic strategies in regenerative medicine.

3. Human pluripotent stem cell-derived fetal hepatic stellate cells promote vascularization and maturation in liver organoids

Yun-Zhong Nie[1], Xia Yang[1], Yang Li[1], Riana Plummer[1], Xiao-Shan Deng[1], Yoshihito Hayashi[1,] Qing-Lin Li[1], Luo Na[1], Toshiharu Kasai[1], Takashi Okumura[1], Naoki Tanimizu[1], Yasuharu Ueno[1], Hideki Taniguchi[1,2]

hiPSC-derived liver organoids (LOs) are valuable for studying human liver organogenesis but face challenges in faithfully recapitulating certain processes, like vasculogenesis, due to the lack of specific cell components. Hepatic stellate cells (HSCs), which are liver-specific pericytes that may play a crucial role in liver vasculogenesis, remain underutilized in developmental studies due to the disease-related status of primary HSCs under conventional culture conditions and the inefficient generation process from human pluripotent stem cells. In this study, we present an efficient method for generating hiPSC-derived HSCs (hiPSC-HSCs) resembling the transcriptomic profiles of fetal human HSCs. These hiPSC-HSCs exhibit exceptional expandability while maintaining essential cellular features. Additionally, in entirely hiPSC-derived LOs consisting of HSCs, hepatic endoderm, and endothelial cells, hiPSC-HSCs play a vital role in LO maturation and vascularization, both *in vitro* and *in vivo*. Our work represents a significant advancement in understanding HSC roles in human liver development, and LOs containing hiPSC-HSCs hold potential in modeling congenital human liver diseases.

4. hiPSC-liver bud *in vitro* growth enhanced by perfusion culture

Yoshiki Kuse[1], Naoki Tanimizu[1], Yasuharu Ueno[1], Nie Yunzhong[1], Takashi Okumura[1], , Soichiro Yamabe[1], Toshiharu Kasai[1], Tomomi Tadokoro[2], Satoshi Okamoto[2], and Hideki Taniguchi[1,2]:

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

To overcome the critical shortage of organ donors, the generation of hiPSC-derived organs with struc-

tures and functions is urgently needed. Although hiPSC-organoid is an innovative technology to reconstitute tissue structure and function, an alternative for organ transplantation. Blood perfusion is a critical event for organ growth by supplying nutrients and oxygen. However, blood perfusion is still lacking in the present organoid culture system. We are developing perfusion culture systems using two approaches; hiPSC-liver organoids (LOs) connected with pefusable hiPSC-blood vessel, and the decellularized liver tissue infused with hiPSC-LOs.

From our first approach, we generated the perfusable hiPSC-derived blood vessel using collagen gels, hiPSC-derived vascular smooth muscle cells (SMC), and vascular endothelial cells (EC). Although we clarified that hiPSC-derived blood vessels are histologically similar to the vascular structure of *in vivo* blood vessels, EC-seeded blood vessels did not show angiogenesis, resulting in no contact and connection with capillaries within hiPSC-LOs under co-culture. Therefore, we established a new culture protocol to differentiate hiPSC into specific EC lineages that exist in the fetal liver. We demonstrated that hiPSC-derived blood vessel containing those specific ECs had higher angiogenic potential. Under an optimized culture condition, we successfully induced the connection between the hiPSC-derived blood vessels with the capillaries within hiPSC-LOs. Therefore, we recently tried to establish the organoid perfusion system. Our perfusion system enabled us to culture the hiPSC-LOs by 14 days from co-culture and increase the area of hepatic progenitors/hepatocyte within hiPSC-LOs. Optimizing the perfusion culture system to mimic *in vivo* blood perfusion during organogenesis, we are trying to enhance hiPSC-LO growth more efficiently.

As a second approach, we utilize decellularized liver (DL) which preserves the *in vivo* vascular structures. The decellularization technique has been established to prepare the scaffold for organ reconstitution. Decellularized organs potentially retain the architecture of the original tissue, including the extracellular matrix. A recent report shows how the recellularized liver using hepatocytes could exert liver-specific functions after transplantation. However, to further enhance the functionality of recellularized livers, challenges remain in optimizing both the cellular sources for infusion and the perfusion protocols, particularly in controlling oxygen concentration in the culture medium. In this study, we successfully generated hiPSC-liver tissue (hiPSC-LT) with enhanced hepatic functions by infusing hiPSC-LOs into DL. In addition, oxygenation using artificial red blood cells (hemoglobin-vesicles) suppressed cell death and further improved the function of hiPSC-LT. Notably, oxygenated hiPSC-LT exhibited significant hepatic function *in vivo* after transplantation—an outcome not achieved with non-oxygenated hiPSC-LT.

5. Generation of 3D cancer tissue using patient-derived pancreatic cancer cells

Kenta Takahashi[1], Shunsuke Tabe[1], Yuya Yamomoto[1], Yasuharu Ueno[1], Hideki Taniguchi[1,2], and Naoki Tanimizu[1]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Pancreatic adenocarcinoma (PDAC) has poor prognosis, with a 5-year survival rate of about 10% due to delayed diagnosis, drug resistance, and recurrence. Organoid technologies have been applied to investigate the properties of PDACs. To recapitulate tumor microenvironment (TME), which is thought to be crucial for the poor prognosis of PDAC, we generated multicellular spheroids consisting of primary PDAC cells isolated from Japanese pancreatic cancer patients with hiPSC-mesenchymal cells (MCs) and -endothelial cells (ECs) and then fused them to construct fused pancreatic cancer organoid (FPCO) (Takeuchi K et al. ***Cell Rep*** 2023). Our FPCO resembles the tissue structure of clinical tissue including PDAC ductal structures, dense deposition of extracellular matrix components compared to conventional organoids, and heterogeneous cancer-related fibroblasts (CAFs) namely immunological CAF (iCAF), myofibroblastic ones (myCAF), and antigen presenting ones (apCAFs). In addition to CAFs, we developed FPCO containing tumor associated macrophages (TAMs) to recapitulate immunosuppressive TME, by incorporating THP1 (Tabe S et al. ***Biomaterials*** 2025) or hiPSC-derived macrophages that recapitulate cellular features of tissue-resident macrophages. Our results indicate that peripheral blood monocyte derived macrophages and tissue-resident ones give rise to distinct subsets of TAMs. Since the PDAC organoid showed strong resistance to anti-cancer drugs, we apply this new cancer organoid in drug screening and biological analysis to develop effective therapies against PDAC.

6. Space Organogenesis (Development of advanced 3D organ culture system utilizing microgravity environment)

Tomomi Tadokoro[2], Tatsuya Kobayashi[1,2], Yoshiki Kuse[1], Yasuharu Ueno[1], Yoshiharu Kasai[1], Takashi Okumura[1], and Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate

School of Medical Science, Yokohama City University

Microgravity in orbit does not cause subsidence or convection and is considered advantageous in expanding cells in three dimensions. Utilizing this microgravity environment, we aim to develop a novel method for generating human iPSCs-derived liver tissue in collaboration with Japan Aerospace Exploration Agency (JAXA). In particular, we attempt to establish a new technique for generating three-dimensional organs containing large blood vessels. The second space experiment was conducted in March 2024 to uncover the effects of microgravity on cell growth and differentiation of hiPSC-derived liver tissue. After we prepared hiPSC-liver buds (LBs) and hiPSC-derived blood vessels (BVs) on the earth, we placed those organoids into the culture container and launched them to the International Space Station "KIBO". We confirmed that hiPSC-LBs were successfully assembled around hiPSC-BVs under microgravity, as how *in silico* simulation suggested. After the perfusion culture of BV-equipped hiPSC-liver organoids (LOs) for a predetermined period in the incubator installed in "KIBO", the samples were transported back to the earth. Adherence of hiPSC-LBs to the BVs and the formation of hiPSC-LOs by fusion of hiPSC-LBs were observed in the post-flight samples. Moreover, endothelial cells formed more vascular networks within the hiPSC-LOs in the post-flight samples than the ground controls. Gene ontology analysis of RNA-seq data revealed that genes associated with hepatic functions—such as complement and coagulation cascades, fat digestion and absorption, bile secretion, steroid hormone biosynthesis, and retinol metabolism—were enriched in the post-flight samples. These findings suggest that the space environment may provide optimal conditions for tissue reconstruction. We anticipate that these results from space experiments will contribute to: (1) the development of novel techniques for human three-dimensional tissue preservation and transportation, which are essential for the practical application of regenerative medicine products; (2) the establishment of innovative methods for generating human organs equipped with large blood vessels; and (3) the creation of advanced three-dimensional culture devices that simulate microgravity conditions on Earth.

7. Reconstitution of the biliary system in hiPSC-liver organoids

Ayumu Okumura[1], Erica Carolina[1], Kenji Aoshima[1], Li Zoushuyang[1], Lai Yunghsun[1], Taichi Tsuyuki[1], Minjia Zhong[1], Kazuki Yanagisawa[1], Yoshiki Kuse[1], Takashi Okumura[1], Toshiharu Kasai[1], and Hideki Taniguchi[1,2], and Naoki Tanimizu[1]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

The biliary system consisting of bile canaliculi of hepatocytes, intrahepatic bile ducts (IHBDs), extrahepatic bile ducts (EHBDs), and gallbladder, is a crucial tissue structure for maintaining liver homeostasis by providing the excretion route for the bile secreted from hepatocytes. Although various types of liver organoids have been established, the generation of hiPSC-liver organoids associated with the bile drainage system consisting of IHBD and EHBD has not been reported so far.

Previous works demonstrate that IHBD formation depends on JAGGED1(JAG1)-NOTCH signal activated by interactions between portal vein and liver progenitor cells. Therefore, to generate liver organoid containing IHBD-like structures, we developed a new co-culture system in which the hiPSC-liver progenitors are located next to the hiPSC-blood vessel (BV) to recapitulate the interaction between portal vein components and liver progenitor cells. In this condition, hiPSC-liver progenitors differentiated into cholangiocytes and formed duct structures. We named this organoid as blood vessel incorporated liver organoid (BVLO). hiPSC-cholangiocytes in BVLO showed secretory functions in vitro and formed duct structures within the recipient liver after organoid transplantation to immunodeficient mice. Furthermore, when BVLO was transplanted to bile duct ligated mice, it temporally attenuated cholestatic symptoms and extended the survival period of injured mice. Finally, we introduced the artificial BV containing mesenchymal cells derived from JAG1 KO hiPSCs and found that bile duct formation was attenuated. This could be an in vitro model for human Alagille syndrome, a human congenital biliary disease caused by JAG1 or NOTCH2 mutations for understating underlying mechanisms. Under collaboration, we have generated iPSCs from patients' peripheral blood cells and differentiated them to mesenchymal cells for constructing artificial blood vessels. Using these cells, BVLOs will be developed to investigate how mutation affects IHBD development.

Now, we focus on the establishment of EHBD organoids. We induced EHBD progenitor cells from hiPSC-definitive endoderm cells and generated 3D cystic structures. Currently, we are further optimizing culture conditions to confer EHBD characteristics on those cystic structures. For this purpose, we analyzed gene expression profiles of mouse fetal tissue containing the EHBD primordium. Using our original transcriptome data, we are searching for molecules that may promote the differentiation and maturation of hiPSC-derived extrahepatic biliary epithelial cells.

Our final goal is to connect these two tubular

structures with hiPSC-derived hepatocytes on a dish to generate Hepatobiliary Tubular Organoids (HBTO) that possess a long-term hepatic function *in vitro* as well as *in vivo*.

8. Generation of a novel treatment for pediatric craniofacial deformity using human auricular perichondrium-derived elastic cartilage devices

Takayoshi Oba[1,2], Satoshi Okamoto[1], Yasuharu Ueno[1], Chie Ikezuki[1,2], Takuya Ohkuma[1], Yuriko Yamakawa[1,2], Konka Boku[1] and Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Auto-transplantation of rib cartilage segments is the current most popular treatment for patients with craniofacial deformity. However, major disadvantages such as limited harvestable amounts and post-operative pain of the donor site remain to be solved. To this end, a none-invasive, morphologically stable scaffold-free elastic cartilage implantation treatment for patients with craniofacial deformity is essential.

Our previous study showed the world's first technology of separating and identifying chondroprogenitor cells from the human auricular perichondrium (Kobayashi S et al. PNAS 2011, Patent registration no. 4748222; PCT/JP2008/051327). We succeeded in developing non-scaffold elastic cartilage, which is obtainable in vitro, by using three-dimensional rotation culture and U–bottomed micropatterned plate culture (Enomura M et al. Int J Mol Sci 2020, Oba T et al. J Tissue Eng 2022, Patent application no. 2021-141210; PCT/JP2022/25582). Furthermore, the size and elasticity of the tissue were maintained after craniofacial transplantation in immunodeficient mice, indicating the tissue to be morphologically stable.

For the clinical application of reconstructed elastic cartilage, we conducted a series of regulatory consultations with the Pharmaceuticals and Medical Devices Agency (PMDA), including consultations on raw materials, overproliferation, general toxicity, quality, and storage stability. Following these consultations, a clinical trial notification was formally submitted to the Ministry of Health, Labour and Welfare (MHLW) and was accepted. Based on this regulatory approval, we successfully achieved the first-in-human (FIH) clinical application of reconstructed elastic cartilage. Based on this first-in-human clinical experience, we will further expand the number of clinical cases with the aim of obtaining regulatory approval for reconstructed elastic cartilage.

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