Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Therapy
幹細胞治療分野

Professor	Hiromitsu Nakauchi, M.D., Ph.D.	教　授　医学博士	中　内　啓　光
Assistant Professor	Satoshi Yamazaki, Ph.D.	助　教　生命科学博士	山　﨑　　聡
Assistant Professor	Tomoyuki Yamaguchi, Ph.D.	助　教　医学博士	山　口　智　之

Recent great progress in stem cell biology has brought about increase in the prospect for application of stem cell-based therapy. Especially the discovery of iPSCs, a great step forward in stem-cell research, holds out the promise of development of novel therapeutic strategies by generating iPSCs from patients. The goal of this laboratory is to provide new insights into stem cell biology as well as approaches to novel therapeutic intervention for various intractable diseases.

1. Clonal Analysis Unveils Self-Renewing Lineage-Restricted Progenitors Generated Directly from Hematopoietic Stem Cells

Ryo Yamamoto[1], Yohei Morita[1,2], Jun Ooehara[1], Sanae Hamanaka[1,3], Masafumi Onodera[4], Karl Lenhard Rudolph[2], Hideo Ema[1,5], Hiromitsu Nakauchi[1,3]: [1]Division of Stem Cell Therapy, Center for Stem Cell Biology and Regeneration Medicine, Institute of Medical Science, University of Tokyo, [2]Leibniz Institute for Age Research, Fritz Lipmann Institute, Jena, [3]Japan Science Technology Agency, ERATO, Nakauchi Stem Cell and Organ Regeneration Project, [4]Department of Human Genetics, National Research Institute for Child Health and Development, [5]Present address: Department of Cell Differentiation, Sakaguchi Laboratory of Developmental Biology, Keio University School of Medicine

Consensus holds that hematopoietic stem cells (HSCs) give rise to multipotent progenitors (MPPs) of reduced self-renewal potential and that MPPs eventually produce lineage-committed progenitor cells in a stepwise manner. Using a single-cell transplantation system and marker mice, we unexpectedly found myeloid-restricted progenitors with long-term repopulating activity (MyRPs), which are lineage-committed to megakaryocytes, megakaryocyte-erythroid cells, or common myeloid cells (MkRPs, MERPs, or CMRPs, respectively) in the phenotypically defined HSC compartment together with HSCs. Paired daughter cell assays combined with transplantation revealed that HSCs can give rise to HSCs via symmetric division or directly differentiate into MyRPs via asymmetric division (yielding HSC-MkRP or HSC-CMRP pairs). These myeloid bypass pathways could be essential for fast responses to ablation stress. Our results show that loss of self-renewal and stepwise progression through specific differentiation stages are not essential for lineage commitment of HSCs and suggest a revised model of hematopoietic differentiation.

2. Generation of Engraftable Hematopoietic Stem Cells From Induced Pluripotent Stem Cells by Way of Teratoma Formation

Nao Suzuki[1,2], Satoshi?Yamazaki[1,3], Tomoyuki Yamaguchi[1,3], Motohito Okabe[1], Hideki Masaki[3], Satoshi Takaki[2], Makoto Otsu[1] and Hiromitsu Nakauchi[1,3]: [1]Division of Stem Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Science, University of

Tokyo, [2]Department of Immune Regulation Research Institute, National Center for Global Health and Medicine, [3]Japan Science Technology Agency, Exploratory Research for Advanced Technology (ERATO) Nakauchi Stem Cell and Organ Regeneration Project

In vitro generation of hematopoietic stem cells (HSCs) from induced pluripotent stem cells (iPSCs) has the potential to provide novel therapeutic approaches for replacing bone marrow (BM) transplantation without rejection or graft versus host disease. Hitherto, however, it has proved difficult to generate truly functional HSCs transplantable to adult host mice. Here, we demonstrate a unique *in vivo* differentiation system yielding engraftable HSCs from mouse and human iPSCs in teratoma-bearing animals in combination with a maneuver to facilitate hematopoiesis. In mice, we found that iPSC-derived HSCs migrate from teratomas into the BM and their intravenous injection into irradiated recipients resulted in multilineage and long-term reconstitution of the hematolymphopoietic system in serial transfers. Using this *in vivo* generation system, we could demonstrate that X-linked severe combined immunodeficiency (X-SCID) mice can be treated by HSCs derived from gene-corrected clonal iPSCs. It should also be noted that neither leukemia nor tumors were observed in recipients after transplantation of iPSC-derived HSCs. Taken our findings together, our system presented in this report should provide a useful tool not only for the study of HSCs, but also for practical application of iPSCs in the treatment of hematologic and immunologic diseases.

3. Search of machinery for efficient reversion from EpiSC to ESC-like cell

Hideyuki Murayama, Hideki Masaki, Hideyuki Sato, Tomoyuki Yamaguchi, and Hiromitsu Nakauchi: Division of Stem Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Science, University of Tokyo

Pluripotent stem cells (PSCs) can be classed as either naïve or primed. Mouse embryonic stem cells (ESCs) are naïve PSCs derived from inner cell mass (ICM) of pre-implantation blastocysts. Their naïve state is maintained in an appropriate culture medium containing leukemia inhibitory factor (LIF). Epiblast stem cells (EpiSCs) are primed PSCs derived from post-implantation epiblasts; their self-renewal ability is maintained by activin A and basic fibroblast growth factor (bFGF) signaling. Naïve and primed PSCs are distinguished from one another by differences in signaling pathways that maintain pluripotency. In contrast to mouse ESCs, however, mouse EpiSCs are barely able to contribute to chimeras when injected into blastocysts, suggesting that a definitive difference between naïve and primed PSCs exists with respect to ability to contribute to chimeras.

Transition of mouse EpiSCs to ES-like cells (rESCs) rarely occurs even after stimulation with LIF-STAT3 signaling. This finding suggest that derivation conditions affect pluripotent features of mouse EpiSCs. However, the cellular mechanisms that limit reversion efficiency remain unclear. Pluripotency in non-rodent PSCs is more like that in rodent primed-PSCs, so that chimeric animals derived from PSCs are reported only in work with rodents. Non-rodent PSCs thus are expected not to contribute to chimeras (one reason why knockout or transgenic studies have not been done using non-rodent mammals). Therefore, we investigated the conditions for efficient reversion of primed PSCs to naïve-like PSCs as part of generation of non-rodent naïve PSCs.

Here we demonstrated dramatic improvement of reversion efficiency from primed to naïve-like PSCs through up-regulation of E-cadherin in the presence of the cytokine LIF. Analysis revealed that attenuation of Wnt/β-catenin signaling with small-molecule inhibitors significantly enhances reversion efficiency of mouse EpiSCs. We showed reverted EpiSCs contributed extensively to development and succeeded in germline transmission. Although activation of Wnt/β-catenin signals has been thought desirable for maintenance of naïve PSCs, this study provides the first evidence that inhibition of Wnt/β-catenin signaling enhances reversion of mouse EpiSCs to naïve-like PSCs (rESCs). Our investigations thus provide insight into the significance of E-cadherin and Wnt/β-catenin signaling as well as into approaches for increasing efficiency of reversion of primed PSCs to naïve-like PSCs.

4. Generation of functional Ovary-like Structure from Fetal Primordial Germ Cells after Ectopic Rat-Mouse Xeno Transplantation in Immune deficient Animals

Tomonari Hayama[1] Tomoyuki Yamaguchi[1] Megumi Kato-Itoh[1] Mami Kawarai[1] Makoto Sanbo[2] Chihiro Tamura[2] Youn-Su Lee[1] Hideyuki Murayama[1] Ayumi Umino[1] Hideyuki Sato[1] Sanae Hamanaka[1] Hideki Masaki[1] Toshihiro Kobayashi[1] Masumi Hirabayashi[2] Hiromitsu Nakauchi[1]: [1]Division of Stem Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Science, University of Tokyo, [2]Center for Genetic Analysis of Behavior, National Institute for Physiological Sciences

Primordial germ cells (PGCs) are stem cells of germ cell lineage in genital ridge of fetus. As female PGCs give rise to definitive oocytes that con-

tribute to next generation. Recently induction of artificial PGC from pluripotent stem cell or generation of gonad-like tissue by co-transplantation of PGCs with fetal gonadal somatic cells has been reported. To apply these technologies to zootechnics, it is necessary to establish xeno transplantation of PGCs.

In this study, we tried to generate ovary-like tissue in immune deficient animals to establish xeno transplantation model. We transplanted rat PGCs together with gonadal somatic cells under the kidney capsule of immune deficient mice and transplanted mouse PGCs together with gonadal somatic cells under the kidney capsule of immune deficient rat. The transplanted cells constructed ovary-like tissues under the kidney capsule. Histological examinations of ovary-like tissues are performed. These tissues are similar to normal gonad in sight of immunohistological examinations that reveals Mouse VASA homologue, Stella. Gene expression profile of rat GV stage oocyte-like cell collected from rat ovary-like tissue in mouse is similar to rat GV stage oocyte. Mouse GV stage oocyte-like cells (OLCs) collected from rat. GV stage OLCs were matured to M2 stage. Intracytoplasmic sperm injection (ICSI) was performed to M2 stage OLCs. Embryo from OLC developed to full term offspring.

Our studies clarify that rat/mouse female PGC and gonadal somatic cells can develop and reconstruct ovary- like structure in ectopic xeno niches.

Publications

1. Hirose S, Takayama N, Nakamura S, Nagasawa K, Ochi K, Hirata S, Yamazaki S, Yamaguchi T, Otsu M, Sano S, Takahashi N, Sawaguchi A, Ito M, Kato T, Nakauchi H, Eto K. Immortalization of Erythroblasts by c-MYC and BCL-XL Enables Large-Scale Erythrocyte Production from Human Pluripotent Stem Cells. *Stem Cell Reports.* 2013 Dec 5; 1(6): 499-508. doi: 10.1016/j.stemcr.2013.10.010.
2. Ito K, Yanagida A, Okada K, Yamazaki Y, Nakauchi H, Kamiya A. Mesenchymal progenitor cells in mouse fetal liver regulate differentiation and proliferation of hepatoblasts. *Liver Int.* 2013 Nov 15. doi: 10.1111/liv.12387. [Epub ahead of print] PMID: 24238062
3. Mochizuki Y, Iida A, Lyons E, Kageyama R, Nakauchi H, Murakami A, Watanabe S. Use of cell type-specific transcriptome to identify genes specifically involved in Müller glia differentiation during retinal development. *Dev Neurobiol.* 2013 Oct 1. doi: 10.1002/dneu.22131. [Epub ahead of print] PMID: 24124169
4. Akiyama K, Shimada Y, Higuchi T, Ohtsu M, Nakauchi H, Kobayashi H, Fukuda T, Ida H, Eto Y, Crawford BE, Brown JR, Ohashi T. Enzyme augmentation therapy enhances the therapeutic efficacy of bone marrow transplantation in mucopolysaccharidosis type II mice. *Mol Genet Metab.* 2013 Sep 21. doi: pii: S1096-7192(13)00325-9. 10.1016/j.ymgme.2013.09.013. [Epub ahead of print] PMID: 24100247
5. Razak SR, Ueno K, Takayama N, Nariai N, Nagasaki M, Saito R, Koso H, Lai CY, Murakami M, Tsuji K, Michiue T, Nakauchi H, Otsu M, Watanabe S. Profiling of MicroRNA in Human and Mouse ES and iPS Cells Reveals Overlapping but Distinct MicroRNA Expression Patterns. *PLoS One.* 2013 Sep 23; 8(9): e73532. doi: 10.1371/journal.pone.0073532. PMID: 24086284
6. Saka K, Kawahara M, Teng J, Otsu M, Nakauchi H, Nagamune T. Top-down motif engineering of a cytokine receptor for directing ex vivo expansion of hematopoietic stem cells. *J Biotechnol.* 2013 Sep 23. doi: pii: S0168-1656(13)00403-3. 10.1016/j.jbiotec.2013.09.012. [Epub ahead of print] PMID: 24070902
7. Saito T, Yano F, Mori D, Ohba S, Hojo H, Otsu M, Eto K, Nakauchi H, Tanaka S, Chung UI, Kawaguchi H. Generation of Col2a1-EGFP iPS Cells for Monitoring Chondrogenic Differentiation. *PLoS One.* 2013 Sep 16; 8(9): e74137. doi: 10.1371/journal. pone.0074137. PMID: 24066106
8. Miyanishi M, Mori Y, Seita J, Chen JY, Karten S, Chan CK, Nakauchi H, Weissman IL. Do pluripotent stem cells exist in adult mice as very small embryonic stem cells? *Stem Cell Reports.* 2013 Jul 24; 1(2): 198-208. doi: 10.1016/j.stemcr.2013.07.001.PMID: 24052953
9. Hirabayashi M, Goto T, Tamura C, Sanbo M, Hara H, Kato-Itoh M, Sato H, Kobayashi T, Nakauchi H, Hochi S. Derivation of Embryonic Stem Cell Lines from Parthenogenetically Developing Rat Blastocysts. *Stem Cells Dev.* 2013 Nov 7. [Epub ahead of print] PMID: 24010570
10. Yamamoto R, Morita Y, Ooehara J, Hamanaka S, Onodera M, Rudolph KL, Ema H, Nakauchi H. Clonal analysis unveils self-renewing lineage-restricted progenitors generated directly from hematopoietic stem cells. *Cell.* 2013 Aug 29; 154(5): 1112-26. doi: 10.1016/j.cell.2013.08.007. PMID: 23993099
11. Kon A, Shih LY, Minamino M, Sanada M, Shiraishi Y, Nagata Y, Yoshida K, Okuno Y, Bando M, Nakato R, Ishikawa S, Sato-Otsubo A, Nagae G, Nishimoto A, Haferlach C, Nowak D, Sato Y, Alpermann T, Nagasaki M, Shimamura T, Tanaka H, Chiba K, Yamamoto R, Yamaguchi T, Otsu M, Obara N, Sakata-Yanagimoto M, Nakamaki T, Ishiyama K, Nolte F, Hofmann WK, Miyawaki S, Chiba S, Mori H,

Nakauchi H, Koeffler HP, Aburatani H, Haferlach T, Shirahige K, Miyano S, Ogawa S. Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms. *Nature Genet.* 2013 Oct; 45(10): 1232-7. doi: 10.1038/ng.2731. Epub 2013 Aug 18. PMID: 23955599

12. Yanagida A, Ito K, Chikada H, Nakauchi H, Kamiya A. An in vitro expansion system for generation of human iPS cell-derived hepatic progenitor-like cells exhibiting a bipotent differentiation potential. *PLoS One.* 2013 Jul 25; 8(7): e67541. doi: 10.1371/journal.pone.0067541. Print 2013. PMID: 23935837
13. Shinji Hirata, Naoya Takayama,Ryoko Jono-Ohnishi, Hiroshi Endo, Sou Nakamura, Takeaki Dohda, Masanori Nishi, Yuhei Hamazaki, Eiichi Ishii, Shin Kaneko, Makoto Otsu, Hiromitsu Nakauchi, Shinji Kunishima, and Koji Eto. CAMT-iPS Cells Exhibiting Defective MPL Signaling Dysregulate Megakaryopoiesis and Erythropoiesis. *J Clin Invest.* 2013. 123(9): 3802-14.
14. Nakagawa Y, Nakamura S, Nakajima M, Endo H, Dohda T, Takayama N, Nakauchi H, Arai F, Fukuda T, Eto K. Two differential flows in a bioreactor promoted platelet generation from human pluripotent stem cell - derived megakaryocytes. *Exp Hematology.* 2013 Apr 22. doi: pii: S0301-472X(13)00165-3. 10.1016/j.exphem.2013.04.007.
15. Suzuki N, Yamazaki S, Yamaguchi T, Okabe M, Masaki H, Takaki S, Otsu M, Nakauchi H. Generation of engraftable hematopoietic stem cells from induced pluripotent stem cells by way of teratoma formation. *Mol Ther.* 2013 Jul; 21(7): 1424-31. doi: 10.1038/mt.2013.71. Epub 2013 May 14.
16. Hamanaka S, Ooehara J, Morita Y, Ema H, Takahashi S, Miyawaki A, Otsu M, Yamaguchi T, Onodera M, Nakauchi H. Generation of transgenic mouse line expressing Kusabira Orange throughout body, including erythrocytes, by random segregation of provirus method. *Biochem Biophys Res Commun.* 2013 Jun 14; 435(4): 586-591. doi: 10.1016/j.bbrc.2013.05.017. Epub 2013 May 17.
17. Matsunari H, Nagashima H, Watanabe M, Umeyama K, Nakano K, Nagaya M, Kobayashi T, Yamaguchi T, Sumazaki R, Herzenberg LA, Nakauchi H. Blastocyst complementation generates exogenic pancreas in vivo in apancreatic cloned pigs. *Proc Natl Acad Sci USA.* 2013 Feb 19. [Epub ahead of print]
18. Kiyohashi K, Kakinuma S, Kamiya A, Sakamoto N, Nitta S, Yamanaka H, Yoshino K, Fijuki J, Murakawa M, Kusano-Kitazume A, Shimizu H, Okamoto R, Azuma S, Nakagawa M, Asahina Y, Tanimizu N, Kikuchi A, Nakauchi H, Watanabe M. Wnt5a signaling mediates biliary differentiation of fetal hepatic stem/progenitor cells. *Hepatology.* 2013 Feb 5. doi: 10.1002/hep.26293.
19. Hiramoto T, Ebihara Y, Mizoguchi Y, Nakamura K, Yamaguchi K, Ueno K, Nariai N, Mochizuki S, Yamamoto S, Nagasaki M, Furukawa Y, Tani K, Nakauchi H, Kobayashi M, Tsuji K. Wnt3a stimulates maturation of impaired neutrophils developed from severe congenital neutropenia patient-derived pluripotent stem cells. *Proc Natl Acad Sci USA*. 2013 Feb 4.
20. Usui A, Mochizuki Y, Iida A, Miyauchi E, Satoh S, Sock E, Nakauchi H, Aburatani H, Murakami A, Wegner M, Watanabe S. The early retinal progenitor-expressed gene Sox11 regulates the timing of the differentiation of retinal cells. *Development.* 2013 Feb; 140(4): 740-50. doi: 10.1242/dev.090274. Epub 2013 Jan 14.
21. Nishimura T, Kaneko S, Kawana-Tachikawa A, Tajima Y, Goto H, Zhu D, Nakayama-Hosoya K, Iriguchi S, Uemura Y, Shimizu T, Takayama N, Yamada D, Nishimura K, Ohtaka M, Watanabe N, Takahashi S, Iwamoto A, Koseki H, Nakanishi M, Eto K, Nakauchi H. Generation of rejuvenated antigen-specific T cells by pluripotency reprogramming and dedifferentiation. *Cell Stem Cell.* 12: 114-126m 2013
22. Oikawa T, Kamiya A, Zeniya M, Chikada H, Hyuck AD, Yamazaki Y, Wauthier E, Tajiri H, Miller LD, Wang XW, Reid LM, Nakauchi H. SALL4, a stem cell biomarker in liver cancers. *Hepatology.* 2012 Nov 23. doi: 10.1002/hep.26159.
23. Nakajima-Takagi Y, Osawa M, Oshima M, Takagi H, Miyagi S, Endoh M, Endo TA, Takayama N, Eto K, Toyoda T, Koseki H, Nakauchi H, Iwama A. Role of SOX17 in hematopoietic development from human embryonic stem cells. *Blood.* 2012 Nov 20.
24. Lin HT, Otsu M, Nakauchi H. Stem cell therapy: an exercise in patience and prudence. *Philos Trans R Soc Lond B Biol Sci.* 2013 Jan 5; 368 (1609): 20110334. doi: 10.1098/rstb.2011.0334.
25. Matsuno N, Yamamoto H, Watanabe N, Uchida N, Ota H, Nishida A, Ikebe T, Ishiwata K, Nakano N, Tsuji M, Asano-Mori Y, Izutsu K, Masuoka K, Wake A, Yoneyama A, Nakauchi H, Taniguchi S. Rapid T-cell chimerism switch and memory T-cell expansion are associated with pre-engraftment immune reaction early after cord blood transplantation. *Br J Haematol.* 2012 Nov 1. doi: 10.1111/bjh.12097.
26. Kamiya A, Nakauchi H. Enrichment and clonal culture of hepatic stem/progenitor cells during mouse liver development. *Methods Mol Biol.* 2013; 945: 273-86. doi: 10.1007/978-1-62703-125-7_16.

Center for Stem Cell Biology and Regenerative Medicine

Laboratory of Diagnostic Medicine
病態解析領域

Project Associate Professor Nobukazu Watanabe, M.D., Ph.D.　特任准教授　医学博士　渡　辺　信　和

The Laboratory of Diagnostic Medicine was established in January 2009 as a division of the Center for Stem Cell Biology and Regenerative Medicine. Our major purpose is to conduct clinical research and develop analytical methods of pathogenic conditions during infectious disease, cancer and hematopoietic stem cell and organ transplantations. Through collaborations with hospitals in Japan, we have performed several problem-based clinical studies to tackle the issues of adult T cell leukemia (ATL) and pathogenic conditions after transplantation, e.g. cytomegalovirus infection, graft failure, acute graft versus host disease (GVHD), relapse of leukemia, and recurrence of hepatitis.

1. Phenotypic analysis of ATL cells and prediction of the onset of ATL from human T-lymphotropic virus type 1 (HTLV-1) asymptomatic carriers

Tomohiro Ishigaki, Seiichiro Kobayashi[1], Nobuhiro Ohno[2], Yuji Zaike[3], Natsuko Sato, Eri Watanabe, Kaoru Uchimaru[2] and Nobukazu Watanabe: [1]Department of Molecular Therapy, [2]Research Hospital, [3]Department of Laboratory Medicine, IMSUT

Among the one million HTLV-1 carriers in Japan, approximately one thousand progress to ATL every year. Through collaborations with the Research Hospital and two laboratories at IMSUT, we are analyzing ATL cells using a flow cytometry-based method of phenotypic analysis [HTLV-1 analysis system (HAS)-Flow] to monitor disease condition. In addition, we are analyzing peripheral blood from HTLV-1 carriers to find a predictable phenotypic change of peripheral blood cells just before ATL onset in order to begin more effective treatment.

2. Analysis of ATL cells and immune cells after hematopoietic cell transplantation, DC therapy and anti-CCR4 antibody therapy in patients with ATL.

Eri Watanabe, Natsuko Sato, Ilseung Choi[4], Yoko Suehiro[4], Nobuaki Nakano[5], Yoshitaka Inoue[6], Seiichiro Kobayashi, Kaoru Uchimaru, Atae Utsunomiya[5], Takahiro Fukuda[6], Naokuni Uike[4] and Nobukazu Watanabe: [4]Department of Hematology, National Kyushu Cancer Center; [5]Department of Hematology, Imamura-bunin Hospital; [6]Stem Cell Transplantation Division, National Cancer Center Hospital

In a Japanese study group of cell therapy for ATL, hematopoietic cell transplantation, DC therapy and anti-CCR4 antibody therapy are planned for patients with acute ATL. We are joining this study group and analyzing engraftment and ATL cells using HLA-Flow and HAS-Flow methods. In addition,we are analyzing ATL cells and normal regulatory T cells with their expression levels of CCR4 which is the target of anti-CCR4 antibody therapy using 12-color flow cytometer.

3. Studies for the mechanisms underlying persistent chimerism and late rejection after cord blood transplantation in patients with severe combined immunodeficiencies (SCID).

Eri Watanabe, Nobukazu Watanabe, Kosuke Imai[7], Tomohiro Morio[7]: [7]Department of Pediatrics, Tokyo Medical Dental University

Although T cells and NK cells are lacked in patients with SCID, persistent chimerism and late rejection sometimes occur after cord blood transplantation. We analyze subpopulation-specific chimerism using HLA-Flow method and investigate the underlying mechanisms of these pathogenic conditions.

4. Studies for the mechanisms underlying recurrence of type C hepatitis and rejection after living-donor liver transplantation

Nobukazu Watanabe, Akinobu Takaki[8], Kazuko Koike[6], Takahito Yagi[9]: [8]Department of Gastroenterology and Hepatology, [9]Department of Gastroenterological Surgery, Transplant and Surgical Oncology, Okayama University Graduate School of Medicine and Dentistry

Since the 2004 approval of insurance coverage for living-donor liver transplantations (LDLT), more than 6,000 LDLTs have been performed in Japan. Although most recipients have a good prognosis, patients with hepatitis C virus (HCV) infection still face the recurrence of hepatitis after transplantation. In addition, rejection is an important issue because immunosuppressive agents are needed to suppress anti-graft immune reactions. Long-term use of immunosuppressants, however, can worsen HCV infections and future malignancies. To understand the mechanism underlying these pathologic conditions, we are investigating the following: chimerism analysis/HLA-Flow method, detection of regulatory T cells and allospecific T cells, and identification of HCV-specific $CD8^+$ T cells using tetramers.

Publications

1. Honda MJ, Watanabe E, Mikami Y, Saito Y, Toriumi T, Shirakawa T, Shimizu N, Watanabe N, Isokawa K. Mesenchymal dental stem cells for tissue regeneration. nt J Oral Maxillofac Implants. 2013 Nov-Dec; 28(6): e451-60. doi: 10.11607/jomi.te25.
2. Ohno N, Kobayashi S, Ishigaki T, Yuji K, Kobayashi M, Sato K, Watanabe N, Tojo A, Uchimaru K, Loss of CCR4 antigen expression after mogamulizumab therapy in a case of adult T-cell leukaemia-lymphoma. Br J Haematol. 163(5): 683-685, 2013.
3. Kobayashi S, Tian Y, Ohno N, Yuji K, Ishigaki T, Isobe M, Tsuda M, Oyaizu N, Watanabe E, Watanabe N, Tani K, Tojo A, Uchimaru K. The CD3 versus CD7 Plot in Multicolor Flow Cytometry Reflects Progression of Disease Stage in Patients Infected with HTLV-I. PLoS One. 2013; 8 (1): e53728. doi: 10.1371/journal.pone.0053728. Epub 2013 Jan 22.
4. Ishigaki T, Isobe M, Kobayashi S, Yuji K, Ohno N, Watanabe N, Tojo A, Uchimaru K. Development of peripheral T-cell lymphoma not otherwise specified in an HTLV-1 carrier. Int. J. Hematol. 97(5): 667-672, 2013.
5. Nishimura T, Kaneko S, Kawana-Tachikawa A, Tajima Y, Goto H, Zhu D, Nakayama-Hosoya K, Iriguchi S, Uemura Y, Shimizu T, Takayama N, Yamada D, Nishimura K, Ohtaka M, Watanabe N, Takahashi S, Iwamoto A, Koseki H, Nakanishi M, Eto K, Nakauchi H. Generation of rejuvenated antigen-specific T cells by reprogramming to pluripotency and redifferentiation. Cell Stem Cell. 12(1): 114-126, 2013.
6. Matsuno N, Yamamoto H, Watanabe N, Uchida N, Ota H, Nishida A, Ikebe T, Ishiwata K, Nakano N, Tsuji M, Asano-Mori Y, Izutsu K, Masuoka K, Wake A, Yoneyama A, Nakauchi H, Taniguchi S. Rapid T-cell chimerism switch and memory T-cell expansion are associated with pre-engraftment immune reaction early after cord blood transplantation. Br. J. Haematol. 160 (2): 255-258, 2013.

Center for Stem Cell Biology and Regenerative Medicine

Stem Cell Bank
ステムセルバンク

Associate Professor Makoto Otsu, M.D., Ph.D.

准教授 医学博士 大 津 真

Stem cells represent a valuable cell source in the field of regenerative medicine. Hematopoietic stem cells provide a good example of such usefulness of stem cell research, showing many successful cases in both hematopoietic cell transplantation and gene therapy. Pluripotent stem cells have become another possibility of cell sources in regenerative medicine that may be utilized either for the basic research or to cure the diseases. Our eventual goal is to establish safe and efficacious treatment for the patients suffering from various types of intractable diseases with no curative treatment available.

1. Recapitulation of pathophysiological features of Wiskott Aldrich Syndrome using induced pluripotent stem cells (iPSCs)

Mozhgan Kharaj, Haruna Takagi, Chieko Konishi, Miki Ando, Huang-Ting Lin, Takashi Ishida, Chen-Yi Lai, Tomoyuki Yamaguchi, Makoto Otsu, Hiromitsu Nakauchi

Wiskott Aldrich Syndrome (WAS) is an X-linked disorder, which is characterized by thrombocytopenia, immunodeficiency, eczema and autoimmunity. Patients have mutations in the gene encoding WAS protein (WASp), leading to the absence or dysfunction of this molecule specifically in their hematopoietic cells. As severe reduction in platelet numbers with their small sizes is one of the most obvious features for the disease, which is associated with a significant risk of life-threatening hemorrhage, elucidation of its precise cause has been the subject of interest for researchers for many years. Although both platelet production and consumption are reportedly affected by WASp-deficiency, it has been an issue of debate whether defective proplatelet release from megakaryocytes and/or destruction of platelets in the spleen constitutes major cause of this phenomena. Because murine WASp-null models are poor phenocopies of the disease, showing relatively unaffected numbers and sizes of platelets comparing to those in wild type mice, more accurate investigation for the mechanisms underlying thrombocytopenia needs a reliable model, which can mimic the disease features as precisely as possible. Induced Pluripotent Stem Cells (iPSC) have recently been successful to model several monogenic diseases *in vitro*, we thus sought to utilize this technology for our purpose. We first established iPSC lines of two WAS patients (with different mutations) using hematopoietic progenitor cells in peripheral blood and the Sendai virus (SeV) vector harboring four reprogramming factors (OCT3/4, SOX2, KLF4 and c-MYC), then investigated possible defects in the processes of megakaryocyte and platelet production in patient cells and their underlying mechanisms. In brief, iPSCs were allowed to differentiate into hematopoietic progenitors (HPCs) for 14 days. Megakaryocyte/erythrocyte progenitor cells (CD34 + CD41 +) were then sorted by flow cytometry, and cultured on feeder cells with fixed input numbers for another 9 days in the presence of appropriate cytokines. Absolute counts of megakaryocytes and platelets were estimated by flow cytometry analysis using fluorescent beads. To avoid the issue of clonal variation that may significantly compromise the results, we have used at least five different iPSC clones from each patient

and those from healthy individuals. First, we did not detect any considerable difference in the efficiency of iPSC generation between patient and control samples. Both patients' iPSCs were shown to exhibit the pluripotent states by detailed characterization, and were confirmed to maintain each patient-specific gene mutation after the process of somatic cell reprogramming. In the process of HPC generation, both patient-iPSCs and control-iPSCs showed comparable capability to form cells with hematopoietic cell lineages. As expected, the numbers of both platelets and megakaryocytes obtained from WAS iPSCs showed a significant decrease comparing to those from healthy iPSCs. Although still preliminary, the data so far demonstrate that there is an intrinsic defect in megakaryocyte and platelet production machineries in WAS patient cells and that the reduced platelet number seems to be due to a proplatelet production deficiency. This finding so far can support the utility of iPSC-based approaches in a pathophysiological studies for WAS, for which proper disease-modeling is otherwise not feasible, thus providing a promising measure to develop effective treatment with maximal safety for this intractable genetic disorder.

2. Alpharetroviral vectors: demonstration of safe and efficacious gene therapy using X-CGD iPSCs.

Huang-Ting Lin, Hideki Masaki, Tomoyuki Yamaguchi, Haruna Takagi, Mozhgan Kharaj, Chieko Konishi, Miki Ando, Takashi Ishida, Chen-Yi Lai, Makoto Otsu, Hiromitsu Nakauchi

Alpharetroviral vectors are of a newly developed design that is both replication-deficient and self-inactivating. Comparative integrome analysis has revealed its integration profile to be more neutral than that of lenti- or gammaretroviral vectors in addition to having minimal genotoxic risk. However, previous studies were carried out using either animal models or immortalized cell lines the physiological relevance of which to humans may be questionable. Using induced pluripotent stem cells (iPSCs) it is possible to recapitulate disease phenotypes whilst permitting precise cell manipulation in vitro. Chronic granulomatous disease (CGD) is a condition characterized by impaired neutrophil (NEU) functionality. It represents a "worst case" scenario in hematopoietic gene therapy given that no clinical trial has successfully achieved sustained persistence gene marked cells and a certain number of patients even developed myelodysplastic changes. In this study, the objective is to demonstrate safe and efficacious alpharetroviral mediated gene therapy using iPSCs as a modeling platform. To generate patient autologous iPSCs, peripheral blood (PB) CD34+ cells were reprogrammed using a Sendai virus vector expressing OCT4/SOX2/KLF4/c-MYC. Alpharetroviral vectors were used to insert codon optimized gp91 cDNA into iPSCs. Neutrophil differentiation was induced using G-CSF. The maturation status was determined by assessing cell morphology and immunophenotype. The ROS generating capacity was determined by the DHR flow cytometry assay and the formation of neutrophil extracellular traps (NETs). Identification of insertion sites was attempted using PCR-based methods and the vector integration profile was assessed through Southern blotting. Overall, the validity of using iPSCs as a disease model was established by demonstrating that differentiated NEUs (control) are functionally comparable to PB NEUs. Addition of the gp91 transgene to CGD-iPSCs led to functional recovery. Using these techniques, it was possible to correlate the efficiency of transduction to the level of transgene expression with the extent of functional recovery. Similar to previous findings, it was found that transgene expression could be sustained in the absence of any clonal expansion. Taken together, it is hope these data may act as an important pre-clinical assessment step that facilitates the application of these vectors in clinical trials. In the future, it is hoped that alpharetroviral vectors may also be utilized to treat other congenital hematological disorders through hematological gene therapy.

Publications

1. Lai CY, Yamazaki S, Okabe M et al. Stage-specific roles for Cxcr4 signaling in murine hematopoietic stem/progenitor cells in the process of bone marrow repopulation. Stem Cells 2014.
2. Suzuki N, Yamazaki S, Yamaguchi T et al. Generation of engraftable hematopoietic stem cells from induced pluripotent stem cells by way of teratoma formation. Mol Ther 2013; 21: 1424-1431.
3. Saka K, Kawahara M, Teng J et al. Top-down motif engineering of a cytokine receptor for directing ex vivo expansion of hematopoietic stem cells. J Biotechnol 2013; 168: 659-665.
4. Saito T, Yano F, Mori D et al. Generation of Col2a1-EGFP iPS cells for monitoring chondrogenic differentiation. PLoS One 2013; 8: e74137.
5. Razak SR, Ueno K, Takayama N et al. Profiling of microRNA in human and mouse ES and iPS cells reveals overlapping but distinct microRNA expression patterns. PLoS One 2013; 8: e73532.
6. Nishimura S, Manabe I, Takaki S et al. Adipose Natural Regulatory B Cells Negatively Control

Adipose Tissue Inflammation. Cell Metab 2013.
7. Lin HT, Otsu M, Nakauchi H. Stem cell therapy: an exercise in patience and prudence. Philos Trans R Soc Lond B Biol Sci 2013; 368: 20110334.
8. Kon A, Shih LY, Minamino M et al. Recurrent mutations in multiple components of the cohesin complex in myeloid neoplasms. Nat Genet 2013; 45: 1232-1237.
9. Hirose S, Takayama N, Nakamura S et al. Immortalization of Erythroblasts by c-MYC and BCL-XL Enables Large-Scale Erythrocyte Production from Human Pluripotent Stem Cells. Stem Cell Reports 2013; 1: 499-508.
10. Hirata S, Takayama N, Jono-Ohnishi R et al. Congenital amegakaryocytic thrombocytopenia iPS cells exhibit defective MPL-mediated signaling. J Clin Invest 2013; 123: 3802-3814.
11. Hamanaka S, Ooehara J, Morita Y et al. Generation of transgenic mouse line expressing Kusabira Orange throughout body, including erythrocytes, by random segregation of provirus method. Biochem Biophys Res Commun 2013; 435: 586-591.

Center for Stem Cell Biology and Regenerative Medicine

Laboratory of Stem Cell Regulation
幹細胞制御領域

Associate Professor (Project) Koichi Hattori, M.D, Ph.D. | 特任准教授 医学博士 服 部 浩 一

The goal of our laboratory is to identify novel therapeutic targets for diseases like cancer or inflammatory diseases by studying the role inflammatory and adult stem cells. Persistent inflammation is associated with diseases, including cancer, atherosclerosis, arthritis and autoimmune diseases. We show that CD11b + Jagged + inflammatory cells aid in the proliferation, survival and epithelial-to-mesenchymal transition process of malignant cells, promoting angiogenesis and metastasis. We identified inflammatory cells as drivers of hematopoietic stem cell transplantation-associated graft-versus-host disease.

1. Bone marrow-derived CD11b＋Jagged2＋ cells promote epithelial-to-mesenchymal transition and metastasization in colorectal cancer.

Francisco Caiado[1], Tânia Carvalho[1], Isadora Rosa[1], Leonor Remédio[1], Ana Costa[2], João Matos[2], Beate Heissig[3,4], Hideo Yagita[3], Koichi Hattori[4,5], João Pereira da Silva[2], Paulo Fidalgo[2], António Dias Pereira[2], Sérgio Dias[1]: [1]Instituto de Medicina Molecular, Edificio Egas Moniz, Faculdade de Medicina da Universida de Lisboa, [2]Portuguese Institute of Oncology, IPOLFG, [3]Division of Stem Cell Dynamics, Institute of Medical Sciences, University of Tokyo, [4]Atopy Center, Juntendo University, and [5]Laboratory of Stem Cell Regulation, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Sciences, University of Tokyo

Timely detection of colorectal cancer metastases may permit improvements in their clinical management. Here, we investigated a putative role for bone marrow-derived cells in the induction of epithelial-to-mesenchymal transition (EMT) as a marker for onset of metastasis. In ectopic and orthotopic mouse models of colorectal cancer, bone marrow-derived CD11b(Itgam)(＋)Jagged2 (Jag2)(＋) cells infiltrated primary tumors and surrounded tumor cells that exhibited diminished expression of E-cadherin and increased expression of vimentin, 2 hallmarks of EMT. In vitro coculture experiments showed that the bone marrow-derived CD11b(＋)Jag2(＋) cells induced EMT through a Notch-dependent pathway. Using neutralizing antibodies, we imposed a blockade on CD11b(＋) cells' recruitment to tumors, which decreased the tumor-infiltrating CD11b(＋)Jag2(＋) cell population of interest, decreasing tumor growth, restoring E-cadherin expression, and delaying EMT. In support of these results, we found that peripheral blood levels of CD11b(＋)Jag2(＋) cells in mouse models of colorectal cancer and in a cohort of untreated patients with colorectal cancer were indicative of metastatic disease. In patients with colorectal cancer, the presence of circulating CD11b(＋)Jag2(＋) cells was accompanied by loss of E-cadherin in the corresponding patient tumors. Taken together, our results show that bone marrow-derived CD11b(＋)Jag2(＋) cells, which infiltrate primary colorectal tumors, are sufficient to induce EMT in tumor cells, thereby triggering onset of metastasis. Furthermore, they argue that quantifying circulating CD11b(＋)Jag2(＋) cells in patients may offer an indicator of colorectal cancer progression to metastatic levels of the disease.

Publications

〈Koichi Hattori Group〉

1. Francisco Caiado, Tânia Carvalho, Isadora Rosa, Leonor Remédio, Ana Costa, João Matos, Beate Heissig, Hideo Yagita, Koichi Hattori, João Pereira da Silva, Paulo Fidalgo, António Dias Pereira, Sérgio Dias. Bone Marrow-Derived CD11b＋Jagged2＋ Cells promote epithelial-to-mesenchymal transition and metastasization in colorectal cancer. Cancer Res. 73: 4233-4246, 2013.
2. 服部浩一，西田知恵美：血液線維素溶解系を起点とした組織再生機構，幹細胞研究と再生医療，南山堂，138–142，2013.
3. 服部浩一，西田知恵美：造血幹細胞の骨髄内再生機構と末梢組織中への動員メカニズム，血液フロンティア，Vol. 23(10)31–39，2013.
4. 佐藤亜紀，高橋聡，服部浩一：免疫・炎症疾患としてのGVHD，血液内科，Vol 67，No 4，479–485，2013.
5. 服部浩一，島津浩：骨髄における巨核球系細胞の成熟分化機構，医薬ジャーナル社，Vol. 23，21–28，2013.
6. 服部浩一，宗像慎也：造血系細胞による血管新生制御機構，血管新生研究の最先端，医薬ジャーナル社，1，56–69，2013.

Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Processing
幹細胞プロセシング分野

Associate Professor	Makoto Otsu	准教授　医学博士	大津　真
Project Assistant Professor	Shinji Mochizuki	特任助教　医学博士	望月慎史

Our major goal is to cure patients suffering from life-threatening diseases by the treatment with processing of various stem cells. Currently our efforts are directed toward the establishment of novel therapies using human pluripotent stem cells (hPSC), such as embryonic stem cells and induced pluripotent stem cells (ESC and iPSC, respectively), and the analysis of pathogenesis of a variety of disorders based on disease-specific iPS cells.

1. Novel method for efficient production of multipotential hematopoietic progenitors from human pluripotent stem cells

Feng Ma, Yasuhiro Ebihara[1], Shinji Mochizuki, Sachiyo Hanada, Emiko Matsuzaka, Yuji Zaike[2], Hiromitsu Nakauchi[3], Kohichiro Tsuji[4], Makoto Otsu; [1]Department of Pediatric Hematology-Oncology, and [2]Department of Laboratory Medicine, Research Hospital, [3]Division of Stem Cell Therapy, Center for Stem Cell Therapy and Regenerative Medicine, [4]Department of Pediatrics, National Hospital Organization Shinshu Ueda Medical Center

ESC are pluripotent cells derived from the inner cell mass of preimplantation embryos, and iPSC are induced from somatic cells by nuclear reprogramming. Since both have the ability to be maintained in culture indefinitely as undifferentiated cells, yet they are capable of forming more differentiated cell types, they are expected as a novel source of human transplantable cells for the regenerative medicine. We then planed to produce hematopoietic stem cells (HSC) for therapeutic HSC transplantation and functional blood cells for transfusion medicine from these human pluripotent stem cells. In result, we developed a novel method for the efficient production of hematopoietic progenitor cells (HPC) from hESC and hiPSC by co-culture with AGMS-3 stromal cells, which originate from murine aorta-gonad-mesonephros (AGM) region at 11 to 12 dpc. In the co-culture, various hematopoietic progenitors were generated, and this hematopoietic activity was concentrated in cobblestone-like (CS) cells within differentiated human ESC or iPSC colonies. A fraction of CS cells expressed CD34 and retained a potential for endothelial cells. They also contained HPC, especially erythroid and multipotential HPC at high frequency. The multipotential HPC abundant among the CS cells produced all types of mature blood cells, including adult type β globin-expressing erythrocytes and tryptase and chymase-double positive mast cells (MC). They showed neither immature properties of PSC nor potentials to differentiate into endoderm and ectoderm at a clonal level. The developed co-culture system of hPSC can provide a novel source for hematopoietic and blood cells applicable to cellular therapies and drug screenings.

2. Derivation of blood cells from human pluripotent stem cells in culture without animal serum or cells

Yasuhiro Ebihara[1], Feng Ma, Shinji Mochizuki,

Sachiyo Hanada, Emiko Matsuzaka, Yuji Zaike[2], Hiromitsu Nakauchi[3], Kohichiro Tsuji[4], Makoto Otsu

It is inevitable to establish an *in vitro* culture method for the induction of hPSC, such as hESC or hiPSC, to differentiate into mature blood cells without animal serum and cells. To achieve this, we first induced hPSC to differentiate into mesenchymal stem cells (MSC). When human ES or iPS cells cultured on murine embryonic fibroblast (MEF) feeder cells were recultured on gelatin-coated culture dishes with platelet lysate (PL)-containing media in the absence of MEF feeder cells. Cells were passaged several times with PL containing media, and then MSC were induced after 6 to 8 weeks. The MSC were spindle-like shaped, revealed a phenotype of CD45−, CD34−, CD14−, CD105+, CD166+, CD31−, and SSEA-4−, and had the ability to differentiate into mesenchymal tissues such as bone, cartilage and fat in vitro. Murine MEF and undifferentiated hPSC were undetectable in the hPSC-derived MSC by reverse transcription polymerase chain reaction analysis. We then cocultured hPSC with MSC derived from hPSC themselves under serum-free condition. Two weeks later, a number of HPC appeared in the coculture. These HPC were cultured in hematopoietic colony assay using human serum. In result, hPSC-derived HPC produced various hematopoietic colonies, such as myeloid, erythroid and multilineage colonies, including all types of blood cells. The novel culture method must be useful for the clinical application of hPSC-derived blood cells.

3. Differential production of connective tissue-type and mucosal mast cells from hESC for anti-allergy drug screening

Feng Ma, Yang Wenyu, Yanzheng Gu, Yasuhiro Ebihara[1], Shinji Mochizuki, Sachiyo Hanada, Emiko Matsuzaka, Hiromitsu Nakauchi[3], Kohichiro Tsuji[4], Makoto Otsu

MC function as effector cells in allergy and atopic disease. Therefore, anti-allergy drugs have been established to diminish MC function. However, since the acquisition of an abundance of human MC (hMC) is difficult because of no culture method producing massive hMC, most anti-allergy drugs targeted animal MC. Thus, efficient discovery of effective anti-allergy drugs needs to establish the culture system of massive hMC. Then, hESC are considered as a potential cell source for hMC. In human, two types of MC have been characterized; connective tissue-type and mucosal MC (CTMC and MMC, respectively). CTMC contain tryptase, chymase, MC carboxypeptidase and cathepsin G in their secretory granules, are predominantly located in normal skin and in intestinal submucosa, and involve in atopic dermatitis. MMC contain tryptase in their secretory granules, but lack the other proteases, are the main type of MC in normal alveolar wall and in small intestinal mucosa, and involve in allergic rhinitis or bronchial asthma. Although MC can be generated from human adult $CD34^+$ HPC *in vitro*, these MC are mainly MMC. So far, there lacks an evidence for the direct derivation of CTMC from adult HPC. We achieved successful production of hESC-derived $CD34^+$ HPC, using coculture with AGMS-3 cells for 1-2 weeks. In suspension culture favoring MC differentiation within 3 weeks, hESC-derived progenitors generated mature MC that shared a chymase/tryptase double positive phenotype and strongly expressed c-Kit, similar to human skin derived CTMC. On the other hand, hESC-derived multipotential hematopoietic progenitors obtained in clonal culture developed into MC for a longer time (over 5 weeks) and only expressed tryptase, with no or few chymase, similar to human $CD34^+$ cell-derived MMC. Since the current culture system of hESC can produce differentially a large number of CTMC and MMC, our study may highlight a new understanding for MC development and finally benefit the screening for anto-allergy drugs.

4. Generation of disease-specific human iPS cells

Yasuhiro Ebihara[1], Shinji Mochizuki, Sachiyo Hanada, Emiko Matsuzaka, Hiromitsu Nakauchi[3], Kohichiro Tsuji[4], Makoto Otsu

Using developmental technics regarding to human pluripotent stem (iPS) cells, disease-specific iPS cells are generating from patients with a variety of disease. We have generated some disease-specific iPS cells. Apart from iPS cells derived from patients with DS or SCN mentioned above, one is from the patient with juvenile myelomonocytic leukemia (JMML). We employed hiPS cells derived from patients with JMML (JMML-hiPS cells). 3 JMML-hiPS cell lines were all created from bone marrow cells and reprogrammed by the defined 4 reprogramming factors (OCT3/4, KLF4, SOX2, and c-MYC). We generated blood cells from JMML-hiPS cells with coculture system using AGMS-3 cells. Hematopoiesis especially myelopoiesis was quite facilitated in cells derived from JMML-iPS cells, and the response to granulocyte macrophage-colony stimulating factor (GM-CSF) for hematopoietic colony formation was highly promoted like the recipient BM cells. These results indicated that JMML-iPS cells might reflect the pathophysiology of JMML. The other is from a patient with acute myeloid leukemia (AML) developed from 8p11 myeloproliferative syndrome (EMS). EMS is an aggressive chronic

myeloproliferative disorder frequently accompanies with T or B lymphoblastic lymphoma, and rapidly transforms into AML. Fibroblast growth factor receptor 1 (FGFR1) has critical role in the pathogenesis of EMS. We produced hiPS cells derived from this patient (EMS-hiPS cells). One EMS-hiPS cell lines was created from bone marrow cells fibroblasts and reprogrammed by the defined 4 reprogramming factors (OCT3/4, KLF4, SOX2, and c-MYC). We generated blood cells from EMS-hiPS cells with coculture system using AGMS-3 cells. EMS-iPS cells produced five-fold more hematopoietic colonies (especially monocyte and erythroid lineage) than control iPS cells. When some of FGFR1 signal inhibitor was added to the hematopoietic culture, colony formation was suppressed with dose increase at 1/7 level. These results indicated that EMS-iPS cells might reflect the pathophysiology of EMS, and EMS-iPS cells might be useful for drug sensitivity test for treatment of EMS.

Publications

1. Ebihara Y, Takedani H, Ishige I, Nagamura-Inoue T, Wakitani S, Tojo A, Tsuji K. Feasibility of autologous bone marrow mesenchymal stem cells cultured with autologous serum for treatment of hemophilic arthropathy. Haemophilia 19: e87-e89, 2013
2. Mae H, Ooi J, Takahashi S, Kato S, Kawakita T, Ebihara Y, Tsuji K, Nagamura F, Echizen H, Tojo A. Acute kidney injury after myeloablative cord blood transplantation in adults: the efficacy of strict monitoring of vancomycin serum trough concentrations. Transpl Infect Dis. 15: 181-186, 2013
3. Yamamoto S, Ebihara Y, Mochizuki S, Kawakita T, Kato S, Ooi J, Takahashi S, Tojo A, Yusa N, Furukawa Y, Oyaizu N, Watanabe J, Sato K, Kimura F, Tsuji K. Quantitative polymerase chain reaction detection of CEP110-FGFR1 fusion gene in a patient with 8p11 myeloproliferative syndrome. Leuk Lymphoma. 54: 2068-2069, 2013
4. Hiramoto T, Ebihara Y, Mizoguchi Y, Nakamura K, Yamaguchi K, Ueno K, Nariai N, Mochizuki S, Yamamoto S, Nagasaki M, Furukawa Y, Tani K, Nakauchi H, Kobayashi M, Tsuji K. Wnt3a stimulates maturation of impaired neutrophils developed from severe congenital neutropenia patient-derived pluripotent stem cells. Proc Natl Acad Sci U S A. 110: 3023-3028, 2013
5. Ebihara Y, Yamamoto S, Mochizuki S, Tsukada M, Taya Y, Kawakita T, Kato S, Ooi J, Takahashi S, Tojo A, Tsuji K. Pneumothorax in an early phase after allogeneic hematopoietic stem cell transplantation. Hematol Rep. 28: 34-35, 2013
6. Ebihara Y, Yamamoto S, Mochizuki S, Tsukada M, Taya Y, Sato A, Kawakita T, Kato S, Ooi J, Takahashi S, Tojo A, Tsuji K. Unusual extramedullary relapse after haploidentical bone marrow transplantation in a patient with acute lymphoblastic leukemia. J Blood Disorders Transf 4: 155, 2013
7. Ebihara Y, Ishikawa K, Mochizuki S, Tanaka R, Manabe A, Iseki T, Maekawa T, Tsuji K. Allogeneic stem cell transplantation for patients with acute myeloid leukemia developing from severe congenital neutropenia. Br J Haematol. 164: 459-461, 2014.
8. Konuma T, Kato S, Ooi J, Oiwa-Monna M, Ebihara Y, Mochizuki S, Yuji K, Ohno N, Kawamata T, Jo N, Yokoyama K, Uchimaru K, Asano S, Tojo A, Takahashi S. Single-unit cord blood transplantation following G-CSF-combined myeloablative conditioning for myeloid malignancies not in remission. Biol Blood Marrow Transplant. In press.
9. Konuma T, Kato S, Ooi J, Oiwa-Monna M, Ebihara Y, Mochizuki S, Yuji K, Ohno N, Kawamata T, Jo N, Yokoyama K, Uchimaru K, Tojo A, Takahashi S. The effect of ABO blood group incompatibility on the outcome of single-unit cord blood transplantation following myeloablative conditioning. Biol Blood Marrow Transplant. In press.
10. Konuma T, Kato S, Ooi J, Oiwa-Monna M, Ebihara Y, Mochizuki S, Yuji K, Ohno N, Kawamata T, Jo N, Yokoyama K, Uchimaru K, Tojo A, Takahashi S. Impact of sex incompatibility on the outcome of single-unit cord blood transplantation for adult patients with hematological malignancies. Bone Marrow Transplant. In press.

Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Transplantation
幹細胞移植分野

Professor	Arinobu Tojo, M.D., D.M.Sc.	教　授　医学博士	東　條　有　伸
Associate Professor	Satoshi Takahashi, M.D., D.M.Sc.	准教授　医学博士	高　橋　　聡

We are conducting clinical stem cell transplantation, especially using unrelated cord blood as a promising alternative donor for clinical use and investigating optimal strategies to obtain the best results in this area. We are also generating preclinical study to utilize virus-specific CTL for immune competent patients such as post-transplantation. Our goal is as allogeneic transplantation to be safer therapeutic option and to extend for older patients.

1. Effect of ABO Blood Group Incompatibility on the Outcome of Single-Unit Cord Blood Transplantation after Myeloablative Conditioning.

Konuma T, Kato S, Ooi J, Oiwa-Monna M, Ebihara Y, Mochizuki S, Yuji K, Ohno N, Kawamata T, Jo N, Yokoyama K, Uchimaru K, Tojo A, Takahashi S.

ABO blood group incompatibility between donor and recipient has been associated with poor transplant outcomes in allogeneic hematopoietic stem cell transplantation. However, its effect on the outcome of cord blood transplantation (CBT) has yet to be clarified. We retrospectively analyzed 191 adult patients who received single-unit CBT after myeloablative conditioning for malignant disease in our institute. Major mismatch showed a significantly lower incidence of platelet engraftment compared with ABO match as a reference (hazard ratio, .57; P=.01). Nevertheless, there was no increase in graft-versus-host disease, transplant-related mortality, and overall mortality after ABO-incompatible CBT. These data suggested that donor-recipient ABO incompatibility does not have a significant impact on outcome after myeloablative CBT for hematological malignancies

2. Single-Unit Cord Blood Transplantation after Granulocyte Colony-Stimulating Factor-Combined Myeloablative Conditioning for Myeloid Malignancies Not in Remission.

Konuma T, Kato S, Ooi J, Oiwa-Monna M, Ebihara Y, Mochizuki S, Yuji K, Ohno N, Kawamata T, Jo N, Yokoyama K, Uchimaru K, Asano S, Tojo A, Takahashi S.

High disease burden in myeloablative allogeneic hematopoietic stem cell transplantation is associated with adverse outcomes in patients with acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS). Quiescent leukemia stem cells could be induced to enter cell cycle by granulocyte colony-stimulating factor (G-CSF) administration and become more susceptible to chemotherapy. We report on the outcome of unrelated cord blood transplantation (CBT) using a conditioning regimen of 12 Gy total body irradiation, G-CSF-combined high-dose cytarabine, and cyclophosphamide in 61 adult patients with AML or advanced MDS not in remission. With a median follow-up of 97 months, the probability of overall survival and cumulative incidence of relapse at 7 years were 61.4% and 30.5%, respectively. In multivariate analysis, poor-risk cytogenetics and high lactate dehydrogenase

values at CBT were independently associated with inferior survival. These data demonstrate that CBT after G-CSF-combined myeloablative conditioning is a promising curative option for patients with myeloid malignancies not in remission.

3. Generation of Multivirus-specificT Cells by a Single Generation of PBMCs with a Peptide Mixture Utilizing Serum-free Medium.

Fujita Y, Takahashi S.

Extension to donors other than HLA-matched siblings following advanced immunosuppressive treatment has resulted in the emergence of viral infections as major contributors to morbidity and mortality after hematopoietic stem cell transplantation (HSCT). While pharmacological agents are standard therapy for some, they have substantial toxicities, generate resistant variants, and are frequently ineffective. Moreover, immune reconstitution is necessary for long-term protection against pathogens after HSCT. Restoration of virus-specific immunity offers an attractive alternative to conventional drugs. Adoptive transfer of virus-specific CTLs from stem cell donors has been proved to be safe and effective in treatment of viral infection. Recently, the system for rapid generation of multivirus-specific T cells has been reported (Gerdemann, U, 2012). With this technique, polyclonal CTLs specific for multivirus antigens can be produced after single stimulation of PBMCs with a peptide mixture spanning the target antigens in the presence of IL4 and IL7. We introduced and verified this system to apply for clinical use in Japan. To confirm regulation by the Japanese FDA, we attempted to generate multivirus-specific T cells in serum-free medium. To meet the requirement for the viral infections after HSCT by broad virus species, we extended the target antigens to cytomegalovirus (CMV), EBV, adenovirus (AdV), HHV-6, BKV, JC virus and VZV. 20×106 of PBMCs were stimulated with peptide mixture spanning the target antigens of 3 (CMV, EBV, AdV) or 7 (CMV, EBV, AdV, HHV-6, BKV, JC virus and VZV) viruses and cultured in serum-free medium with cocktail of IL4 and IL7 for 9-12 days. 20×106 PBMCs were stimulated with each antigen of CMV, EBV and AdV and cultured for 9-12 days in serum free medium or in RPMI1640 + 5% human serum (HS). We obtained average of 144.9×106 cells in RPMI with 5% HS and average of 92.0×106 cells in serum-free medium (n=4), which were statistically significant (p= 0.025). Most of the prepared cells were positive for CD3, mainly consisted of CD4+ central memory cells in each condition. The average percentage of T cell subsets were as follows; CD3+ 97.7%, CD4+ 84.8%, CD8+ 11.7%, CD3+CD62L+CD45RO+ 89.0% in RPMI+5% HS and CD3+98.9%, CD4+ 74.4%, CD8+ 14.8%, CD3+CD62L+CD45RO+ 93.2% in serum-free medium. We observed no significant difference in specificity toward the CMV, EBV and AdV antigens between the cells cultured in medium with HS and in serum-free medium. IFNγ production was observed in average of 9.83% of the prepared cells (19.1 ×106 cells) in RPMI+5% HS and in average of 7.93% (10.0 ×106 cells) in serum-free medium when assessed by intracellular cytokine staining (ICS). We next tried to generate 7 viruses-specific T cells in the serum-free culture system. 20×106 PBMCs were stimulated with each antigens of CMV, EBV, AdV, HHV-6, BKV, JC virus and VZV and cultured for 10-12 days yielding 151.9±39.6×106 cells (n=5). 95.6% of the cells after the expansion were CD3+ and contained both CD4 + and CD8+ cells. The average percentage of CD4 positivity was 74.1% and that for CD8 was 20.8%. 80% of CD3+ cells expressed central memory phenotype. These single stimulated and cultured cells contained average of 21.2% CD4+IFNγ+ cells and 5.1% CD8+IFNγ+ cells measured by ICS and showed specificity toward all the 7 virus antigens (n=4).

Publications

Mizuta S, Matsuo K, Nishiwaki S, Imai K, Kanamori H, Ohashi K, Fukuda T, Yasushi O, Miyamura K, Takahashi S Onizuka M, Atsuta Y, Suzuki R, Morishima Y, Kato K, Sakamaki H, Tanaka J. Pre-transplant administration of imatinib for allogeneic hematopoietic stem cell transplantation in patients with BCR-ABL-positive acute lymphoblastic leukemia. Blood. 2014 Mar 3. [Epub ahead of print]

Konuma T, Kato S, Oiwa-Monna M, Tojo A, Takahashi S. Pretransplant hyperferritinemia has no effect on the outcome of myeloablative cord blood transplantation for acute leukemia and myelodysplastic syndrome. Ann Hematol. 2013 Oct 19. [Epub ahead of print]

Konuma T, Kato S, Ooi J, Oiwa-Monna M, Ebihara Y, Mochizuki S, Yuji K, Ohno N, Kawamata T, Jo N, Yokoyama K, Uchimaru K, Tojo A, Takahashi S. Effect of ABO Blood Group Incompatibility on the Outcome of Single-Unit Cord Blood Transplantation after Myeloablative Conditioning. *Biol Blood Marrow Transplant.* 20: 577-581, 2014

Konuma T, Kato S, Ooi J, Oiwa-Monna M, Ebihara Y, Mochizuki S, Yuji K, Ohno N, Kawamata T, Jo N, Yokoyama K, Uchimaru K, Asano S, Tojo A, Takahashi S. Single-Unit Cord Blood Transplanta-

tion after Granulocyte Colony-Stimulating Factor-Combined Myeloablative Conditioning for Myeloid Malignancies Not in Remission. *Biol Blood Marrow Transplant*. 20: 396-401, 2014

Atsuta Y, Suzuki R, Yamashita T, Fukuda T, Miyamura K, Taniguchi S, Iida H, Uchida T, Ikegame K, Takahashi S, Kato K, Kawa K, Nagamura-Inoue T, Morishima Y, Sakamaki H, Kodera Y; Japan Society for Hematopoietic Cell Transplantation. Continuing increased risk of oral/esophageal cancer after allogeneic hematopoietic stem cell transplantation in adults in association with chronic graft-versus-host disease. *Ann Oncol.* 25 (2): 435-41, 2014

Kurosawa S, Yakushijin K, Yamaguchi T, Atsuta Y, Nagamura-Inoue T, Akiyama H, Taniguchi S, Miyamura K, Takahashi S, Eto T, Ogawa H, Kurokawa M, Tanaka J, Kawa K, Kato K, Suzuki R, Morishima Y, Sakamaki H, Fukuda T. Recent decrease in non-relapse mortality due to graft-versus-host disease and infection after allogeneic hematopoietic cell transplantation in non-remission acute leukemia. *Bone Marrow Transplant*. 48 (9): 1198-204 2013

Nishiwaki S, Miyamura K, Ohashi K, Kurokawa M, Taniguchi S, Fukuda T, Ikegame K, Takahashi S, Mori T, Imai K, Iida H, Hidaka M, Sakamaki H, Morishima Y, Kato K, Suzuki R, Tanaka J; Adult Acute Lymphoblastic Leukemia Working Group of the Japan Society for Hematopoietic Cell Transplantation. Impact of a donor source on adult Philadelphia chromosome-negative acute lymphoblastic leukemia: a retrospective analysis from the Adult Acute Lymphoblastic Leukemia Working Group of the Japan Society for Hematopoietic Cell Transplantation. *Ann Oncol.* 24(6): 1594-602, 2013

Atsuta Y, Kanda J, Takanashi M, Morishima M, Taniguchi S, Takahashi S, Ogawa H, Ohashi K, Ohno Y, Onishi Y, Aotsuka N, Nagamura-Inoue T, Kato K, Kanda Y, on behalf of the HLA Working Group of the Japan Society for Hematopoietic Cell Transplantation. Different effects of HLA disparity on transplant outcomes after single-unit cord blood transplantation between pediatric and adult patients with leukemia. *Hematologica.* 98(5): 814-22, 2013

Kurosawa S, Yakushijin K, Yamaguchi T, Atsuta Y, Nagamura-Inoue T, Akiyama H, Taniguchi S, Miyamura K, Takahashi S, Eto T, Ogawa H, Kurokawa M, Tanaka J, Kawa K, Kato K, Suzuki R, Morishima Y, Sakamaki H, Fukuda T. Changes in incidence and causes of non-relapse mortality after allogeneic hematopoietic cell transplantation in patients with acute leukemia/myelodysplastic syndrome: an analysis of the Japan Transplant Outcome Registry. *Bone Marrow Transplant*. 48(4): 529-36, 2013

Mori T, Tanaka M, Kobayashi T, Ohashi K, Fujisawa S, Yokota A, Fujita H, Nakaseko C, Sakura T, Nannya Y, Takahashi S, Kanamori H, Kanda Y, Sakamaki H, Okamoto S; Kanto Study Group for Cell Therapy. Prospective multicenter study of single-unit cord blood transplantation with myeloablative conditioning for adult patients with high-risk hematologic malignancies. *Biol Blood Marrow Transplant*. 19(3): 486-91, 2013

Doki N, Miyawaki S, Tanaka M, Kudo D, Wake A, Oshima K, Fujita H, Uehara T, Hyo R, Mori T, Takahashi S, Okamoto S, Sakamaki H; Kanto Study Group for Cell Therapy. Visceral varicella zoster virus infection after allogeneic stem cell transplantation. *Transpl Infect Dis*. 15(3): 314-8, 2013

Mae H, Ooi J, Takahashi S, Kato S, Kawakita T, Ebihara Y, Tsuji K, Nagamura F, Echizen H, Tojo A. Acute kidney injury after myeloablative cord blood transplantation in adults: the efficacy of strict monitoring of vancomycin serum trough concentrations. *Transpl Infect Dis*. 15(2): 181-6, 2013

Ebihara Y, Yamamoto S, Mochizuki S, Tsukada M, Taya Y, Kawakita T, Kato S, Ooi J, Takahashi S, Tojo A, Tsuji K. Pneumothorax in an early phase after allogeneic hematopoietic stem cell transplantation. *Hematol Rep*. 5(2): 34-5, 2013

Tanaka J, Morishima Y, Takahashi Y, Yabe T, Oba K, Takahashi S, Taniguchi S, Ogawa H, Onishi Y, Miyamura K, Kanamori H, Aotsuka N, Kato K, Kato S, Atsuta Y, Kanda Y. Effects of KIR ligand incompatibility on clinical outcomes of umbilical cord blood transplantation without ATG for acute leukemia in complete remission. *Blood Cancer J.* 3: e164, 2013

Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Signaling
幹細胞シグナル制御部門

Professor	Toshio Kitamura, M.D., D.M.Sc.		教　授	医学博士	北　村　俊　雄
Project Research Associate	Toshihiko Oki, M.D., D.M.Sc.		特任助教	医学博士	沖　　俊　彦

Our major interest is to elucidate the mechanisms of pluripotency, self-renewal and the control of cell division and differentiation of stem cells like ES cells, iPS cells, and hematopoietic stem cells. We have developed the retrovirus-mediated efficient gene transfer and several functional expression cloning systems, and utilized these system to our experiment. We are now conducting several projects related to stem cells to characterize stem cells, clarify underling mechanisms of reprogramming, maintenance of pluripotency, and differentiation, and eventually to develop new strategies for regenerative medicine.

1. RasGRP family proteins and Leukemia

Toshihiko Oki, Jiro Kitaura, Koutarou Nishimura, Akie Maehara, Tomoyuki Uchida, Fumio Nakahara, and Toshio Kitamura

The Ras guanyl nucleotide-releasing proteins (RasGRPs) are a family of guanine nucleotide-exchange factors, with four members (RasGRP1-4), which positively regulate Ras and related small GTPases. In the previous study, we identified RasGRP4 using expression cloning as a gene that fully transformed IL-3-dependent HF6 cells, and demonstrated that in a mouse bone marrow transplantation (BMT) model, RasGRP4 induced acute myeloid leukemia (AML) and/or T-ALL. On the other hand, it has been reported that RasGRP1 transgenic mice developed thymic lymphoma or skin tumors.

However, the roles of RasGRP family proteins in leukemogenesis have not been investigated in detail. We have recently characterized leukemogenicity of RasGRP1 and 4 in details using a BMT model (Oki et al. Leukemia 2012).

RasGRP1 exclusively induced T-cell acute lymphoblastic leukemia/lymphoma (T-ALL) after a shorter latency when compared to RasGRP4. Accordingly, Ba/F3 cells transduced with RasGRP1 survived longer under growth factor withdrawal or phorbol ester stimulation than those transduced with RasGRP4, presumably due to the efficient activation of Ras. Intriguingly, *NOTCH1* mutations resulting in a gain of function were found in 77% of the RasGRP1-mediated mouse T-ALL samples. In addition, gain-of-function *NOTCH1* mutation was found in human T-cell malignancy with elevated expression of RasGRP1. Importantly, RasGRP1 and NOTCH1 signaling cooperated in the progression of T-ALL in the murine model. The leukemogenic advantage of RasGRP1 over RasGRP4 was attenuated by the disruption of a PKC phosphorylation site (RasGRP1(Thr184)) which RasGRP4 is lacking. In summary, cooperation between aberrant expression of RasGRP1, a strong activator of Ras, and secondary gain-of-function mutations of *NOTCH1* plays an important role in T-cell leukemogenesis.

2. Development of new retroviral vectors.

Toshikhiko Oki, Jiro Kitaura, Tomoyuki Uchida, Fumi Shibata-Minoshima, and Toshio Kitamura:

We previously developed an effective retroviral transduction system consisted of vectors named as

pMXs, pMYs, pMZs and pMCs and packaging cells named as PLAT-E, PLAT-A, and PLAT-F. Based on this system, we developed new vectors including vectors with luciferase maker (pMX-IL), vectors for GFP or RFP fusion proteins, vectors with lox sequences for deletion of inserted genes with Cre-loxP, Tet-On and Tet-Off systems, vectors for expression, inhibition, and monitoring the expression of miroRNA (pMXe series). We utilized these vectors in studying stem cell biology and also in developing the innovative tools for regenerative medicine

3. Co-ordinate control of cell division and cell fate of by the Rho family small GTPases.

Toshihiko Oki, Kohtaro Nishimura, Toshiyuki Kawashima, and Toshio Kitamura:

We previously identified MgcRacGAP through functional cloning as a protein that enhances or induces macrophage differentiation of leukemic cell lines M1 and HL60. Interestingly, MgcRacGAP plays distinct roles depending on the cell cycle. In the interphase, it plays critical roles in activation and nuclear translocation of STAT3 and STAT5 as a Rac-GAP. In the mitotic phase, MgcRacGAP plays essential roles in completion of cytokinesis as a Rho-GAP. Interestingly, Aurora B-mediated phosphorylation of S387 converts MgcRacGAP from Rac-GAP to Rho-GAP.

We have recently shown that expression of MgcRacGAP is regulated by a cell cycle- dependent manner: MgcRacGAP expression increases in S/G2/M phase and decreases in early G1 phase, suggesting that MgcRacGAP may play some roles in G1 check point. In addition to the transcriptional control, MgcRacGAP protein levels are controlled by ubiquitin-dependent degradation, leading to its decrease in G1 phase. Using the proteome analysis and retroviral transduction, we identified APC/CDH1 as an E3 ligase involved in regulation of MgcRacGAP and the degron in MgcRacGAP. Now we are investigating the physiological roles of this regulation. In summary, our results implicate MgcRacGAP in coordination of cell cycle progression and cell fate determination.

4. Molecular therapy targeting signal transduction pathways using small molecule compounds

Toshiyuki Kawashima, Akiho Tsuchiya, Toshihiko Oki, Jiro Kitaura, and Toshio Kitamura:

STAT3 is frequently activated in many cancers and leukemias, and is required for transformation of NIH3T3 cells. Therefore, we have started searching for STAT3 inhibitors. We already established an efficient screening protocol for identification of STAT3 inhibitors, and identified several compounds that inhibit STAT3 activation. Through the screening of a library of small molecule compounds, we found the compounds RJSI-1 and RJSI-2 that inhibited STAT3 activation. RJSI-2 also inhibited activation of STAT1, STAT5, JAK1 and JAK2, however RJSI-2 is not a kinase inhibitor. On the other hand, RJSI-1 inhibited nuclear transport of phosphorylated STAT proteins, implicating a novel mechanism in inhibiting STAT proteins. We have also shown that these compounds are effective in a tumor-burden mouse model. In addition, we collaborate with a US biotech venture company in modification of RSJI-1 for optimization to develop anticancer drugs, and have developed JP1156 which kill the tumor cells with much lower IC50.

5. Development of G0 indicator

Toshihiko Oki, Kotarou Nishimura, Jiro Kitarura, Fumio Nakahara, Asako Sakaue-Sawano[2], Atsushi Miyawaki[2], Toshio Kitamura: [2]Laboratory for Cell Function Dynamics, RIKEN, Wako, Saitama and ERATO Miyawaki Life Function Dynamics Project, JST.

One of the common features of the stem cells is that they are in quiescent (G0) phase of cell cycle. Several reports indicate that tissue specific stem cells like hematopietic stem cells and cancer stem cells with tumor initiating potentials are in G0 phase.

Recently we have developed the system to indicate cells in G0 phase. It is a system to monitor the amount of p27, which is destructed during G0 to G1 phase and is not expressed in S/G2/M phase, using the cells retrovirally trasduced with the fusion protein between a fluorescent protein like mVenus and p27K- (a p27 mutant lacking CDK inhibitory activities) as a similar cell cycle indicator system, fluorescent, ubiquitination-based cell cycle indicator, (Fucci). mVenus-p27K- positive cells are Ki67 negative quiescent cells and mVenus-p27K- signals are enhanced when the cycling cell enter G0 phase in response to serum starvation or contactinhibition.

Using this system, we identified genetic signatures of G0 cells. Several genes specifically expressed in G0 cells are now being investigated in terms of their functions and biological significance in G0 phase. The mVenus-p27K- trasgenic mice have also been generated to track several kinds of tissue specific stem cells in vivo.

Publications

Ishihara, J., Umemoto, T., Yamato, M., Shiratsuchi, Y., Takaki, S., Petrich, B.G., Nakauchi, H., Eto, K., Kitamura, T., and Okano T. (2014) Nov/CCN regulates long term repopulation activity of murine hematopietic stem cells via integrin avb3. Int. J. Hematol. in press.

Oki, T., Nishimura, K., Kitaura, J., Togami, K., Maehara, A., Izawa, K., Sakaue-Sawano. A., Niida, A., Miyano, S., Aburatani, H., Kiyonari, H., Miyawaki, A. and Kitamura, T. (2014) A novel cell-cycle indicator, mVenus-p27K-, identifies quiescent cells and visualizes G0-G1 transition. Scientific Reports 4: 4012.

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. (2014) Ezh2 loss promotes development of myelodysplastic syndrome but attenuates its predisposition to leukemic transformation. J. Exp. Med. in press.

Uchida, T., Kitaura, J., Nakahara, F., Togami, K., Inoue, D., Maehara, A., Nishimura, K., Kawabata, C.K., Doki, N., Kakihana, K., Yoshioka, K., Izawa, K., Oki, T., Sada, A., Harada, Y., Ohashi, K., Katayama, Y., Matsui, T., Harada, H., and Kitamura, T. (2014) Hes1 up-regulation contributes to the development of FIP1L1-PDGFRA-positive leukemia in blast crisis. Exp. Hematol. in press.

Maegawa, S., Gough, S., Watanabe-Okochi, N., Lu, Y., Zhang, N., Castoro, R.J., Estecio, M.R.H., Jelinek, J., Liang, S., Kitamura, T., Aplan, P., and Issa, J.P. (2014) Age-related epigenetic drift in the pathogenesis of MDS and AML. Genome Research. in press.

Izawa, K., Isobe, M., Matsukawa, T., Ito, S., Maehara, A., Takahashi, M., Yamanishi, Y., Kaitani, A., Oki, T., Okumura, K., Kitamura, T., and Kitaura, J. (2014) Sphingomyelin and ceramide are physiological ligands for human LMIR3/CD300f, inhibiting FceRI-mediated mast cell activation. J. Allergy. Clin. Immunol. 133: 270-273.

Ono, R., Masuya, M., Nakajima, H., Enomoto, Y., Miyata, E., Nakamura, A., Ishii, S., Suzuki, K., Shibata-Minoshima, F., Katayama, N., Kitamura, T., and Nosaka, T. (2013) Plzf drives MLL-fusion-mediated leukemogenesis specifically in long-term hematopoietic stem cells. Blood 122: 1271-1283.

Wada, R., Muraoka, N., Inagawa, K., Yamakawa, H., Miyamoto, K., Sadahiro, T., Umei, T., Kaneda, R., Suzuki, T., Kamiya, K., Tohyama, S., Yuasa, S., Kokaji, K., Aeba, R., Yozu, R., Yamagishi, H., Kitamura, T., Fukuda, K. and Ieda, M. (2013) Induction of human cardiomyocyte-like cells from fibroblasts by defined factors. Proc. Natl. Acad. Sci. USA. 110: 12667-12672.

Inoue, D., Kitaura, J., Togami, K., Nishimura, K., Enomoto, Y., Uchida, T., Kagiyama, Y., Kawabata, K.C., Nakahara, F., Izawa, K., Oki, T., Maehara, A., Isobe, M., Tsuchiya, A., Harada, Y., Harada, H., Ochiya, T., Aburatani, A., Kimura, H., Thol, F., Heuser, M., Levine, R.L., Abdel-Wahab, O. and Kitamura, T. (2013) Myelodysplastic syndromes are induced by histone methylation-altering ASXL1 mutations. J. Clin. Invest. 123: 4627-4640.

Okochi, N., Yoshimi, A., Sato, T., Ikeda, T., Kumano, K., Taoka, K., Sato, Y., Shinohara, A., Tsuruta, A., Masuda, A., Yokota, H., Yatomi, Y., Takahashi, K., Kitaura, J., Kitamura, T. and Kurokawa, M. (2013) The shortest isoform of C/EBPβ, Liver inhibitory protein (LIP), collaborates with Evi1 to induce AML in a mouse BMT model. Blood 121: 4142-4155.

Nishimura, K., Oki, T., Kitaura, J., Kuninaka, S., Saya, H., Sakaue-Sawano, A., Miyawaki, A. and Kitamura, T. (2013) APCCDH1 targets MgcRacGAP for destruction in the late M phase. PLOS One 8: e63001.

Harada, Y., Inoue, D., Ding, Y., Imagawa, J., Doki, N., Matsui, H., Yahata, T., Matsushita, H., Ando, K., Shashida, G., Iwama, A., Kitamura, T. and Harada, H. (2013) Runx1/AML1 mutant collaborates with Bmi1 overexpression in the development of human and mouse myelodysplastic syndromes. Blood 13: 3434-3446.

Takahashi, M., Izawa, K., Kashiwakura, J., Yamanishi, Y., Enomoto, Y., Kaitani, A., Maehara, A., Isobe, M., Ito, S., Matsukawa, T., Nakahara, F., Oki, T., Kajikawa, M., Ra, C., Okayama, Y., *Kitamura, T. and *Kitaura, J. (2013) Human CD300C delivers an Fc receptor-g-dependent activating signal in mast cells and monocytes and differs from CD300A in ligand recognition. J. Biol. Chem. 288: 7662-6775.

Kitamura, T. (2013) Genetic and epigenetic alterations in hematopoietic malignancies. Int. J. Hematol. 97: 163-164.

Sakane, A., Alamir Mahomoud Abdallah, A., Nakano, K., Honda, K., Kitamura, T., Imoto, I., Matsushita, N. and Sasaki, T. (2013) Junctional Rab13-binding protein (JRAB) requires cell spreading via filamins. Genes Cells 18: 810-822.

Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Dynamics
幹細胞ダイナミクス解析分野

Associate Professor　Beate Heissig, M.D.

准教授　医学博士　　ハイジッヒ，ベアーテ

Proteases perform highly selective and limited cleavage of specific substrates including growth factors and their receptors, cell adhesion molecules, cytokines, apoptotic ligands and angiogenic factors. We demonstrated that protease activation is necessary for myeloid cell recruitment into ischemic tissues and cancer tissues. In colorectal cancers, we identified bone marrow-derived CD11b + Jagged2 + cells as key regulators for the epithelial-to-mesenchymal transition process and showed that they are indicative of metastasis.

Bone marrow-derived CD11b＋Jagged2＋ cells promote epithelial-to-mesenchymal transition and metastasization in colorectal cancer.

Francisco Caiado[1], Tânia Carvalho[1], Isadora Rosa[1], Leonor Remédio[1], Ana Costa[2], João Matos[2], Beate Heissig[3,4], Hideo Yagita[3], Koichi Hattori[4,5], João Pereira da Silva[2], Paulo Fidalgo[2], António Dias Pereira[2], Sérgio Dias[1]: [1]Instituto de Medicina Molecular, Edificio Egas Moniz, Faculdade de Medicina da Universidade de Lisboa, [2]Portuguese Institute of Oncology, IPOLFG, [3]Division of Stem Cell Dynamics, Institute of Medical Sciences, University of Tokyo, [4]Atopy Center, Juntendo University, and [5]Laboratory of Stem Cell Regulation, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Sciences, University of Tokyo

Timely detection of colorectal cancer metastases may permit improvements in their clinical management. Here, we investigated a putative role for bone marrow-derived cells in the induction of epithelial-to-mesenchymal transition (EMT) as a marker for onset of metastasis. In ectopic and orthotopic mouse models of colorectal cancer, bone marrow-derived CD11b(Itgam)(＋)Jagged2 (Jag2)(＋) cells infiltrated primary tumors and surrounded tumor cells that exhibited diminished expression of E-cadherin and increased expression of vimentin, 2 hallmarks of EMT. In vitro coculture experiments showed that the bone marrow-derived CD11b(＋)Jag2(＋) cells induced EMT through a Notch-dependent pathway. Using neutralizing antibodies, we imposed a blockade on CD11b(＋) cells' recruitment to tumors, which decreased the tumor-infiltrating CD11b(＋)Jag2(＋) cell population of interest, decreasing tumor growth, restoring E-cadherin expression, and delaying EMT. In support of these results, we found that peripheral blood levels of CD11b(＋)Jag2(＋) cells in mouse models of colorectal cancer and in a cohort of untreated patients with colorectal cancer were indicative of metastatic disease. In patients with colorectal cancer, the presence of circulating CD11b(＋)Jag2(＋) cells was accompanied by loss of E-cadherin in the corresponding patient tumors. Taken together, our results show that bone marrow-derived CD11b(＋)Jag2(＋) cells, which infiltrate primary colorectal tumors, are sufficient to induce EMT in tumor cells, thereby triggering onset of metastasis. Furthermore, they argue that quantifying circulating CD11b(＋)Jag2(＋) cells in patients may offer an indicator of colorectal cancer progression to metastatic levels of the disease.

Publications

1. Caiado F, Carvalho T, Rosa I, Remédio L, Costa A, Matos J, Heissig B, Yagita H, Hattori K, da Silva JP, Fidalgo P, Pereira AD, Dias S. Bone Marrow-Derived CD11b + Jagged2 + Cells promote epithelial-to-mesenchymal transition and metastasization in colorectal cancer. Cancer Res. 73: 4233-4246, 2013.