Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Therapy
幹細胞治療分野

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Recent great progress in stem cell biology has brought about an increase in the prospects for application of stem cell-based therapy. Especially the discovery of iPSCs, a great step forward in stem-cell research, holds out the promise for 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. Generation of Rejuvenated Antigen-Specific T Cells by Reprogramming to Pluripotency and Redifferentiation

Toshinobu Nishimura[1], Shin Kaneko[1,9], Ai Kawana-Tachikawa[2], Yoko Tajima[1], Haruo Goto[1], Dayong Zhu[2], Kaori Nakayama-Hosoya[2], Shoichi Iriguchi[1], Yasushi Uemura[6], Takafumi Shimizu[1], Naoya Takayama[3,10], Daisuke Yamada[7], Ken Nishimura[8], Manami Ohtaka[8], Nobukazu Watanabe[4], Satoshi Takahashi[5], Aikichi Iwamoto[2], Haruhiko Koseki[7], Mahito Nakanishi[8], Koji Eto[3,10] and Hiromitsu Nakauchi[1]: [1]Division of Stem Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, [2]Division of Infectious Diseases, Advanced Clinical Research Center, [3]Stem Cell Bank, Center for Stem Cell Biology and Regenerative Medicine, [4]Laboratory of Diagnostic Medicine, Center for Stem Cell Biology and Regenerative Medicine, [5]Division of Molecular Therapy, Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [6]Division of Immunology, Aichi Cancer Center Research Institute, Nagoya, Aichi, Japan, [7]Laboratory for Lymphocyte Development, RIKEN Center for Allergy and Immunology, Yokohama, Japan, [8]Research Center for Stem Cell Engineering, National Institute of Advanced Industrial Science and Technology, Ibaraki, Japan, [9]Present address: Department of Fundamental Cell Technology, Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan, [10]Present address: Department of Clinical Application, CiRA, Kyoto University, Kyoto, Japan

Adoptive immunotherapy with functional T cells is potentially an effective therapeutic strategy for combating many types of cancer and viral infection. However, exhaustion of antigen-specific T cells represents a major challenge to this type of approach. In an effort to overcome this problem, we reprogrammed clonally expanded antigen-specific $CD8^+$ T cells from an HIV-1-infected patient to pluripotency. The T cell-derived induced pluripotent stem cells were then redifferentiated into $CD8^+$ T cells that had a high proliferative capacity and elongated telomeres. These "rejuvenated" cells possessed antigen-specific killing activity and exhibited T cell receptor gene-rearrangement patterns identical to those of the original T cell clone from the patient. We also found that this method can be effective for generating specific T cells for other pathology-associated antigens. Thus, this type of approach may have broad applications in the field of adoptive im-

munotherapy.

2. Earliest lineage commitment of hematopoietic stem cell revealed by in vivo five lineage tracing system

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While hematopoiesis, one of the best-characterized cell differentiation systems, has provided important conceptual models for basic processes of cell differentiation, early differentiation pathways of hematopoietic stem cells (HSCs) remain obscure. Consensus holds that 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. However, without an assay system that permits clonal assay of potentials for differentiation into all types of hematopoietic progenitor and lineage cells, definitive experimental evidence for hierarchical relationships among HSCs, MPPs, and lineage-committed progenitor cells is lacking.

The Ly5 antigen, which is only expressed by leukocytes, is commonly used to distinguish between donor, recipient and competitor cells. However it is not expressed by platelets and erythrocytes hence the contribution of these populations are not accounted for when assessing multi-lineage reconstitution.

To address this issue, we generated a transgenic mouse line in which platelets and erythrocytes in addition to neutrophils/monocytes and T and B cells express Kusabira-Orange fluorescent protein. Highly enriched HSCs, $CD150^+CD41^-CD34^{-/low}$c-$Kit^+Sca\text{-}1^+Lin^-$ ($CD150^+CD41^-CD34^-$KSL) cells and $CD150^-CD41^-CD34^-$KSL cells were individually isolated from KuO transgenic mice. These were transplanted into lethally irradiated mice (B6-Ly5.2) along with 2×10^5 competitor bone marrow cells (B6-Ly5.1/Ly5.2). Secondary transplantations were carried out by transplanting 5×10^6 bone marrow cells from the primary recipient mice into another lethally irradiated recipient. The peripheral blood cells in both primary and secondary recipient mice were periodically analyzed.

Unexpectedly, in the phenotypically-defined HSC compartment we could detect not only HSCs but also progenitors of megakaryocytes, of common myeloid cells, and of megakaryocyte-erythroid cells and these progenitors exhibited long-term repopulation activity (rMkPs, rCMPs, and rMEPs respectively). This indicates that loss of self-renewal activity in HSCs is not an essential step for lineage commitment.

These results also suggested that these myeloid committed progenitors (rCMPs, rMEPs, and rMkPs) were close to HSCs in the developmental pathway.

To determine whether HSCs directly give rise to these progenitors, we next performed paired daughter cell assays. After a single $CD150^+CD41^-CD34^-$KSL cells divided once in culture, daughter cells were separated by micromanipulation techniques and individually transplanted into lethally irradiated mice along with 2×10^5 competitor cells. Interestingly, we detected HSC-HSC, HSC-rMkP, and HSC-rCMP pairs in the paired daughter cells, suggesting that MkP and CMP commitment may occur at the level of HSCs through a single division of HSCs.

In conclusion, our results demonstrated that the $CD150^+CD41^-CD34^-$KSL population contains rMkPs and rCMPs, which HSCs can give rise to via one division. Here, we propose a revision of the hierarchical overview of normal hematopoiesis, namely myeloid bypass pathway from HSCs into myeloid lineage committed progenitors (rMkPs and rCMPs) without passing through conventional MPP status.

3. Mesenchymal progenitor cells in mouse fetal liver regulate differentiation and proliferation of hepatoblasts.

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Hepatoblasts are somatic progenitor cells of the fetal liver that possess high proliferative capacity and bi-potency for differentiation into both hepatocytes and cholangiocytes. Although mesenchymal cells are known to be important for liver ontogeny, current understanding of their physiological role in the fetal liver remains obscure. Here, mesenchymal

cell populations in the developing liver were purified and their potential to support proliferation and differentiation of hepatoblasts was examined. Fetal liver cells were fractionated with a flow cytometer using antibodies against cell surface markers. Gene expression of mesenchymal-specific transcripts and morphological characteristics were analyzed. The ability of the mesenchymal cells to support hepatoblast function was analyzed using a co-culture system. $CD45^{-}Ter119^{-}CD71^{-}Dlk1^{mid}PDGFRalpha^{+}$ cells from the mid-fetal stage liver expressed the mesenchymal cell-specific transcription factors H 2.0-like homeobox 1 and LIM homeobox 2 at high levels. Fetal mesenchymal cells make contact with hepatoblasts *in vivo* and possess the potential to differentiate into chondrocytes, osteocytes, and adipocytes under appropriate cell culture conditions, indicating that these cells are possible candidates for mesenchymal stem/progenitor cells. Fetal mesenchymal cells expressed pleiotrophin, hepatocyte growth factor, and midkine 1, which are involved in growth of hepatoblasts. Using the co-culture system with hepatoblasts and fetal mesenchymal cells, these cells were shown to support proliferation and maturation of hepatoblasts through indirect and direct interactions, respectively. These data suggested that $Dlk1^{mid}PDGFRalpha^{+}$ cells in non-hematopoetic fraction derived from the fetal liver exhibit mesenchymal stem/progenitor cell characteristics and form a niche to support the development of hepatoblasts during liver ontogeny.

4. An *in vitro* expansion system for generation of human iPS cell-derived hepatic progenitor-like cells exhibiting a bipotent differentiation potential

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Hepatoblasts, hepatic stem/progenitor cells in liver development, have a high proliferative potential and the ability to differentiate into both hepatocytes and cholangiocytes. In regenerative medicine and drug screening for the treatment of severe liver diseases, human induced pluripotent stem (iPS) cell-derived mature functional hepatocytes are considered to be a potentially good cell source. However, induction of proliferation of these cells is difficult *ex vivo*. To circumvent this problem, we generated hepatic progenitor-like cells from human iPS cells using serial cytokine treatments *in vitro*. Highly proliferative hepatic progenitor-like cells were purified by fluorescence-activated cell sorting using antibodies against CD13 and CD133 that are known cell surface markers of hepatic stem/progenitor cells in fetal and adult mouse livers. When the purified $CD13^{high}CD133^{+}$ cells were cultured at a low density with feeder cells in the presence of suitable growth factors and signaling inhibitors (ALK inhibitor A-83-01 and ROCK inhibitor Y-27632), individual cells gave rise to relatively large colonies. These colonies consisted of two types of cells expressing hepatocytic marker genes (hepatocyte nuclear factor 4alpha and alpha-fetoprotein) and a cholangiocytic marker gene (cytokeratin 7), and continued to proliferate over long periods of time. In a spheroid formation assay, these cells were found to express genes required for mature liver function, such as cytochrome P450 3A4 and 7 A1. When these cells were cultured in a suitable extracellular matrix gel, they eventually formed a cholangiocytic cyst-like structure with epithelial polarity, suggesting that human iPS cell-derived hepatic progenitor-like cells have a bipotent differentiation ability. Collectively these data indicate that this novel procedure using an *in vitro* expansion system is useful for not only liver regeneration but also for the determination of molecular mechanisms that regulate liver development.

5. Development of an all-in-one inducible lentiviral vector for gene specific analysis of reprogramming.

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Fair comparison of reprogramming efficiencies and in vitro differentiation capabilities among induced pluripotent stem cell (iPSC) lines has been hampered by the cellular and genetic heterogeneity of de novo infected somatic cells. In order to address this problem, we constructed a single cassette all-in-one inducible lentiviral vector (Ai-LV) for the expression of three reprogramming factors (Oct3/4, Klf4 and Sox2). To obtain multiple types of somatic cells having the same genetic background, we generated reprogrammable chimeric mice using iPSCs derived from Ai-LV infected somatic cells. Then,

hepatic cells, hematopoietic cells and fibroblasts were isolated at different developmental stages from the chimeric mice, and reprogrammed again to generate 2nd iPSCs. The results revealed that somatic cells, especially fetal hepatoblasts were reprogrammed 1200 times more efficiently than adult hepatocytes with maximum reprogramming efficiency reaching 12.5%. However, we found that forced expression of c-Myc compensated for the reduced reprogramming efficiency in aged somatic cells without affecting cell proliferation. All these findings suggest that the Ai-LV system enables us to generate a panel of iPSC clones derived from various tissues with the same genetic background, and thus provides an invaluable tool for iPSC research.

6. Generation of Rat Gonad-like Structure from Fetal Primordial Germ Cells by Ectopic Xeno Transplantation in Immune deficient Mice

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Primordial germ cells (PGCs) are stem cells of germ cell lineage in genital ridge of fetus. As PGCs give rise to definitive oocytes and spermatozoa that contribute to new life in the next generation, they have been actively studied. However because of their rare population, detailed mechanisms of important events (epigenetic modification, mobilization, proliferation and meiosis) are not clear yet. On the other hand, 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 recently. To apply these new technologies to zootechnics and human treatment, it is necessary to establish ectopic xeno transplantation of PGCs or artificial PGCs to generate definitive oocytes or spermatozoa in terms of ethics and safety issue.

Publications

1. Toshinobu Nishimura, Shin Kaneko, Ai Kawana-Tachikawa, Yoko Tajima, Haruo Goto, Dayong Zhu, Kaori Nakayama, Shoichi Iriguchi, Yasushi Uemura, Takafumi Shimizu, Naoya Takayama, Daisuke Yamada, Ken Nishimura, Manami Ohtaka, Nobukazu Watanabe, Satoshi Takahashi, Aikichi Iwamoto, Haruhiko Koseki, Mahito Nakanishi, Koji Eto and Hiromitsu Nakauchi. Generation of rejuvenated antigen-specific T cells by pluripotency reprogramming and dedifferentiation. *Cell Stem Cell.* (accepted for publication)
2. 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.
3. 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.
4. 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.
5. 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.
6. 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.
7. Hirabayashi M, Tamura C, Sanbo M, Kato-Itoh M, Kobayashi T, Nakauchi H, Hochi S. A retrospective analysis of germline competence in rat embryonic stem cell lines. *Transgenic Res.* 2012 Aug 9. [Epub ahead of print]
8. Yamaguchi T, Hamanaka S, Kamiya A, Okabe M, Kawarai M, Wakiyama Y, Umino A, Hayama T, Sato H, Lee YS, Kato-Itoh M, Masaki H, Kobayashi T, Yamazaki S, Nakauchi H. Development of an All-in-One Inducible Lentiviral Vector for Gene Specific Analysis of Reprogramming. *PLoS One.* 2012; 7(7): e41007. Epub 2012 Jul 18.
9. Nakamura S, Oshima M, Yuan J, Saraya A, Miyagi S, Konuma T, Yamazaki S, Osawa M, Nakauchi H, Koseki H, Iwama A. Bmi1 confers resistance to oxidative stress on hematopoietic stem cells. *PLoS One*. 2012; 7(5): e36209. Epub 2012 May 11.
10. Kumano K, Arai S, Hosoi M, Taoka K, Takayama N, Otsu M, Nagae G, Ueda K, Nakazaki K, Kamikubo Y, Eto K, Aburatani H, Nakauchi H, Kurokawa M. Generation of in-

duced pluripotent stem cells from primary chronic myelogenous leukemia patient samples. *Blood*. 2012 Jun 28; 119(26): 6234-42. Epub 2012 May 16.

11. Tashiro Y, Nishida C, Sato-Kusubata K, Ohki-Koizumi M, Ishihara M, Sato A, Gritli I, Komiyama H, Sato Y, Dan T, Miyata T, Okumura K, Tomiki Y, Sakamoto K, Nakauchi H, Heissig B, Hattori K. Inhibition of PAI-1 induces neutrophil-driven neoangiogenesis and promotes tissue regeneration via production of angiocrine factors in mice. *Blood.* 2012 Jun 28; 119(26): 6382-93. Epub 2012 May 9.
12. Sekine K, Takebe T, Suzuki Y, Kamiya A, Nakauchi H, Taniguchi H.Highly efficient generation of definitive endoderm lineage from human induced pluripotent stem cells. *Transplant Proc*. 2012 May; 44(4): 1127-9.
13. Kobayashi T, Kato-Itoh M, Yamaguchi T, Tamura C, Sanbo M, Hirabayashi M, Nakauchi H. Identification of Rat Rosa26 Locus Enables Generation of Knock-in Rat Lines Ubiquitously Expressing tdTomato. *Stem Cells Dev*. 2012 Jun 11. [Epub ahead of print]
14. Nishida C, Kusubata K, Tashiro Y, Gritli I, Sato A, Ohki-Koizumi M, Morita Y, Nagano M, Sakamoto T, Koshikawa N, Kuchimaru T, Kizaka-Kondoh S, Seiki M, Nakauchi H, Heissig B, Hattori K. MT1-MMP plays a critical role in hematopoiesis by regulating HIF-mediated chemokine/cytokine gene transcription within niche cells. *Blood.* 2012 Jun 7; 119(23): 5405-16. Epub 2012 Apr 27.
15. Usui J, Kobayashi T, Yamaguchi T, Knisely AS, Nishinakamura R, Nakauchi H. Generation of Kidney from Pluripotent Stem Cells via Blastocyst Complementation. *Am J Pathol.* 2012 Jun; 180(6): 2417-26. Epub 2012 Apr 14.
16. Hirabayashi M, Tamura C, Sanbo M, Goto T, Kato-Itoh M, Kobayashi T, Nakauchi H, Hochi S. Ability of tetraploid rat blastocysts to support fetal development after complementation with embryonic stem cells. *Mol Reprod Dev*. 2012 Jun; 79(6): 402-12. doi: 10.1002/mrd.22043. Epub 2012 May 4.
17. Ghosn EE, Yamamoto R, Hamanaka S, Yang Y, Herzenberg LA, Nakauchi H, Herzenberg LA. Distinct B-cell lineage commitment distinguishes adult bone marrow hematopoietic stem cells. *Proc Natl Acad Sci U S A*. 2012 Apr 3; 109 (14): 5394-8. Epub 2012 Mar 19.
18. Wang J, Sun Q, Morita Y, Jiang H, Gros A, Lechel A, Hildner K, Guachalla LM, Gompf A, Hartmann D, Schambach A, Wuestefeld T, Dauch D, Schrezenmeier H, Hofmann WK, Nakauchi H, Ju Z, Kestler HA, Zender L, Rudolph KL. A Differentiation Checkpoint Limits Hematopoietic Stem Cell Self-Renewal in Response to DNA Damage. *Cell*. 2012. 148(5): 1001-1014.
19. Oguro H, Yuan J, Tanaka S, Miyagi S, Mochizuki-Kashio M, Ichikawa H, Yamazaki S, Koseki H, Nakauchi H, Iwama A. Lethal myelofibrosis induced by Bmi1-deficient hematopoietic cells unveils a tumor suppressor function of the polycomb group genes. *J Exp Med*. 209: 445-454, 2012.
20. Ito H, Kamiya A, Ito K, Yanagida A, Okada K, Nakauchi H. In vitro expansion and functional recovery of mature hepatocytes from mouse adult liver. *Liver Int*. 2012 Jan 4. doi: 10.1111/j.1478-3231.2011.02741.x. [Epub ahead of print]
21. Umeyama K, Saito H, Kurome M, Matsunari H, Watanabe M, Nakauchi H, Nagashima H. Characterization of the ICSI-mediated gene transfer method in the production of transgenic pigs. *Mol Reprod Dev*. 2012 Mar; 79(3): 218-28.

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 analyzing system (HAS)-Flow method] to monitor disease condition. In addition, we are analyzing peripheral blood from HTLV-1 carriers to find a predictable phenotypic change of immune 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 method and HAS-Flow method. In addition, we are analyzing ATL cells and normal regulatory T cells with their expression levels of CCR4 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

Mikami Y, Suzuki S, Ishii Y, Watanabe N, Takahashi T, Isokawa K, Honda MJ. The p75 neurotrophin receptor regulates MC3T3-E1 osteoblastic differentiation. Differentiation. 84(5): 392-399, 2012.

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.

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Stem cells represent a precious cell source usable in various types of regenerative medicine. Hematopoietic stem cells provide a good example of the potential of stem cells as seen in successful cases in both the hematopoietic transplantation and gene therapy. Pluripotent stem cells are the emerging cell sources that may be utilized either for the basic research or to cure the diseases. Our aim is to establish safe and efficacious treatment for the patients suffering from diseases with no curative treatment available.

1. Pleiotropic nature of hematopoietic stem cell responses to an inflammatory niche environment

Makoto Otsu, Huang-Ting Lin, Jun Ooehara, Chen-Yi Lai, Mozhgan Kharaj, Takashi Ishida, Naoaki Mizuno, Hiromitsu Nakauchi

Hematopoietic stem cells (HSCs) have been utilized in transplantation settings to treat a range of intractable disorders. These include hematopoietic malignancies and primary immunodeficiencies, whereby successful outcomes are defined as sustained reconstitution of functional hematopoiesis by transplanted HSCs. Central to this success is the bone marrow "niche" and the microenvironment regulated by its constituent cells. It is an anatomical location that transplanted HSCs ultimately reside in hence their behavior can be highly susceptible to environmental changes. Therefore, niche influences may strongly impact upon HSC functions and the success of clinical outcomes. As such, worth serious consideration are incidences of negative responses in the form of "inflammation" following preconditioning treatment and/or allogeneic reactions in relation to transplantation. In such cases, inflammatory cytokines are secreted into the microenvironment, which could exert detrimental effects on HSCs. From initial screening experiments, it was found that interleukin (IL)-1 and tumor necrosis factor (TNF)-α were rapidly produced within murine BM in response to total body irradiation (TBI). The detrimental effects of these cytokines on donor HSCs were substantiated by demonstrating loss of function in both in vivo and ex vivo studies. These results highlighted the pleiotropic nature of HSC functional responses to these cytokines. For example, the pleiotropism became greater with factors such as age (donor and recipient) and the oxygen tension of culture.

In this presentation, two factors "age" and "inflammation" shall be discussed in depth particularly in the context of transplantation. These are important considerations that may be critical in developing new strategies to optimize clinical outcomes for transplanted patients.

2. Stage-specific roles for Cxcr4 signaling in murine hematopoietic stem/progenitor cells in the process of bone marrow repopulation

Chen-Yi Lai, Satoshi Yamazaki, Sachie Suzuki, Shigeru Kakuta, Yoichiro Iwakura, Makoto Otsu, Hiromitsu Nakauchi

Hematopoietic cell transplantation has proven beneficial for various intractable diseases, but how hematopoietic stem/progenitor cells (HSPCs) home

to the bone marrow (BM) microenvironment and initiate hematopoietic reconstitution remains unclear. The use of newly elucidated molecular determinants for HSPC engraftment should benefit patients. Here we report that modification of Cxcr4 signaling in murine HSPCs does not significantly affect initial homing/lodging events, but leads to alteration in subsequent BM repopulation kinetics. By using C-terminal truncated Cxcr4 as a gain-of-function effector, we demonstrated that signal augmentation likely led to favorable in vivo retention/expansion of primitive cell populations, possibly in part through alterations in integrin expression profile. Unexpectedly however, sustained signal enhancement even with wild-type Cxcr4 overexpression resulted in impaired peripheral blood (PB) reconstitution, most likely by preventing release of mature leukocytes from the marrow environment. We thus conclude that timely regulation of Cxcr4/CXCR4 signaling is key in providing HSPCs with enhanced repopulation potential following transplantation, whilst preserving the ability to release HSPC progeny into PB for improved transplantation outcomes.

3. Protection of hematopoietic stem cells from stress-induced functional impairment by very low-dose interleukin-1 stimulation

Jun Ooehara, Chen-Yi Lai, Huang-Ting Lin, Satoshi Yamazaki, Makoto Otsu, Hiromitsu Nakauchi

Background: Hematopoietic stem cell (HSC) gene therapy is a treatment option that potentially provides life-long immune reconstitution for patients with primary immunodeficiency diseases. Virus-mediated gene transfer is the measure currently used to confer functionality on patients' blood CD34+ cells (HSPCs). Because this procedure typically requires ex vivo stimulation of HSPCs for 4-5 days, it is essential to optimize the cocktail of cytokines so that the transduced cells retain the best reconstitution capability. Here we sought to revisit the use of interleukin-1 (IL-1), which is the well-known cytokine, but its positive effects on highly purified HSCs remain doubtful.

Results: By analyzing IL-1 receptor knockout mice, we found impaired reconstitution ability in their HSCs, suggesting the positive effect of IL-1 at the stem cell level. We then tested in vitro effects of IL-1 in a 7-day serum-free culture and observed its dose-dependent biphasic effects on HSCs: while it forced HSC differentiation at the concentrations higher than ~10 ng/ml, it clearly enhanced proliferative response of the primitive cells at low concentrations around 0.05 ng/ml. Consistent with this, addition of IL-1 at a limited dose improved in vivo reconstitution capability of cultured HSCs in both primary and secondary recipients, and the positive effects became more evident with certain stress conditions including hypoxia and aging.

Conclusions: This study provides the possible revival of one of the oldest cytokine as a positive amplifier/protector of HSCs, potentially culminating in the improvement in long-term hematopoiesis that is relevant to clinical gene therapy trials.

In vitro studies: HSCs overexpressing wt- or ΔC-CXCR4 exhibited enhanced chemotaxis and proliferation in response to SDF1, confirming the gain-of-function effects of these modifications. CA forming ability was greater in HSCs overexpressing ΔC-CXCR4 than control counterparts and absent in CXCR4-KO HSCs, suggesting the critical role of CXCR4-signaling in HSC proliferation in the presence of stromal support.

In vivo studies: 1) the homing/lodging phase. Unexpectedly, we did not find significant alteration in the numbers of early progenies detectable in recipient BM 3 days after transplantation of HSCs receiving either loss- or gain-of-function modification to CXCR4, indicating that this signaling is indispensable in HSC homing. 2) the early repopulation phase. Impairment of hematopoietic repopulation in BM became evident for CXCR4-KO HSCs through 2-3 wks. On the other hand, HSCs overexpressing CXCR4, more remarkably of ΔC-mutation, showed enhanced BM repopulation kinetics at ~3 wks post transplantation, suggesting the importance of CXCR4 signaling in HSC amplification at this post-transplantation phase. 3) long-term hematopoiesis. CXCR4-KO-HSCs showed poor hematopoietic reconstitution potentials, consistent with previous observations. Interestingly, impaired peripheral repopulation was also observed with HSCs overexpressing wt- or ΔC-CXCR4. Further characterization revealed that the recipients of CXCR4-overexpressing HSCs did retain their progenies, which showed multilineage differentiation, but exhibited impaired release of mature leukocytes from the BM to the peripheral blood. Most importantly, however, test-cell chimerism in the long-term HSC fraction was significantly higher in the mice receiving HSCs overexpressing CXCR4, especially of ΔC-type, than those transplanted with control HSCs, indicating that the augmentation of CXCR4 signaling enhanced competitive repopulation ability of HSCs. These modified HSCs demonstrated repopulation abilities also in secondary recipients.

We demonstrated that CXCR4 signaling is indispensible for HSC homing and that continuous overexpression of CXCR4 cannot benefit the peripheral reconstitution in contrary to the expectation. More importantly, our studies showed that augmentation of CXCR4 signaling leads to HSC expansion in vivo. We thus conclude that CXCR4 signaling has a role in HSC self-renewal and that its regulation may find the approach that will improve HSC

transplantation outcomes.

Publications

1. Nishimura S, Manabe I, Nagasaki M, Kakuta S, Iwakura Y, Takayama N, Ooehara J, Otsu M, Kamiya A, Petrich BG, Urano T, Kadono T, Sato S, Aiba A, Yamashita H, Sugiura S, Kadowaki T, Nakauchi H, Eto K, Nagai R. In vivo imaging visualizes discoid platelet aggregations without endothelium disruption and implicates contribution of inflammatory cytokine and integrin signaling. Blood. 2012; 119(8): e45-56.
2. Nakanishi M, Otsu M. Development of Sendai Virus Vectors and Their Potential Applications in Gene Therapy and Regenerative Medicine. Current gene therapy. 2012. Epub 2012/08/28.
3. Kumano K, Arai S, Hosoi M, Taoka K, Takayama N, Otsu M, Nagae G, Ueda K, Nakazaki K, Kamikubo Y, Eto K, Aburatani H, Nakauchi H, Kurokawa M. Generation of induced pluripotent stem cells from primary chronic myelogenous leukemia patient samples. Blood. 2012; 119(26): 6234-42.

Center for Stem Cell Biology and Resenerative Medicine

Laboratory of Stem Cell Regulation
幹細胞制御領域

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The major goal of our laboratory is to identify novel therapeutic targets for diseases like cancer or inflammatory diseases. We study the role of inflammatory cells and adult stem cells in regenerative and cancer biology. Stem cells from adult tissues have the unique capacity to repair damaged tissue, a process controlled in part by the microenvironment. We currently focused our scientific efforts on understanding the mechanism how inflammatory cells control blood vessel formation thereby promoting cancer growth or tissue regeneration (1-3), and which factors can alter the generation of blood cells (4,5).

1. Inhibition of PAI-1 induces neutrophil-driven neoangiogenesis and promotes tissue regeneration via production of angiocrine factors in mice

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Plasminogen activator inhibitor-1 (PAI-1), an endogenous inhibitor of a major fibrinolytic factor, tissue-type plasminogen activator, can both promote and inhibit angiogenesis. However, the physiologic role and the precise mechanisms underlying the angiogenic effects of PAI-1 remain unclear. In the present study, we report that pharmacologic inhibition of PAI-1 promoted angiogenesis and prevented tissue necrosis in a mouse model of hind-limb ischemia. Improved tissue regeneration was due to an expansion of circulating and tissue-resident granulocyte-1 marker (Gr-1(+)) neutrophils and to increased release of the angiogenic factor VEGF-A, the hematopoietic growth factor kit ligand, and G-CSF. Immunohistochemical analysis indicated increased amounts of fibroblast growth factor-2 (FGF-2) in ischemic gastrocnemius muscle tissues of PAI-1 inhibitor-treated animals. Ab neutralization and genetic knockout studies indicated that both the improved tissue regeneration and the increase in circulating and ischemic tissue-resident Gr-1(+) neutrophils depended on the activation of tissue-type plasminogen activator and matrix metalloproteinase-9 and on VEGF-A and FGF-2. These results suggest that pharmacologic PAI-1 inhibition activates the proangiogenic FGF-2 and VEGF-A pathways, which orchestrates neutrophil-driven angiogenesis and induces cell-driven revascularization and is therefore a potential therapy for ischemic diseases.

2. New functions of the fibrinolytic system in bone marrow cell-derived angiogenesis.

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Angiogenesis is a process by which new blood vessels form from preexisting vasculature. This process includes differentiation of angioblasts into endothelial cells with the help of secreted angiogenic factors released from cells such as bone marrow (BM)-derived cells. The fibrinolytic factor plasmin, which is a serine protease, has been shown to promote endothelial cell migration either directly, by degrading matrix proteins such as fibrin, or indirectly, by converting matrix-bound angiogenic growth factors into a soluble form. Plasmin can also activate other pericellular proteases such as matrix metalloproteinases (MMPs). Recent studies indicate that plasmin can additionally alter cellular adhesion and migration. We showed that factors of the fibrinolytic pathway can recruit BM-derived hematopoietic cells into ischemic/hypoxic tissues by altering the activation status of MMPs. These BM-derived cells can function as accessory cells that promote angiogenesis by releasing angiogenic signals. This review will discuss recent data regarding the role of the fibrinolytic system in controlling myeloid cell-driven angiogenesis. We propose that plasmin/plasminogen may be a potential target not only for development of effective angiogenic therapeutic strategies for the treatment of cancer, but also for development of strategies to promote ischemic tissue regeneration.

3. Plasmin inhibitor reduces lymphoid tumor growth by suppressing matrixmetallproteinase-9 dependent CD11b+/F4/80+ myeloid cell recruitment.

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Activation of the fibrinolytic system during lymphoma progression is a well-documented clinical phenomenon. But the mechanism by which the fibrinolytic system can modulate lymphoma progression has been elusive. The main fibrinolytic enzyme, plasminogen (Plg)/plasmin (Plm), can activate matrix metalloproteinases (MMPs), like MMP-9, which has been linked to various malignancies. Here we provide the evidence that blockade of Plg reduces lymphoma growth by inhibiting MMP-9-dependent recruitment of $CD11b^+F4/80^+$ myeloid cells locally within the lymphoma tissue. Genetic plasminogen deficiency and drug-mediated Plm blockade delayed lymphoma growth and diminished MMP-9 dependent $CD11b^+F4/80^+$ myeloid cell infiltration into lymphoma tissues. A neutralizing antibody against CD11b inhibited lymphoma growth *in vivo*, which indicates that $CD11b^+$ myeloid cells play a role in lymphoma growth. Plg deficiency in lymphoma-bearing mice resulted in reduced plasma levels of the growth factors vascular endothelial growth-A and Kit ligand, both of which are known to enhance myeloid cell proliferation. Collectively, the data presented in this study demonstrate a previously undescribed role of Plm in lymphoproliferative disorders and provide strong evidence that specific blockade of Plg represents a promising approach for the regulation of lymphoma growth.

4. MT1-MMP plays a critical role in hematopoiesis by regulating HIF-mediated chemokine/cytokine gene transcription within niche cells.

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HSC fate decisions are regulated by cell-intrinsic and cell-extrinsic cues. The latter cues are derived from the BM niche. Membrane-type 1 matrix metalloproteinase (MT1-MMP), which is best known for its proteolytic role in pericellular matrix remodeling, is highly expressed in HSCs and stromal/niche cells. We found that, in MT1-MMP(−/−) mice, in addition to a stem cell defect, the transcription and release of kit ligand (KitL), stromal cell-derived factor-1 (SDF-1/CXCL12), erythropoietin (Epo), and IL-

7 was impaired, resulting in a trilineage hematopoietic differentiation block, while addition of exogenous KitL and SDF-1 restored hematopoiesis. Further mechanistic studies revealed that MT1-MMP activates the hypoxia-inducible factor-1 (HIF-1) pathway via factor inhibiting HIF-1 (FIH-1) within niche cells, thereby inducing the transcription of HIF-responsive genes, which induce terminal hematopoietic differentiation. Thus, MT1-MMP in niche cells regulates postnatal hematopoiesis, by modulating hematopoietic HIF-dependent niche factors that are critical for terminal differentiation and migration.

5. Plasminogen deficiency attenuates post-natal erythropoiesis in male C57BL/6 mice through decreased activity of the LH-testosterone axis

Yurai Okaji[1], Yoshihiko Tashiro, Ismael Gritli, Chiemi Nishida, Aki Sato, Yoko Ueno[1], Sandra Del Canto Gonzalez[1], Makiko Ohki-Koizumi, Haruyo Akiyama, Hiromitsu Nakauchi[2], Koichi Hattori[3], Beate Heissig[1,3]: [1]Frontier Research Initiative, [2]Division of Stem cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Sciences, University of Tokyo, [3]Atopy Center, Juntendo University

Novel roles for the serine protease plasmin have been recently implicated in physiological and pathological processes. However, whether plasmin is involved in erythropoiesis, is not known. In the present study, we studied the consequences of plasminogen deficiency on erythropoiesis in plasminogen deficient (Plg KO) mice. Erythroid differentiation was attenuated in male Plg KO mice and resulted in erythroblastic accumulation within the spleen and bone marrow, with increased apoptosis in the former, erythrocytosis and splenomegaly, whereas similar erythropoietic defect was less prominent in female Plg KO mice. In addition, erythrocyte lifespan was shorter in both male and female Plg KO mice. Erythropoietin levels were compensatory increased in both male and female Plg KO mice, and resulted in a higher frequency of BFU-E within the spleen and bone marrow. Surprisingly, we found that male Plg KO mice but not their female counterparts exhibited normochromic normocytic anemia. The observed gender-linked erythropoietic defect was attributed to decreased serum testosterone levels in Plg KO mice, as a consequence of impaired secretion of the pituitary luteinizing hormone (LH) under steady state condition. Surgical castration, causing testosterone deficiency and stimulating LH release, attenuated erythroid differentiation and induced anemia in WT animals, but did not further decrease the hematocrit levels in Plg KO mice. In addition, complementation of LH using human choriogonadotropin, which increases testosterone production, improved the erythropoietic defect and anemia in Plg KO mice. The present results identify a novel role for plasmin in the hormonal regulation of post-natal erythropoiesis by the LH-testosterone axis.

Publications

〈Koichi Hattori Group〉

1. Yoshihiko Tashiro, Chiemi Nishida, Kaori Sato-Kusubata, Makiko Ohki-Koizumi, Makoto Ishihara, Aki Sato, Ismael Gritli, Hiromitsu Komiyama, Yayoi Sato, Yuichi Tomiki, Hirokazu Sakamoto, Takashi Dan, Toshio Miyata, Ko Okumura, Hiromitsu Nakauchi, Beate Heissig, and Koichi Hattori: Inhibition of PAI-1 induces neutrophil-driven neoangiogenesis and promotes tissue regeneration via production of angiocrine factors in mice. Blood. l19(26) 6382-6393. 2012
2. Beate Heissig, Makiko Ohki-Koizumi, Yoshihiko Tashiro, Ismael Gritli, Kaori Sato-Kusubata and Koichi Hattori. New functions of the fibrinolytic system in bone marrow cell-derived angiogenesis. International Journal of Hematology 95(2). 131-137. 2012
3. Ishihara M, Nishida C, Tashiro Y, Gritli I, Rosenkvist J, Koizumi M, Yamamoto R, Yagita H, Okumura K, Nishikori M, Wanaka K, Tsuda Y, Okada Y, Nakauchi H, Heissig B, Hattori K:, Plasmin inhibitor reduces T-cell lymphoid tumor growth by suppressing matrix metalloproteinase-9-dependent $CD11b^{+}/F4/80^{+}$ myeloid cell recruitment. Leukemia, 26. 332-339. 2012
4. Chiemi Nishida, Kaori Kusubata, Yoshihiko Tashiro, Ismael Gritli, Aki Sato, Makiko Ohki-Koizumi, Yohei Morita, Makoto Nagano, Takeharu Sakamoto, Naohiko Koshikawa, Takahiro Kuchimaru, Shinae Kizaka-Kondoh, Motoharu Seiki, Hiromitsu Nakauchi, Beate Heissig, and Koichi Hattori. MT1-MMP plays a critical role in hematopoiesis by regulating HIF-mediated chemo-/cytokine gene transcription within niche cells. Blood. 119(23) 5405-5416. 2012
5. Okaji Y, Tashiro Y, Gritli I, Nishida C, Sato A, Ueno Y, Del Canto Gonzalez S, Ohki-Koizumi M, Akiyama H, Nakauchi H, Heissig B, Hattori K: Plasminogen deficiency attenuates post-natal erythropoiesis in male C57BL/6 mice through decreased activity of the LH-testosterone axis. Exp Hematol, Vol 40, 143-154, 2012
6. 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，2013 印刷中

7. 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，56–69，2013
8. 服部浩一，島津浩：がん微小環境形成に関与する骨髄由来細胞と治療戦略，細胞，ニューサイエンス社，Vol 44.（11），2012
9. 服部浩一，佐藤亜紀：血液線維素溶解系因子による造血系細胞の動態制御機構，臨床血液，日本血液学会，Vol 53. 680–685，2012
10. 服部浩一，佐藤亜紀：造血幹細胞動態と血管新生制御機構，診断と治療社，血管再生治療，38–45，2012

Center for Stem Cell Biology and Regenerative Medicine

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

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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 hematopoetic progenitors from human pluripotent stem cells

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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 originates from mirine aorta-gonad-mesoneephros (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 ES or iPS cell colonies. The 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. Generation of functional erythrocytes from human ES or iPS cell-derived definitive hematopoiesis

Feng Ma, Yasuhiro Ebihara[1], Shinji Mochizuki, Shohei Yamamoto[1], Sachiyo Hanada, Sahoko Matsuzaka, Yuji Zaike[2], Hiromitsu Nakauchi[3],

Kohichiro Tsuji

A critical issue for utilization of hESC or hiPSC in possible clinical use is whether they can derive terminally mature progenies with the normal function. To solve this, we examined hESC or hiPSC-derived erythroid cells in coculture with mFLSC or AGM cells. By the coculture, large quantity of hESC or hiPSC-derived erythroid progenitors allowed us to analyze the development of erythropoiesis at a clone level and to investigate their function as oxygen carrier. The results showed that the globin expression in the erythroid cells in individual clones changed in a time-dependent manner. In particular, embryonic ε globin positive erythrocytes decreased, while adult-type β globin positive cells increased to almost 100% in all single clones we examined, indicating they had already been fated to definitive hematopoiesis. Enucleated erythrocytes also appeared in the clonal erythroid progenies. A comparison analysis showed that hESC-derived erythroid cells took a similar pathway in differentiation to human cord blood $CD34^+$ progenitor-derived erythrocytes when traced by glycophorin A, CD71 and CD81. Furthermore, these hESC-derived erythroid cells could function as oxygen carrier, and had a sufficient glucose-6-phosphate dehydrogenase activity. The present study provided an experimental model to explore early development of human erythropoiesis, hemoglobin switching, erythroid pathogenesis, and to discover drugs for hereditary diseases in erythrocyte development. The quantitative production and their functional maturation indicate that hPSC-derived erythrocytes can be a novel potential source for therapeutic transfusion.

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

Yasuhiro Ebihara[1], Feng Ma, Shinji Mochizuki, Shohei Yamamoto[1], Sachiyo Hanada, Sahoko Matsuzaka, Yuji Zaike[2], Hiromitsu Nakauchi[3], Kohichiro Tsuji

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 SEA-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.

4. 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, Shohei Yamamoto[1], Sachiyo Hanada, Sahoko Matsuzaka, Hiromitsu Nakauchi[3], Kohichiro Tsuji

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 3weeks, 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 ob-

tained 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.

5. Generation of mature eosinophils from human pluripotent stem cells

Feng Ma, Yang Wenyu, Yanzheng Gu, Yasuhiro Ebihara[1], Shinji Mochizuki, Shohei Yamamoto[1], Sachiyo Hanada, Sahoko Matsuzaka, Hiromitsu Nakauchi[3], Kohichiro Tsuji

Eosinophils are multifunctional leukocytes implicated in the pathogenesis of numerous inflammatory processes. As the major effectors, eosinophils function in a variety of biological responses, allergic diseases and helminth infections. It is generally accepted human eosinophils develop through a pathway initially sharing common feature with basophils. However, there lacks a clear chart for early development of human eosinophils, such as during embryonic or fetal stages. We established an efficient method for producing eosinophils from hESC and hiPSC. By a two-step induction, we first generated multipotential HPC by co-culturing hPSC with AGMS-3 cells for 2 weeks. Then, total co-culture cells were transferred into suspension culture favoring eosinophil development with addition of IL-3 and other factors (stem cell factor, interleukin-6, thrombopoietin, Flt-3 ligand) . The maturation of hPSC-derived eosinophils was shown in a time-dependent manner, first co-expressing eosinophil-and basophil-specific markers [eosinophil peroxidase (EPO), and 2D7, respectively], then the portion of eosinophil markers gradually increased while that of basophil markers decreased, typically mimicking the development of eosinophils from human adult hematopoietic progenitors. By flowcytometric analysis, an eosinophil-specific surface marker, Siglec-8, was also expressed on these hESC/iPSC-derived eosinophils in a time-dependent manner, paralleling to those with EPO. The expression of eosinophil-specific granule cationic proteins (EPO, MBP, ECP, EDN) and IL-5 receptor mRNA was also detected by RT-PCR. Furthermore, transmission electron microscopy (TEM) observation confirmed the eosinophil property. Eosinophils derived from hiPSCs hold similar characteristics as those from hESCs. Our study provides an experimental model for exploring early genesis of eosinophils, especially in uncovering the mechanisms controlling the development of the initial innate immune system of human being in normal and diseased individuals.

6. Hematopoiesis of human induced pluripotent stem cells derived from patients with Down syndrome

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Trisomy 21, genetic hallmark of Down syndrome, is the most frequent human chromosomal abnormality. Infants and children with Down Syndrome (DS) are known to have some hematological disorders with an increased risk of developing leukemia. Ten to 20% of newborn with DS are diagnosed as neonatal preleukemic status, transient myeloproliferative disorder (TMD), and approximately 30% of TMD patients are predisposed to acute megakaryoblastic leukemia (AMKL). Recently, acquired mutations in the N-terminal activation domain of the *GATA1* gene, leading to expression of a shorter GATA1 isoform (GATA1s), have been reported in AMKL and TMD, but neither patients nor mice with germline mutations leading to expression of GATA1s developed AMKL and TMD in the absent of trisomy 21. These findings suggested that trisomy 21 itself directly contributes to the development of AMKL and TMD. However, the role of trisomy 21 in hematopoiesis, particularly in the human fetus remains poorly understood. To better understand the effects of trisomy 21 on hematopoiesis in embryonic stage and leukemogenesis, we employed hiPSC derived from patients with DS (DS-hiPSC). Six DS-hiPSC and 5 hiPSC lines (control) from healthy donors were all created from skin fibroblasts and reprogrammed by the defined 3 or 4 reprogramming factors (OCT3/4, KLF4, and SOX2, or c-MYC in addition to the 3 factors, respectively). We generated blood cells from DS-hiPSC and controls with coculture system using AGMS-3 cells. The cells from hiPSC were harvested at day 11 or 12 of coculture and analyzed the presence of hematopoietic markers and the potentials of hematopoietic colony formation. In the experiments using hiPSC reprogrammed by 3 factors, human CD34 expression in harvested cells from DS-hiPSC or controls were detected 10.06 ± 4.35% and 3.04%, respectively. We next examined the hematopoietic colony formation. Both myeloid and erythroid colonies were detected. Number of colonies formed from DS-hiPSC was 43.7 ± 11.1 to 74.3 ± 11.2 per an iPS cell colony, which was approximately 2 to 3.5 folds the number of control. Similar results were

obtained in the experiments using hiPSC reprogrammed by 4 factors. These results indicated that hiPSC derived from patients with DS could differentiate into multiple hematopoietic cell lineages and the differentiation into hematopoietic lineage was promoted in DS patients.

7. Wnt3a stimulates maturation of impaired neutrophils developed from severe congenital neutropenia patient-derived pluripotent stem cells

Takafumi Hiramoto, Yasuhiro Ebihara[1], Mochizuki, Shohei Yamamoto[1], Masao Nagasaki[6], Yoichi Furukawa[4], Hiromitsu Nakauchi[3], Masao Kobayashi[7], Kohichiro Tsuji: [6]Functional Genomics, Human Genome Center, [7]Department of Pediatrics, Hiroshima University Graduate School of Biomedical Sciences

The derivation of induced pluripotent stem cells (iPS cells) from individuals of genetic disorders offers new opportunities for basic research into these diseases and the development of therapeutic compounds. Severe congenital neutropenia (SCN) is a serious disorder characterized by severe neutropenia at birth. SCN is associated with heterozygous mutations in the neutrophil elastase (ELANE) gene, but the mechanisms that disrupt neutrophil development have not yet been clarified because of the current lack of an appropriate disease model. Here, we generated iPS cells from an individual with SCN (SCN-iPS cells). Granulopoiesis from SCN-iPS cells revealed neutrophil maturation arrest and little sensitivity to granulocyte-colony stimulating factor, reflecting a disease status of SCN. Molecular analysis of the granulopoiesis from the SCN-iPS cells versus control iPS cells showed reduced expression of genes related to the Wnt3a/β-catenin pathway, (e.g., lymphoid enhancer-binding factor (LEF)-1), whereas Wnt3a administration induced elevation LEF-1 expression and the maturation of SCN-iPS cell-derived neutrophils. These results indicate that SCN-iPS cells provide a useful disease model for SCN, and the activation of the Wnt3a/β-catenin pathway may offer a novel therapy for SCN with ELANE mutation.

8. Generation of disease-specific human iPS cells

Shohei Yamamoto[1], Mai Nanya, Yasuhiro Ebihara[1], Shinji Mochizuki, Sachiyo Hanada, Sahoko Matsuzaka, Hiromitsu Nakauchi[3], Kohichiro Tsuji

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 patients (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.

9. The effect of SR1 on hematopoietic cells derived from human iPS cells

Mai Nanya, Yasuhiro Ebihara[1], Shohei Yamamoto[1], Shinji Mochizuki, Sachiyo Hanada, Sahoko Matsuzaka, Hiromitsu Nakauchi[3], Kohichiro Tsuji

Recently StemRegenin 1 (SR1), an aryl hydrocarbon receptor (AhR) antagonist was found to expand human CD34+ cells, which indicated that SR1 acted with hematopoietic stem cells (HSCs). To examine the effect of SR1 on hematopoietic cells derived from human iPS cells, we added SR1 when human iPS cells were cocultured with AGM-3 cells. CD34+ cells generated in coculture system at day 14 were expanded two-fold more than control. Hematopoietic cell in suspension culture induced from CD34+ cells expanded more than control at five-

fold increase. The cultured cells from iPS cells in condition with SR1 formed three-fold more hematopoietic colonies than control. These results indicated that SR1 has capability to increase CD34+ cells including hematopoietic progenitor cells from iPS cells.

Publications

Ebihara, Y., Takahashi, S., Mochizuki, S., Kato, S., Kawakita, T., Ooi, J., Yokoyam, K., Nagamura, F., Tojo A, Asano, S. and Tsuji, K. Unrelated cord blood transplantation after myeloablative conditioning regimen in adolescent and young adult patients with hematologic malignancies: a single institute analysis. Leuk. Res., 36: 128-131, 2012

Ebihara Y, Ma F, Tsuji K. Generation of red blood cells from human embryonic/induced pluripotent stem cells for blood transfusion. Int J Hematol. 95: 610-616, 2012

Yamamoto S, Akiyama K, Oyama N, Hayashi M, Fujimoto Y, Ikeda H, Isoyama K. Fatal hepatic sinusoidal obstruction syndrome in a child with primary CNS lymphoma during induction therapy. Int J Hematol. 96: 284-6, 2012

Yamamoto S, Ebihara Y, Mochizuki S, Tsuda M, Yuji K, Uchimaru K, Tojo A, Tsuji K. Acute lymphoblastic leukemia with t(1;19)(q23;p13)/TCF3-PBX1 fusion in an adult male with Down syndrome. Acta Haematol. 128: 242-243, 2012

Yamamoto S, Yagawa A, Toyama D, Akiyama K, Hayashi M, Mabuchi M, Shimizu T, Ikeda H, Isoyama K. Successful treatment of hepatic sinusoidal obstructive syndrome after hematopoietic stem cell transplantation in a child using recombinant thrombomodulin. Acta Haematol. 129: 62-64, 2012

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 in press.

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. In press.

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 PCR detection of CEP110-FGFR1 fusion gene in a patien with 8p11 syndrome. Leuk Lymhoma in press.

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, Kohichiro Tsuji. Wnt3a stimulates maturation of impaired neutrophils developed from severe congenital neutropenia patient-derived pluripotent stem cells. Proc Natl Acad Sci U S A. in press.

Singh VK, Tsuji K, Sharma PB, Chandra R. Multidimensional role of CD34 protein in hematopoietic stem cell biology. Int J Sci Tech Man. in press.

Center for Stem Cell Biology and Regenerative Medicine

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We are conducting clinical stem cell transplantation, especially using unrelated cord blood as a promising alternative donor in IMSUT research hospital. We are also engaged in the clinical and basic research for promotion of transplantation as well as regenerative medicine.

(1) Hematopoietic Stem Cell Transplantation (HSCT)
Our facility is a main hub of hematopoietic stem cell transplantation (HSCT) centers in Japan. In close association with Department of Hematology/Oncology in the IMSUT research hospital, as many as 600 cases of allogeneic HSCT have been performed and HSCT-related complications including acute/ chronic GVHD and opportunistic infection have been treated until now. Recent years unrelated cord blood has turned to be our major stem cell source in HSCT. Since 1998 we have performed more than 260 cases of cord blood Transplantation (CBT) in adults and demonstrated outstanding clinical results among domestic and overseas HSCT centers. During such a transition of our stem cell source, immunological reconstitution from the CB graft, optimal use of immunosuppressive agents as well as viral infection/ reactivation are becoming our main theme to be elucidated, and we are now approaching these issues in collaboration with other divisions in the center.

(2) iPS cell and hematopoietic stem cell (HSC) research
Recent development of induced pluripotent stem (iPS) cells has suggested the possible application of reprogrammed somatic cells to individualized therapy for intractable disorders. We are trying to generate iPS cells using lentiviral vector and tetracycline-inducible gene expression system for introducing and expressing 3 or 4 factors required for generation of iPS cells with relatively homogeneous genetic background. We are also challenging to reprogram mature blood cells into HSC according to the similar strategy used for iPS cells.

1. Matched HLA haplotype contributes to reduce severe acute GVHD with conserving GVL effect in HLA-mismatched cord blood transplantation.

Takahashi S, Ooi J, Kato S, Kawakita T, Tojo A

We studied the clinical outcomes of 170 consecutive adult patients who received unrelated CBT between August 1998 and January 2011 in the institute of medical Science, University of Tokyo. Patients received previous allogeneic transplants were excluded from this study. All patients received myeloablative regimens including 12 Gy of total

body irradiation, cyclosporine plus short term methotraxate for GVHD prophylaxis and almost same supportive care by the institutional protocol. By low-resolution typing method for HLA-A, -B and -DR loci, 6 patients received matched grafts, 57 received 1 antigen-mismatched and 107 received 2 antigens-mismatched grafts in the graft-versus-host (GvH) direction. We have determined the HLA haplotype based on common haplotypes in Japanese population referred from the 11th International Histocompatibility Workshop and other previous reports. We evaluated the impact of haplotype matching on cumulative incidences of hematopoietic recovery, of GVHD, of relapse and of non-relapse mortality (NRM) using the Pepe and Mori's test. Estimates of overall and disease-free survivals were calculated using the Kaplan-Meier method and analyzed by the log-rank test. Thirty-three among all 170 pairs were defined as the haplotype-matched pairs sharing same haplotypes in both grafts and recipients. The age, sex, cytomegalovirus serological status, diagnosis, risk of the disease at the transplant, numbers of total nucleated cells and $CD34^+$ cells at the cryopreserved were not significantly different between both groups with and without matched haplotypes. Engraftment of platelet after CBT tended to be earlier in haplotype-matched group compared with control group among the 1 antigen-mismatched pairs in the host-versus-graft direction (median: 38 days versus 44 days) and among the 2 antigens-mismatched pairs (median: 38 days versus 42 days), but those were not significant. The cumulative incidences of grades III and IV acute GVHD in patients with haplotype-matched (7%) were significantly lower than non-matched group (9%) among 2 antigens-mismatched pairs in the GvH direction (P=0.033). Notably, cumulative incidences of relapse tended to be lower in haplotype-matched patients among this group (3 years cumulative incidences were 7% in haplotype-matched patients versus 21% in non-matched patients, P=0.086). The haplotype matching effects were not observed in survival rates, cumulative incidences of NRM among any HLA-mismatched pairs. Those data suggest that untyped variation carried on the HLA haplotytpe might be better to be matched. The haplotype matching seemed to effect on lower risk of sever acute GVHD, on the other hand, graft-versus-leukemia effect was conserved in the setting of HLA-mismatched CBT.

2. Second myeloablative allogeneic stem cell transplantation (SCT) using cord blood for leukemia relapsed after initial allogeneic SCT.

Ooi J, Takahashi S, Tsukada N, Kato S, Sato A, Uchimaru K, Tojo A

There are many reports of second allogeneic stem cell transplantation (allo-SCT) using cord blood (CB) for graft failure after initial allo-SCT. However, the efficacy of second allo-SCT using CB for patients with leukemia relapsed after initial allo-SCT is unknown. We report the results of second allo-SCT using CB in seven adult patients with leukemia relapsed after initial allo-SCT. All patients received a myeloablative conditioning regimen including oral busulfan 16 mg/kg, intravenously fludarabine 100mg/m(2) and cyclophosphamide 120 mg/kg. All but one patient had myeloid reconstitution and four patients remain alive at between 4 and 40 months after second SCT. We conclude that second myeloablative allo-SCT using CB may be feasible in selected patients with the relatively younger age, less organ damage and longer time interval between first and second allo-SCT.

3. Establishment of murine iPSC-derived hematopoietic progenitor cell lines which can yield mature blood cells.

Izawa K, Yamaguchi K, Furukawa Y, Yamamoto M, Tojo A

Hematopoietic stem/progenitor cells (HS/PCs) constitute a quite minor part of bone marrow (BM) nucleated cells and cannot be expanded *in vitro* with sustained hematopoietic ability for a long time. It is also difficult to efficiently direct pluripotent stem cells including ESCs and iPSCs toward the stage of HSCs. GATA2 is an essential transcription factor for hematopoietic development and is expressed in HS/PCs. We generated iPSCs (GGKI-iPSCs) from GFP+ BM cells from heterozygous GATA2-GFP knock-in mice in which GFP cDNA was inserted into exon 2 of the GATA2 gene. Colony forming assays confirmed that HPCs exist in a GFP+ fraction of BM cells in those mice, suggesting that HS/PCs derived from GGKI-iPSCs may be identified according to GFP expression. First, GGKI-iPSCs were induced toward hematopoietic differentiation over an OP9 cell monolayer with a cocktail of cytokines. Interestingly, after two months of culture, we found that GFP+ cells were continuously proliferating. They increased in number by 100-fold per week and were sustained for at least 3 months without loss of their properties. Next, GFP+ cells were FACS-sorted according to its expression level. In a methylcellulose assay, the number of colonies from GFP^{low} cells is 100 times higher than that from GFP^{high} cells. The microarray and RT-PCR analysis suggested that GFP^{low} fraction includes myeloid signatures, while GFP^{high} fraction has early erythroid signatures. Furthermore, transplantation experiments revealed that CD11b+ mature myeloid cells in PB could be detected in mice transplanted with GFP^{low}, but not GFP^{high}, cells. As a result, we succeeded in establishment of murine

iPSC-derived hematopoietic progenitor cell lines which can yield mature blood cells.

Publications

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 PCR detection of CEP110-FGFR1 fusion gene in a patient with 8p 11 syndrome (letter to the editor). Leuk Lymphoma. In press, 2013 Jan 18. [Epub ahead of print]

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. Transplant Infectious Disease. In press, 2012 Dec 20. doi: 10.1111/tid.12038. [Epub ahead of print]

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. Hemophilia. 2012 Dec 4. doi: 10.1111/hae.12056. [Epub ahead of print]

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. In press, 2012 Sep 10

Kanda J, Ichinohe T, Kato S, Uchida N, Terakura S, Fukuda T, Hidaka M, Ueda Y, Kondo T, Taniguchi S, Takahashi S, Nagamura-Inoue T, Tanaka J, Atsuta Y, Miyamura K, Kanda Y. Unrelated cord blood transplantation vs related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction. Leukemia. 27(2): 286-94, 2013

Kanda J, Atsuta Y, Wake A, Ichinohe T, Takanashi M, Morishima Y, Taniguchi S, Takahashi S, Ogawa H, Ohashi K, Ohno Y, Aotsuka N, Onishi Y, Kato K, Nagamura-Inoue T, Kanda Y. Impact of the direction of HLA mismatch on transplant outcome in single unrelated cord blood transplantation. Biol Blood Marrow Transplant., S 1083-8791, 2012.

Ebihara Y, Takahashi S, Mochizuki S, Kato S, Kawakita T, Ooi J, Yokoyama K, Nagamura F, Tojo A, Asano S, Tsuji K. Unrelated cord blood transplantation after myeloablative conditioning regimen in adolescent patients with hematologic malignancies: a single institute analysis. Leuk Res. 6: 128-31, 2012

Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Signaling
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Our major interest is to elucidate the mechanisms of pluripotency, self-renewal and the control of cell division and differentiation of stem cells such as 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. Screening of surface antigens of iPS cells using a retrovirus-mediated signal transduction method SST-REX.

Toshikhiko Oki, Jiro Kitaura, Masunori Kajikawa[1], and Toshio Kitamura: [1]ACTGen, Komagane, Nagano.

We previously developed a retrovirus-mediated signal sequence trap method SST-REX as a screening method for surface and secreted proteins. We searched surface antigens of cancer cells or immune cells. Here we used SST-REX to iPS to identify iPS-specific surface antigens, surface antigen "catalog" of iPS cells, and attempted to develop iPS-specific antibodies. So far, we have identified 40 iPS cell antigens, found that at least 3 of them were expressed rather specifically in iPS cells and ES cells, and developed specific antibodies to these 3 antigens and investigated expressions of these antigens in iPS cells. We also investigated the effects of transduction of these antigens on iPS induction, and transduction of one of the antigen enhanced reprogramming process, though the precise mechanisms remain to be investigated.

2. 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 lym-

phoblastic leukemia/lymphoma (T-ALL) after a shorter latency when compared with 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)) not present on RasGRP4. In conclusion, 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.

3. Development of new retroviral vectors.

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

We 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. We developed new vectors like, 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

4. 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 metaphase, MgcRacGAP plays some roles in the segregation of chromosomes probably as Cdc42-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 cell-cycle dependent mechanism: increase in S/G2/M phase and decrease in early G1 phase, suggesting that MgcRacGAP may play some roles in G1 check point. The ubiquitin-dependent degradation of MgcRacGAP is one of the mechanisms that account for its decrease in G1 phase. Using the proteome analysis and retroviral transduction, we identified the 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.

5. 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.

6. 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 such as hematopietic stem cells and cancer

stem cells with tumor initiating potentials are in G0 phase.

Recently we have developed the system to indicate the cells in G0 phase. It is a system to monitor the amount of the protein X, which is destructed during G0 to G1 phase and is not expressed in S/G 2/M phase, using the cells retrovirally trasduced with the fusion protein between a fluorescent protein like mVenus and protein X (mVenus-X), as a similar cell cycle indicator system, fluorescent, ubiquitination-based cell cycle indicator, (Fucci). mVenus-X positive cells are Ki67 negative quiescent cells and mVenus-X signals are enhanced when the cycling cell enter G0 phase in response to serum starvation or contact inhibition.

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-X trasgenic mice have also been generated to track several kinds of tissue specific stem cells in vivo.

Publications

Nishimura, K., Oki, T., Kitaura, J., Kuninaka, S., Saya, H., Sakaue-Sawano, A., Miyawaki, A. and Kitamura, T. APCCDH1 targets MgcRac GAP for destruction in the late M phase. PLOS One in press.

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. Human CD300C delivers an Fc receptor-g-dependent activating signal in mast cells and monocytes and differs from CD300 A in ligand recognition. J. Biol. Chem. in press.

Sakane, A., Almair, A., Abdallah, M., Nakano, K., Honda, K., Ikeda, W., Nishikawa, Y., Matsumoto, M., Matsushita, N., Kitamura, T. and Sasaki, T. (2012) Rab13 small G protein and Junctional Rab 13-binding protein (JRAB) orchestrate actin cytoskeletal organization during epithelial junctional development. J. Biol. Chem. 287, 42455-42968.

Enomoto, Y., Kitaura, J., Shimanuki, M., Kato, N., Nishimura, K., Takahashi, M., Nakamura, H., Kitamura, T., and Sonoki T. (2012) MicroRNA-125b-1 accelerates a C-terminal mutant of C/EBPα (C/EBPα-C(m))-induced myeloid leukemia. Int. J. Hematol. 96, 334-341.

Shibata, T., Takamura, N., Motoi, Y., Goto, Y., Karuppuchamy, T., Izawa, K., Akashi-Takamura, S., Tanimura, N., Kunisawa, J., Kiyono, H., Akira, S., Kitamura, T., Kitaura, J., and Miyake K. (2012) PRAT4A-dependent expression of cell surface TLR5 on neutrophils, classical monocytes and dendritic cells. Int Immunol. 24; 613-623.

Izawa, K., Yamanishi, Y., Maehara, A., Takahashi, M., Isobe, M., Ito, S., Kaitani, A., Matsukawa, T., Matsuoka, T., Nakahara, F., Oki, T., Kiyonari, H., Abe, T., Okumura, K., Kitamura, T., and Kitaura, J. (2012) LMIR3 negatively regulates mast cell activation and allergic responses by binding to extracellular ceramide. Immunity 37: 827-839.

Yamanishi, Y., Takahashi, M., Izawa, K., Isobe, M., Ito, S., Tsuchiya, A., Maehara, A., Uchida, T., Togami, K., Enomoto, Y., Nakahara, F., Oki, T., Kajikawa, M., Kurihara, H., Kitamura, T., and Kitaura, J. (2012) A soluble form of LMIR5/CD300b amplifies lipppolisaccharide-induced lethal inflammation in sepsis. J. Immunol. 189, 1773-1779.

Kitamura, T., and Inoue, D. (2012) HDAC inhibitor-induced thrombocytepenia is caused by its unexpected target. Exp. Hematol. 40, 695-697.

Komori, T., Doi, A., Nosaka, T., Furuta, H., Akamizu, T., Kitamura, T., Senba, E. and Morikawa, Y. (2012) Regulation of AMP-activated protein kinase signaling by AFF4 protein, member of AF 4 (ALL-fused gene from chromosome 4) family of transcription factors, in hypothalamic neurons. J. Biol. Chem. 287, 19985-19996.

Doki, N., Kitaura, J., Inoue, D., Kato, N., Kagiyama, Y., Uchida, T., Togami, K., Isobe, M., Ito, S., Maehara, A., Izawa, K., Oki, T., Harada, Y., Nakahara, F., Harada, H., and Kitamura, T. (2012) Fyn is not essential for Bcr-Abl-induced leukemogenesis in mouse bone marrow transplantation models. Int. J. Hematol. 95: 167-175.

Oki, T., Kitaura, J., Watanabe-Okochi, N., Nishimura, K., Maehara, A., Uchida, T., Komeno, Y., Nakahara, F., Harada, Y., Sonoki, T., Harada, H., and Kitamura, T. (2012) Aberrant expression of RasGRP1 cooperates with gain-of-function NOTCH1 mutations in T-cell leukemogenesis. Leukemia 26: 1038-1045.

Nakamura, M., Kitaura, J., Enomoto, Y., Lu, Y., Nishimura, K., Isobe, M., Ozaki, K., Komeno, Y., Nakahara, F., Oki, T., Kume, H., Homma, Y., and Kitamura, T. (2012) TSC-22 is a negative-feedback regulator of Ras/Raf signaling: Implications for tumorigenesis. Cancer Science 103: 26-33.

Suzuki, K., Ono, R., Ohishi, K., Masuya, M., Kataoka, I., Liu, B., Nakamori, Y., Ino, K., Monma, F., Hamada, H., Kitamura, T., Katayama, N., and Nosaka, T. (2012) IKAROS isoform 6 enhances BCR-ABL-mediated proliferation of human CD34+ hematopoietic cells on stromal cells. Int J Oncology 40: 53-62.

Shibata-Minoshima, F., Oki, T., Doki, N., Nakahara, F., Kageyama, S., Kitaura, J., Fukuoka, J. and Kitamura, T. (2012) Identification of RHOXF2

(PEPP2) as a cancer-promoting gene by expression cloning. Int J Oncology 40: 93-98.

Kawamura, S., Sato, I., Wada, T., Yamaguchi, K., Li, Y., Li, D., Zhao, X., Ueno, S., Aoki, H., Tochigi, T., Kuwahara, M., Kitamura, T., Takahashi, K., Moriya, S., and Miyagi, T. (2012) Plasma membrane-associated sialidase (NEU3) regulates progression of prostate cancer to androgen-independent growth through modulation of androgen receptor signaling. Cell Death and Differentiation 19: 170-179.

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

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Proteases can perform highly selective and limited cleavage of specific substrates including growth factors and their receptors, cell adhesion molecules, cytokines, apoptotic ligand and angiogenic factors. To understand the molecular mechanism underlying hematopoietic stem cell differentiation, we investigated the role of proteases in the regulation of blood cell formation and blood vessel formation using gene deficient mice.

1. Plasminogen deficiency attenuates post-natal erythropoiesis in male C57BL/6 mice through decreased activity of the LH-testosterone axis

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Novel roles for the serine protease plasmin have been recently implicated in physiological and pathological processes. However, whether plasmin is involved in erythropoiesis, is not known. In the present study, we studied the consequences of plasminogen deficiency on erythropoiesis in plasminogen deficient (Plg KO) mice. Erythroid differentiation was attenuated in male Plg KO mice and resulted in erythroblastic accumulation within the spleen and bone marrow, with increased apoptosis in the former, erythrocytosis and splenomegaly, whereas similar erythropoietic defect was less prominent in female Plg KO mice. In addition, erythrocyte lifespan was shorter in both male and female Plg KO mice. Erythropoietin levels were compensatory increased in both male and female Plg KO mice, and resulted in a higher frequency of BFU-E within the spleen and bone marrow. Surprisingly, we found that male Plg KO mice but not their female counterparts exhibited normochromic normocytic anemia. The observed gender-linked erythropoietic defect was attributed to decreased serum testosterone levels in Plg KO mice, as a consequence of impaired secretion of the pituitary luteinizing hormone (LH) under steady state condition. Surgical castration, causing testosterone deficiency and stimulating LH release, attenuated erythroid differentiation and induced anemia in WT animals, but did not further decrease the hematocrit levels in Plg KO mice. In addition, complementation of LH using human choriogonadotropin, which increases testosterone production, improved the erythropoietic defect and anemia in Plg KO mice. The present results identify a novel role for plasmin in the hormonal regulation of post-natal erythropoiesis by the LH-testosterone axis.

2. MT1-MMP plays a critical role in hematopoiesis by regulating HIF-mediated chemokine/cytokine gene transcription within niche cells

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HSC fate decisions are regulated by cell-intrinsic and cell-extrinsic cues. The latter cues are derived from the BM niche. Membrane-type 1 matrix metalloproteinase (MT1-MMP), which is best known for its proteolytic role in pericellular matrix remodeling, is highly expressed in HSCs and stromal/niche cells. We found that, in MT1-MMP(−/−) mice, in addition to a stem cell defect, the transcription and release of kit ligand (KitL), stromal cell-derived factor-1 (SDF-1/CXCL12), erythropoietin (Epo), and IL-7 was impaired, resulting in a trilineage hematopoietic differentiation block, while addition of exogenous KitL and SDF-1 restored hematopoiesis. Further mechanistic studies revealed that MT1-MMP activates the hypoxia-inducible factor-1 (HIF-1) pathway via factor inhibiting HIF-1 (FIH-1) within niche cells, thereby inducing the transcription of HIF-responsive genes, which induce terminal hematopoietic differentiation. Thus, MT1-MMP in niche cells regulates postnatal hematopoiesis, by modulating hematopoietic HIF-dependent niche factors that are critical for terminal differentiation and migration.

3. Inhibition of PAI-1 induces neutrophil-driven neoangiogenesis and promotes tissue regeneration via production of angiocrine factors in mice

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Plasminogen activator inhibitor-1 (PAI-1), an endogenous inhibitor of a major fibrinolytic factor, tissue-type plasminogen activator, can both promote and inhibit angiogenesis. However, the physiologic role and the precise mechanisms underlying the angiogenic effects of PAI-1 remain unclear. In the present study, we report that pharmacologic inhibition of PAI-1 promoted angiogenesis and prevented tissue necrosis in a mouse model of hind-limb ischemia. Improved tissue regeneration was due to an expansion of circulating and tissue-resident granulocyte-1 marker (Gr-1(+)) neutrophils and to increased release of the angiogenic factor VEGF-A, the hematopoietic growth factor kit ligand, and G-CSF. Immunohistochemical analysis indicated increased amounts of fibroblast growth factor-2 (FGF-2) in ischemic gastrocnemius muscle tissues of PAI-1 inhibitor-treated animals. Ab neutralization and genetic knockout studies indicated that both the improved tissue regeneration and the increase in circulating and ischemic tissue-resident Gr-1(+) neutrophils depended on the activation of tissue-type plasminogen activator and matrix metalloproteinase-9 and on VEGF-A and FGF-2. These results suggest that pharmacologic PAI-1 inhibition activates the proangiogenic FGF-2 and VEGF-A pathways, which orchestrates neutrophil-driven angiogenesis and induces cell-driven revascularization and is therefore a potential therapy for ischemic diseases.

4. New functions of the fibrinolytic system in bone marrow cell-derived angiogenesis

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Angiogenesis is a process by which new blood vessels form from preexisting vasculature. This process includes differentiation of angioblasts into endothelial cells with the help of secreted angiogenic factors released from cells such as bone marrow (BM)-derived cells. The fibrinolytic factor plasmin, which is a serine protease, has been shown to promote endothelial cell migration either directly, by degrading matrix proteins such as fibrin, or indirectly, by converting matrix-bound angiogenic growth factors into a soluble form. Plasmin can also activate other pericellular proteases such as matrix metalloproteinases (MMPs). Recent studies indicate that plasmin can additionally alter cellular adhesion and migration. We showed that factors of the fibri-

nolytic pathway can recruit BM-derived hematopoietic cells into ischemic/hypoxic tissues by altering the activation status of MMPs. These BM-derived cells can function as accessory cells that promote angiogenesis by releasing angiogenic signals. This review will discuss recent data regarding the role of the fibrinolytic system in controlling myeloid cell-driven angiogenesis. We propose that plasmin/plasminogen may be a potential target not only for development of effective angiogenic therapeutic strategies for the treatment of cancer, but also for development of strategies to promote ischemic tissue regeneration.

5. Plasmin inhibitor reduces lymphoid tumor growth by suppressing matrixmetallproteinase-9 dependent CD11b+/F4/80+ myeloid cell recruitment

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Activation of the fibrinolytic system during lymphoma progression is a well-documented clinical phenomenon. But the mechanism by which the fibrinolytic system can modulate lymphoma progression has been elusive. The main fibrinolytic enzyme, plasminogen (Plg)/plasmin (Plm), can activate matrix metalloproteinases (MMPs), like MMP-9, which has been linked to various malignancies. Here we provide the evidence that blockade of Plg reduces lymphoma growth by inhibiting MMP-9-dependent recruitment of $CD11b^+F4/80^+$ myeloid cells locally within the lymphoma tissue. Genetic plasminogen deficiency and drug-mediated Plm blockade delayed lymphoma growth and diminished MMP-9 dependent $CD11b^+F4/80^+$ myeloid cell infiltration into lymphoma tissues. A neutralizing antibody against CD11b inhibited lymphoma growth *in vivo*, which indicates that $CD11b^+$ myeloid cells play a role in lymphoma growth. Plg deficiency in lymphoma-bearing mice resulted in reduced plasma levels of the growth factors vascular endothelial growth-A and Kit ligand, both of which are known to enhance myeloid cell proliferation. Collectively, the data presented in this study demonstrate a previously undescribed role of Plm in lymphoproliferative disorders and provide strong evidence that specific blockade of Plg represents a promising approach for the regulation of lymphoma growth.

Publications

1. Okaji Y, Tashiro Y, Gritli I, Nishida C, Sato A, Ueno Y, Del Canto Gonzalez S, Ohki-Koizumi M, Akiyama H, Nakauchi H, Hattori K, Heissig B. Plasminogen deficiency attenuates post-natal erythropoiesis in male C57BL/6 mice through decreased activity of the LH-testosterone axis. Exp Hematol. 40: 143-154, 2012.
2. Nishida C, Kusubata K, Tashiro Y, Gritli I, Sato A, Ohki-Koizumi M, Morita Y, Nagano M, Sakamoto T, Koishikawa N, Kuchimaru T, Kizaka-Kondoh S, Seiki M, Nakauchi H, Heissig B, Hattori K. MT1-MMP plays a critical role in hematopoiesis by regulating HIF - mediated chemokine/cytokine gene transcription within niche cells. Blood. 119: 5405-16. 2012.
3. Tashiro Y, Nishida C, Sato-Kusubata K, Ohki-Koizumi M, Ishihara M, Sato A, Gritli I, Komiyama H, Sato Y, Dan T, Miyata T, Okumura K, Tomiki Y, Sakamoto K, Nakauchi H, Heissig B, Hattori K. Inhibition of PAI-1 induces neutrophil-driven neoangiogenesis and promotes tissue regeneration via production of angiocrine factors in mice. Blood. 119: 6382-93, 2012.
4. Heissig B, Ohki-Koizumi M, Tashiro Y, Gritli I, Sato-Kusubata K, Hattori K. New functions of the fibrinolytic system in bone marrow cell-derived angiogenesis. Int J Hematol. 95: 131-7, 2012.
5. Ishihara M, Nishida C, Tashiro Y, Gritli I, Rosenkvist J, Koizumi M, Okaji Y, Yamamoto R, Yagita H, Okumura K, Nishikori M, Wanaka K, Yuko Tsuda Y, Okada Y, Nakauchi H, Hattori K, Heissig B. Plasmin inhibitor reduces lymphoid tumor growth by suppressing matrix metallproteinase-9 dependent CD11b+/F4/80+ myeloid cell recruitment. Leukemia. 26: 332-9, 2012.