1. Long-term, immunosuppression free glycemic control by transplantation of islets generated by inter-species organogenesis

Yamaguchi T, Sato H, Kato-Itoh M, Goto T, Hara H, Sanbo M, Kobayashi T, Mizuno N, Yanagida A, Umino A, Ota Y, Hamanaka S, Masaki H, Tamir R D, Hirabayashi M, Nakauchi H: 1Division of Stem Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Science, University of Tokyo, 2Center for Genetic Analysis of Behavior, National Institute for Physiological Sciences, 3Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, 4Centre of Stem Cells and Regenerative Medicine and Institute of Liver Studies, King's College London, 5Department of Pathology, Research Hospital, Institute of Medical Science, University of Tokyo, 6Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine

The islet transplantation is one of the most promising treatments for the type 1 diabetes patients. Although chronic immunosuppression is necessary to achieve successful engraftment and long-term function of allogenic islets, immunosuppression reagent has beta-cell toxic effects and diabetogenicity. Therefore the generation of pancreas from patient derived pluripotent stem cells for islet transplantation is ideal strategy for immunosuppression free treatment and this is an ultimate goal for regenerative medicine.

To achieve this, we have recently reported "blastocyst complementation" for in vivo generation of organs which is completely derived from PSCs using chimera forming ability of PSCs. In the report, we have generated rat pancreas in mouse by interspecific blastocyst complementation and we found that this pancreas was structurally and functionally comparable to in vivo pancreas. However, in other our report, we have found that the supporting tissues such as blood vessels or nerve cells were chimeric in the organ which was generated by blastocyst complementation. The effect of these xenogenic supporting tissues on engraftment of organ has not been elucidated yet.

To analyze this issue, we transplanted mouse PSCs derived islets which were generated in apancreatic rat by interspecific blastocyst complementation into STZ induced diabetic mouse and measured the blood glucose level over time.
The blood glucose level of the interspecific chimera which possess mouse PSCs derived pancreas was controlled normally and glucose tolerance test revealed no abnormality in glucose clearance. As we expectedly, the endocrine and the exocrine cells in pancreas in interspecific chimera were entirely derived from mouse PSCs, on the other hand, the FACS analysis of enzymatically dispersed islets revealed that vascular endothelial cells and other supporting cells in islets were chimeric. We transplanted these islets that contain "xenogenic supporting cells" into diabetic mice and we found that the control of normal blood glucose level can be achieved over 370 days without chronic immunosuppression.

These results indicate that "xenogenic supporting cells" do not influence the successful engraftment and function of islets which are generated in xenogenic animal.

2. Inhibition of Apoptosis Overcomes Stage-Related Compatibility Barriers to Chimera Formation in Mouse Embryos

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Cell types more advanced in development than embryonic stem cells, such as EpiSCs, fail to contribute to chimeras when injected into pre-implantation-stage blastocysts, apparently because the injected cells undergo apoptosis. Here we show that transient promotion of cell survival through expression of the anti-apoptotic gene BCL2 enables EpiSCs and Sox17+ endoderm progenitors to integrate into blastocysts and contribute to chimeric embryos. Upon injection into blastocyst, BCL2-expressing EpiSCs contributed to all bodily tissues in chimeric animals while Sox17+ endoderm progenitors specifically contributed in a region-specific fashion to endodermal tissues. In addition, BCL2 expression enabled rat EpiSCs to contribute to mouse embryonic chimeras, thereby forming interspecies chimeras that could survive to adulthood. Our system therefore provides a method to overcome cellular compatibility issues that typically restrict chimera formation. Application of this type of approach could broaden the use of embryonic chimeras, including region-specific chimeras, for basic developmental biology research and regenerative medicine.

Publications


1. An assessment of the effects of ectopic gp91phox expression in XCGD iPSC-derived neutrophils

Huan-Ting Lin, Makoto Otsu, Hideki Masaki, Tomoyuki Yamaguchi, Taizo Wada, Akihiro Yachie, Ken Nishimura, Manami Ohtaka, Mahito Nakashima, Hiromitsu Nakauchi

For the treatment of monogenetic hematological disorders, restoration of transgene expression in affected cell populations is generally considered to have beneficial effects. However, X-linked chronic granulomatous disease (XCGD) is unique since the appearance of functional neutrophils in the peripheral blood following hematopoietic stem cell gene therapy is transient only. One contributing factor could be the occurrence of detrimental effects secondary to ectopic gp91phox expression in neutrophils, which has not been formally demonstrated previously. This study uses iPSCs to model XCGD, which allows the process of differentiation to be studied intensely in vitro. Alpharetroviral vectors carrying a ubiquitous promoter were used to drive the "ectopic" expression of codon optimized gp91phox cDNA. In the mature fraction of neutrophils differentiated from transduced XCGD-iPSCs, cellular recovery in terms of gp91phox expression and ROS production was abruptly lost before cells had fully differentiated. Most critically, ectopic gp91phox expression could be identified clearly in the developing fraction of the transduced groups, which appeared to correspond with reduced cell viability. It is possible that this impedes further differentiation of developing neutrophils. Therefore affording cellular protection from the detrimental effects of ectopic gp91phox expression may improve XCGD clinical outcomes.

2. Application of droplet digital PCR for estimating vector copy number states in stem cell gene therapy

Huan-Ting Lin, Takashi Okumura, Yukinori Yasuda, Satoru Ito, Hiromitsu Nakauchi, and Makoto Otsu

Stable gene transfer into target cell populations via integrating viral vectors is widely used in stem cell gene therapy (SCGT). Accurate vector copy number (VCN) estimation has become increasingly important. However, existing methods of estimation such as real-time quantitative PCR are more restricted in practicality especially during clinical tri-
als, given the limited availability of sample materials from patients. This study demonstrates the application of an emerging technology called droplet digital PCR (ddPCR) in estimating VCN states in the context of SCGT. Induced pluripotent stem cells (iPSCs) derived from an X-linked chronic granulomatous disease patient were used as clonable target cells for transduction with alpharetroviral vectors harboring codon-optimized CYBB cDNA. Precise primer-probe design followed by multiplex analysis conferred assay specificity. Accurate estimation of per cell VCN values was possible without reliance upon a reference standard curve. Sensitivity was high and the dynamic range of detection was wide. Assay reliability was validated by observation of consistent, reproducible, and distinct VCN clustering patterns for clones of transduced iPSCs with varying numbers of transgene copies. Taken together, use of ddPCR appears to offer a practical and robust approach to VCN estimation with a wide range of clinical and research applications.

3. Multiple allogeneic progenitors in combination function as a unit to support early transient hematopoiesis in transplantation

Takashi Ishida, Satoshi Takahashi, Chen-Yi Lai, Masanori Nojima, Ryo Yamamoto, Emiko Takeuchi, Yasuo Takeuchi, Masaaki Higashihara, Hiromitsu Nakauchi, and Makoto Otsu

Cord blood (CB) is a valuable donor source in hematopoietic cell transplantation. However, the initial time to engraftment in CB transplantation (CBT) is often delayed because of low graft cell numbers. This limits the use of CB. To overcome this cell dose barrier, we modeled an “insufficient dose” CBT setting in lethally irradiated mice and then added hematopoietic stem/progenitor cells (HSCs/HPCs; HSPCs) derived from 4 mouse allogeneic strains. The mixture of HSPCs rescued recipients and significantly accelerated hematopoietic recovery. Including T cells from one strain favored single-donor chimerism through graft versus graft reactions, with early hematopoietic recovery unaffected. Furthermore, using clinically relevant procedures, we successfully isolated a mixture of CD34+ cells from multiple frozen CB units at one time regardless of HLA-type disparities. These CD34+ cells in combination proved transplantable into immunodeficient mice. This work provides proof of concept that when circumstances require support of hematopoiesis, combined multiple units of allogeneic?HSPCs are capable of early hematopoietic reconstitution while allowing single-donor hematopoiesis by a principal graft.

4. Pre-transplantation blockade of TNF-α-mediated oxygen species accumulation protects hematopoietic stem cells

Takashi Ishida, Sachie Suzuki, Chen-Yi Lai, Satoshi Yamazaki, Shigeru Kakuta, Yoichiro Iwakura, Masanori Nojima, Yasuo Takeuchi, Masaaki Higashihara, Hiromitsu Nakauchi, and Makoto Otsu

Hematopoietic stem cell (HSC) transplantation (HSCT) for malignancy requires toxic pre-conditioning to maximize anti-tumor effects and donor-HSC engraftment. While this induces bone marrow (BM)-localized inflammation, how BM environmental change affects transplanted HSCs in vivo remains largely unknown. We here report that, depending on interval between irradiation and HSCT, residence within lethally irradiated recipient BM compromises donor-HSC reconstitution ability. Both in vivo and in vitro we demonstrate that, among inflammatory cytokines, TNF-α plays a role in HSC damage: TNF-α stimulation leads to accumulation of reactive oxygen species (ROS) in highly purified hematopoietic stem/progenitor cells (HSCs/ HSPCs). Transplantation of flow-cytometry - sorted murine HSCs reveals damaging effects of accumulated ROS on HSCs. Short-term incubation either with an specific inhibitor of tumor necrosis factor receptor 1 signaling or an antioxidant N-acetyl-L-cysteine (NAC) prevented TNF-α-mediated ROS accumulation in HSCs. Importantly, pre-transplantation exposure to NAC successfully demonstrated protective effects in inflammatory BM on graft-HSCs, exhibiting better reconstitution capability than that of non-protected control grafts. We thus suggest that in vivo protection of graft-HSCs from BM inflammation is a feasible and attractive approach, which may lead to improved hematopoietic reconstitution kinetics in transplantation with myeloablative conditioning that inevitably causes inflammation in recipient BM.

Publications


The FACS Core Laboratory provides high quality, cost effective state-of-art flow cytometry services for internal and external researchers. The facility has three BD FACSAria cell sorters, a SONY SH800 cell sorter for sorting, a BD FACSCalibur and a BD FACSVerse for analysis. We offer assistance in the following areas: (1) initial project planning, (2) antibody panel design and optimization, (3) instrument operation and maintenance, and (4) data analysis.
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application of an emerging technology called droplet digital PCR (ddPCR) in estimating VCN states in the context of SCGT. Induced pluripotent stem cells (iPSCs) derived from an X-linked chronic granulomatous disease patient were used as clonal target cells for transduction with alpharetroviral vectors harboring codon-optimized CYBB cDNA. Precise primer-probe design followed by multiplex analysis conferred assay specificity. Accurate estimation of per cell VCN values was possible without reliance upon a reference standard curve. Sensitivity was high and the dynamic range of detection was wide. Assay reliability was validated by observation of consistent, reproducible, and distinct VCN clustering patterns for clones of transduced iPSCs with varying numbers of transgene copies. Taken together, use of ddPCR appears to offer a practical and robust approach to VCN estimation with a wide range of clinical and research applications.

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4. Pre-transplantation blockade of TNF-α-mediated oxygen species accumulation protects hematopoietic stem cells

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Publications

3. Koso H, Tsuhako A, Lai CY, Baba Y, Otsu M,


1. Second allogeneic transplantation using unrelated cord blood for relapsed hematological malignancies after allogeneic transplantation.


The efficacy of second allogeneic stem cell transplantation (SCT2) using cord blood (CB) for patients with relapsed hematological malignancies after initial allogeneic stem cell transplantation (SCT1) is unknown. We analyzed the results of SCT2 using single-unit unrelated CB in 34 adult patients with relapsed hematological malignancies after SCT1 in our institution. The patients had acute myeloid leukemia (n=23), acute lymphoblastic leukemia (n=7), chronic myelogenous leukemia (n=2), and myelodysplastic syndrome (n=2). The cumulative incidence of neutrophil and platelet engraftment was 81.6% at 30 days and 68.5% at 100 days, respectively. With a median follow-up of 40 months, the probability of overall survival at 3 years was 29.0%. The cumulative incidence of relapse and transplant-related mortality at 3 years were 60.7% and 27.2%, respectively. The use of CB could offer the opportunity to receive SCT2 for patients who experienced disease relapse after SCT1 without HLA-identical related or unrelated donors.

2. Breakthrough fungemia due to Candida fermentati with fks1p mutation under micafungin treatment in a cord blood transplant recipient.


The prophylactic use of antifungal drugs in allogeneic hematopoietic cell transplant recipients has revealed that the rate of non-albicans candidemia has increased. We herein report the case of a patient with adult T-cell leukemia who developed candidemia due to Candida fermentati during micafungin treatment after cord blood transplantation. The isolate was identified on day 47 by sequencing of the internal transcribed spacer region of the ribosomal RNA gene. The sequencing of the hot spot region of fks1p of isolate revealed naturally occurring amino acid substitutions, which conferred reduced echinocandin susceptibility. This case highlights that breakthrough candidemia due to C. fermentati occurred in a patient receiving micafungin treatment.
3. Generation of multivirus-specific T cells by a single stimulation of PBMCs with a peptide mixture utilizing serum free medium

Fujita Y, Tanaka Y, Takahashi S.

Extension to donors other than HLA-matched siblings following advanced immunosuppressive treatment has resulted in the emergence of viral infections as major contributors to morbidity and mortality after hematopoietic stem cell transplantation (HSCT). The degree of risk for infection is dictated by the degree of tissue mismatch between donor and recipient, and the resultant degree of immunosuppression. Reactivation of latent viruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex and herpes zoster are common and often cause symptomatic disease. Respiratory viruses such as adenovirus, influenza and respiratory symptomatic virus also frequently cause infection. While pharmacological agents are standard therapy for some, they have substantial toxicities, generate resistant variants, and frequently ineffective and costly. Moreover, immune reconstitution is necessary for long-term protection especially after HSCT. As the delay in recovery of virus-specific cellular immune response is clearly associated with viral reactivation and disease, cellular immunotherapy to restore virus-specific immunity offers an attractive alternative to conventional drugs. Adoptive transfer of virus-specific lymphocytes (VSTs) from stem cell donors has been proved to be safe and effective to treat viral infection.

Manufacture of VSTs requires preparation of specialized antigen presenting cells (APCs), uses viruses or viral vectors to provide viral antigens to present on APCs. Recent report from a group of Baylor College of Medicine has introduced a new method to generate multiple VSTs by direct stimulation of peripheral blood mononuclear cells (PBMCs) with peptides to replace the complex and lengthy process above.

By this method, VSTs can be prepared by single stimulation with non-viral products and contain polyclonal mixture of T cells specific for a large number of epitopes in a multiple pathogenic viruses, which reduces the risk of immune escape by viral escape mutants and meet the requirement to treat viral infection after HSCT which occurs by broad pathogens. Moreover this method is as simple and fast as possible and takes only 10-14 days for preparation which makes it clinically useful. With this method, polyclonal CTLs specific for multivirus antigens can be produced after single stimulation of PBMCs with a peptide mixture spanning the target antigens in the presence of IL4 and IL7.

We have introduced and verified this system to apply for clinical use in Japan.

The culture media for T cell expansion used in the studies reported by Baylor College of Medicine are supplemented with fetal bovine serum (FBS). While these serum products are traditionally used to expand T cells to promote cell growth and viability, there are some countries which the use of serum product is not allowed. Also, uncharacterized elements contained in the serum products may cause inconsistency in results from batch to batch. Cell expansion in serum-free media would therefore be preferable.

To meet the requirement for the viral infections after HSCT by broad viral antigens and in terms of regulation by the Japanese FDA, we established the method to generate multivirus-specific T cells targeting 7 viruses (CMV, EBV, AdV, HHV-6, BKV, JCV, and VZV) in serum-free medium.

Publications


Yosuke Tanaka, Takeshi Fukushima, Toshihiko Oki, Kotarou Nishimura, Asako Sakaue-Sawano, Atsushi Miyawaki: Laboratory for Cell Function Dynamics, RIKEN, Wako, Saitama and ERATO Miyawaki Life Function Dynamics Project, JST.

Previously, we reported that MgcRacGap is a marker for midbody and that MgcRacGap-mVenus fusion protein visualized asymmetric inheritance and release of midbody during cytokinesis (Nishimura et al., 2013). We retrovirally introduced MRG-hmKuO2 into hematopoietic stem cells (HSCs), in order to examine whether midbody asymmetric inheritance and release is involved with asymmetric division of HSCs. HSCs showed high frequency of midbody release during cytokinesis. Interestingly, one daughter cell releasing midbody differentiated earlier than the other daughter cell inheriting midbody. We generated Cre-inducible MRG-hmKuO2 mouse line. Briefly, the MRG-hmKuO2 fusion gene is inserted into Rosa26 locus following a loxP-NEO-STOP-loxP cassette, in order to visualize asymmetric inheritance and release of midbody in vivo without retroviral infection. Crossing MRG-hmKuO2 mice with Vav-Cre mice, MRG-hmKuO2 nicely marked midbody asymmetric inheritance and release in HSCs in culture. We are now planning to do paired-daughter assay using HSCs from MRG-hmKuO2 mice to examine whether inheritance and release of midbody link to asymmetric division of HSCs.


Takeshi Fukushima, Yosuke Tanaka, Toshihiko Oki, Kotarou Nishimura, Asako Sakaue-Sawano, Atsushi Miyawaki, Toshio Kitamura: Laboratory for Cell Function Dynamics, RIKEN, Wako, Saitama and ERATO Miyawaki Life Function Dynamics Project, JST.

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

We have developed a novel G0 marker, mVenus-p27K- (Oki et al, 2013). The mVenus-p27K- clearly
marked G0 and very early G1 in NIH3T3 cells. To examine G0 status in HSCs, we generated a Cre-inducible mVenuse-p27K- fusion gene in Rosa26 locus following a loxP-NEO-STOP-loxP cassette. After crossing with Vav-Cre mice, we analyzed mVenuse-p27K- expression in HSCs. We expected most of the HSCs are mVenuse-p27K- positive because most of the HSCs are dormant. However, three different populations (mVenuse-p27K-high (70%), mVenuse-p27K-low (20%), mVenuse-p27K-negative (10%)) were given from HSC fraction (CD150 CD48-cKit Sca-1 Lineage-). These three populations were in G0 stage judged by Pyronin Y/Hoechst double-staining. To examine difference among these three populations, we performed bone marrow reconstitution assay. To our surprise, only mVenuse-p27K-high population showed bone marrow reconstitution ability. Moreover, mVenuse-p27K-high population was only detected in HSC fraction in the bone marrow, not in other hematopoietic fractions. This indicates that our mVenuse-p27K- marker could be a good marker for isolation of transplantable HSCs. To characterize differences among the three fractions in HSCs, we are now performing single-cell RNASeq analysis. We plan to do small-cell Mass Spec and metabolome analysis for these cell fractions. We also plan to examine where mVenuse-p27K-high HSCs locate in bone marrow using 4D imaging.

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

We previously identified MgcRacGAP through functional cloning as a protein that enhances or induces macrophage differentiation of leukemic cell lines M1 and HL60. Interestingly, MgcRacGAP plays distinct roles depending on the cell cycle. In the interphase, it plays critical roles in activation and nuclear translocation of STAT3 and STAT5 as a Rac-GAP. In the mitotic phase, MgcRacGAP plays essential roles in completion of cytokinesis as a Rho-GAP. Interestingly, Aurora B-mediated phosphorylation of S387 converts MgcRacGAP from Rac-GAP to Rho-GAP. We have recently shown that expression of MgcRacGAP is regulated by a cell cycle-dependent manner: MgcRacGAP expression increases in S/G2/M phase and decreases in early G1 phase, suggesting that MgcRacGAP may play some roles in G1 check point. In addition to the transcriptional control, MgcRacGAP protein levels are controlled by ubiquitin-dependent degradation, leading to its decrease in G1 phase. Using the proteome analysis and retroviral transduction, we identified APC/CDH1 as an E3 ligase involved in regulation of MgcRacGAP and the degron in MgcRacGAP. Now we are investigating the physiological roles of this regulation. In summary, our results implicate MgcRacGAP in coordination of cell cycle progression and cell fate determination.

Publications


critical transporter in the response to tunicamycin. Human Cell in press.
1. Fibrinolytic crosstalk with endothelial cells expands murine mesenchymal stromal cells

Douaa Dhahri, Kaori Sato-Kusubata, Makiko Ohki-Koizumi, Chiemi Nishida, Yoshi Tashiro, Shinya Munakata, Hiroshi Shimazu, Yousef Salama, Salita Eiamboonsert, Hiromitsu Nakauchi, Koichi Hattori, and Beate Heissig.

Division of Stem Cell Dynamics, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo.

Tissue plasminogen activator (tPA), aside from its vascular fibrinolytic action, exerts various effects within the body, ranging from synaptic plasticity to control of cell fate. Here, we observed that by activating plasminogen and matrix metalloproteinase-9, tPA expands murine bone marrow-derived CD45⁻TER119⁻Sca-1⁻PDGFRα⁺ mesenchymal stromal cells (PoS-MSCs) in vivo through a crosstalk between PoS-MSCs and endothelial cells. Mechanistically, tPA induces the release of Kit ligand from PoS-MSCs, which activates c-Kit(⁺) endothelial cells to secrete MSC growth factors: platelet-derived growth factor-BB (PDGF-BB) and fibroblast growth factor 2 (FGF2). In synergy, FGF2 and PDGF-BB upregulate PDGFRα expression in PoS-MSCs, which ultimately leads to PoS-MSC expansion. These data show a novel mechanism by which the fibrinolytic system expands PoS-MSCs through a cytokine crosstalk between niche cells.

2. Cancer therapy targeting the fibrinolytic system

Beate Heissig, Salita Eiamboonsert, Yousef Salama, Horoshi Shimazu, Douaa Dhahri, Shinya Munakata, Yoshi Tashiro, Koichi Hattori.

Division of Stem Cell Dynamics, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo.

Proteases perform highly selective and limited cleavage of specific substrates including growth factors and their receptors, cell adhesion molecules, cytokines, apoptotic ligand and angiogenic factors. The goal of our laboratory is to identify novel therapeutic targets for diseases like cancer or inflammatory diseases by studying the role of proteases and microenvironmental cells for inflammation, tissue regeneration and adult stem cells. Blood cell generation (hematopoiesis), tissue regeneration during wound healing or cancer growth depend on the establishment of a cellular microenvironment. Mesenchymal stem cells (MSC) are part of this cellular microenvironment, where they functionally modulate cell homing, angiogenesis, and immune modulation. We demonstrated that the fibrinolytic system with its main representative, the serine protease plasmin modulates the hematopoietic microenvironment by expanding MSC and endothelial cells, and thereby is an active "niche modulator".
The tumor microenvironment is recognized as a key factor in the multiple stages of cancer progression, mediating local resistance, immune-escape and metastasis. Cancer growth and progression require remodeling of the tumor stromal microenvironment, such as the development of tumor-associated blood vessels, recruitment of bone marrow-derived cells and cytokine processing. Extracellular matrix breakdown achieved by proteases like the fibrinolytic factor plasmin and matrix metalloproteases is necessary for cell migration crucial for cancer invasion and metastasis. Key components of the fibrinolytic system are expressed in cells of the tumor microenvironment. Plasmin can control growth factor bioavailability, or the regulation of other proteases leading to angiogenesis, and inflammation. In this review, we will focus on the role of the fibrinolytic system in the tumor microenvironment summarizing our current understanding of the role of the fibrinolytic factors for the modulation of the local chemokine/cytokine milieu, resulting in myeloid cell recruitment, which can promote neoangiogenesis.

**Publications**

**<Beate Heissig Group>**


1. Probing the Metabolic Heterogeneity of Live Euglena gracilis with Stimulated Raman Scattering Microscopy

Yoshifumi Wakisaka, Yuta Suzuki, Osamu Iwata, Ayaka Nakashima, Takuro Ito, Misa Hirose, Ryota Domon, Mai Sugawara, Norimichi Tsumura, Hiroshi Watarai, Tomoyoshi Shimobaba, Kengo Suzuki, Keisuke Goda and Yasuyuki Ozeki:

1. Department of Chemistry, University of Tokyo, 2. Department of Electrical and Information Systems, University of Tokyo, 3. Research & Development Department, Euglena Co., Ltd, 4. Institute for Advanced Biosciences, Keio University, 5. Graduate School of Media and Governance, Keio University, 6. Department of Information Processing and Computer Science, Chiba University, 7. Division of Stem Cell Cellomics, Center for Stem Cells and Regenerative Medicine, University of Tokyo, 8. Department of Electrical and Electronics Engineering, Chiba University, 9. Department of Electrical Engineering, University of California, 10. Japan Science and Technology Agency.

Understanding metabolism in live microalgae is crucial for efficient biomaterial engineering, but conventional methods fail to evaluate heterogeneous populations of motile microalgae due to the labelling requirements and limited imaging speeds. Here, we demonstrate label-free video-rate metabolite imaging of live Euglena gracilis and statistical analysis of intracellular metabolite distributions under different culture conditions. Our approach provides further insights into understanding microagal heterogeneity, optimizing culture methods and screening mutant microalgae.


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Zinc transporters play a critical role in spatiotemporal regulation of zinc homeostasis. Although disruption of zinc homeostasis has been implicated in disorders such as intestinal inflammation and aberrant epithelial morphology, it is largely unknown which zinc transporters are responsible for the intestinal epithelial homeostasis. Here, we show that Zrt-Irt-like protein (ZIP) transporter ZIP7, which is highly expressed in the intestinal crypt, is essential for intestinal epithelial proliferation. Mice lacking Zip7 in intestinal epithelium triggered endoplasmic reticulum (ER) stress in proliferative progenitor cells, leading to significant cell death of progenitor cells. Zip7 deficiency led to the loss of Olfm4+ intestinal stem cells and the degeneration of post-mitotic Paneth cells, indicating a fundamental requirement for Zip7 in homeostatic intestinal regeneration. Taken together, these findings provide evidence for the importance of ZIP7 in maintenance of intestinal epithelial homeostasis through the regulation of ER function in proliferative progenitor cells and maintenance of intestinal stem cells. Therapeutic targeting of ZIP7 could lead to effective treatment of gastrointestinal disorders.

3. Invariant Natural Killer T Cells Play Dual Roles in the Development of Experimental Autoimmune Uveoretinitis.

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Experimental autoimmune uveoretinitis (EAU) represents an experimental model for human endogenous uveitis, which is caused by Th1/Th17 cell-mediated inflammation. Natural killer T (NKT) cells recognize lipid antigens and produce large amounts of cytokines upon activation. To examine the role of NKT cells in the development of uveitis, EAU was elicited by immunization with a peptide from the human interphoto receptor retinoid-binding protein (hIRBP1-20) in complete Freund’s adjuvant and histopathology scores were evaluated in C57BL/6 (WT) and NKT cell-deficient mice. NKT cell-deficient mice developed more severe EAU pathology than WT mice. When WT mice were treated with ligands of the invariant subset of NKT cells (α-GalCer or RCAI-56), EAU was ameliorated in mice treated with RCAI-56 but not α-GalCer. IRBP-specific Th1/Th17 cytokines were reduced in RCAI-56-treated compared with vehicle-treated mice. Although the numbers of IRBP-specific T cells detected by hIRBP1-20/I-A^d tetramers in the spleen and the draining lymph node were the same for vehicle and RCAI-56 treatment groups, RORγt expression by tetramer-positive cells in RCAI-56-treated mice was lower than in control mice. Moreover, the eyes of RCAI-56-treated mice contained fewer IRBP-specific T cells compared with control mice. These re-
results suggest that invariant NKT (iNKT) cells suppress the induction of Th17 cells and infiltration of IRBP-specific T cells into the eyes, thereby reducing ocular inflammation. However, in sharp contrast to the ameliorating effects of iNKT cell activation during the initiation phase of EAU, iNKT cell activation during the effector phase exacerbated disease pathology. Thus, we conclude that iNKT cells exhibit dual roles in the development of EAU.


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A specialized bone marrow microenvironment (niche) regulates hematopoietic stem cell (HSC) self-renewal and commitment. For successful donor-HSC engraftment, the niche must be emptied via myeloablative irradiation or chemotherapy. However, myeloablation can cause severe complications and even mortality. Here we report that the essential amino acid valine is indispensable for the proliferation and maintenance of HSCs. Both mouse and human HSCs failed to proliferate when cultured in valine-depleted conditions. In mice fed a valine-restricted diet, HSC frequency fell dramatically within 1 week. Furthermore, dietary valine restriction emptied the mouse bone marrow niche and afforded donor-HSC engraftment without chemoirradiative myeloablation. These findings indicate a critical role for valine in HSC maintenance and suggest that dietary valine restriction may reduce iatrogenic complications in HSC transplantation.

5. Elucidation and Control of the Mechanisms Underlying Chronic Inflammation Mediated by Invariant Natural Killer T Cells.

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Invariant natural killer T (iNKT) cells are a subpopulation of T lymphocytes with unique specificities against (glyco) lipids presented in the context of CD1d belonging to major histocompatibility complex (MHC) class Ib. They recognize microbiologically encoded or synthetic glycolipids directly through their CD1d-restricted T-cell receptors (TCRs) other than microbial components or lipids including pathogen-associated molecular patterns (PAMPs) and bacterial superantigens. Once activated, iNKT cells participate as early effectors and/or regulators of immune responses. Immunoregulatory cytokines produced in copious amounts by these cells target a wide range of downstream effectors and help shape the ensuing immune responses. Mammals comprise various numbers and isoforms of CD1d molecules, suggesting that (glyco) lipid presentation is a rapidly evolving component of the immune system, which adapts to environmental threats. Recent progress in our understanding of CD1d-restricted iNKT cells contributes to their true potentials in immunotherapeutic applications for various diseases. Recent findings about iNKT cell subtypes, iNKT1, 2, 17, 10, and their roles in pathological inflammation are also introduced and discussed.

Publications


4. Taya, Y., Ota, Y., Wilkinson, A.C., Kanazawa, A.,