Stem Cells and Regenerative Medicine

Program

November 9, 2008

Venue: National Kyushu Medical Center

9:00

Opening Remarks

Yukio Fujiki

(Leader of Global COE Program, Kyushu University)

9:10-12:00

Session 1: Progress in Global COE

Chair: Yukio Fujiki, Keiichi Nakayama,

S1-1 (9:10-9:40)

GCOE: Two ubiquitin ligases control cell cycle in stem, progenitor, and differentiated cells

Keiichi Nakayama (Medical Institute of Bioregulation, Kyushu University)

S1-2 (9:40-10:10)

GCOE : Peroxisome: biogenesis, dysfunctions, and homeostasis

Yukio Fujiki (Graduate School of Systems Life Sciences, Kyushu University)

S1-3 (10:10-10:40)

GCOE: Receptors for bioactive lipid mediators

Takehiko Yokomizo (Graduate School of Medical Sciences, Kyushu University)

10:40-11:00 Coffee break

S1-4 (11:00-11:30)

GCOE: Recent progress of cancer stem cell research in gastrointestinal tumors

Hideshi Ishii (Medical Institute of Bioregulation, Kyushu University)

S1-5(11:30-12:00)

Reconstitution of human immunity and modeling human diseases in humanized mouse

Fumihiko Ishikawa (RIKEN Research Center for Allergy and Immunology, Yokohama)

12:00-13:30 Lunch

13:30-16:30

Session 2: Basic Aspects of Stem Cells I

Chair: Noriyuki Sagata

S2-1(13:30-14:10)

How selfish retrotransposons are silenced in Drosophila germline and somatic cells

Mikiko C. Siomi (Keio University School of Medicine)

S2-2(14:10-14:50)

Transcription factor network to maintain pluripotency

Hitoshi Niwa (RIKEN Center for Developmental Biology, Kobe)

14:50-15:10 Coffee break

S2-3 (15:10-15:50)

Mesoangioblasts for the cell therapy of muscular dystrophy

Giulio Cossu (Stem Cell Research Institute, San Raffaele Del Monte Tabor Foundation, Italy)

S2-4 (15:50-16:30)

Transcription factor C/EBP alpha regulates hematopoietic stem cell proliferation and maintenance

Daniel G. Tenen (Harvard Medical School, USA)

16:30-17:30

Young Investigators Forum (Poster Preview)

Chair: Hideki Sumimoto

Venue: National Kyushu Medical Center

Y-1: Comparing 2 profiles to predict functional sites

Hiroyuki Oda (Medical Institute of Bioregulation, Kyushu University)

Y-2: Ageing-related telomeric change and its acceleration by disease conditions

Toyoki Maeda (Medical Institute of Bioregulation, Kyushu University)

Y-3: Molecular evolutionary study of the ionotropic glutamate-receptor gene family as schizophrenia susceptibility genes: human-specific balancing selection in *GRIN2B* upstream region

Hiroki Shibata (Medical Institute of Bioregulation, Kyushu University)

Y-4: Architecture of the DNA ligase-PCNA-DNA complex and implications for mechanism assembling replication machinery

Kouta Mayanagi (Medical Institute of Bioregulation, Kyushu University)

Y-5: Structural and functional analyses of an ER-resident protein disulfide reductase, ERdj5
Kenji Inaba (Medical Institute of Bioregulation, Kyushu University)

Y-6: Phosphorylation-dependent activation of the mammalian formin FHOD1
Ryu Takeya (School of Medical Sciences, Kyushu University)

Y-7: Activity of c-*myc* intron binding protein 1 (MIBP1) as transcription factor and its modulation by O-GlcNAc transferase (OGT)
Yuji Iwashita (Medical Institute of Bioregulation, Kyushu University)

Y-8: Nemo-like kinase suppresses Notch signaling by interfering with formation of Notch active transcription complex
Tohru Ishitani (Medical Institute of Bioregulation, Kyushu University)

Y-9: Functional regulation of fatty acyl-CoA reductase 1 in plasmalogen biosynthesis
Masanori Honsho (Kyushu University Graduate School of Systems Life Sciences)

Y-10: Apparatus for proliferation and division of peroxisomes
Satoru Mukai (Kyushu University Graduate School of Systems Life Sciences)

Y-11: The ERK-MAPK pathway phosphorylates and targets Cdc25A for $SCF^{BETA\text{-}TRCP}$-dependent degradation for cell cycle arrest
Yoshinori Kanemori (Graduate School of Sciences, Kyushu University)

Y-12: Cell cycle inhibitor p57 is essential for cerebellar development: analysis of neuron-specific p57 conditional knockout mice
Akinobu Matsumoto (Medical Institute of Bioregulation, Kyushu University)

Y-13: Comprehensive identification of substrates for ubiquitin ligases that control cell-cycle using quantitative proteomics
Kanae Yumimoto (Medical Institute of Bioregulation, Kyushu University)

Y-14: Reversal of left-right asymmetry induced by ectopic expression of *Lefty* in mouse embryos
Shinya Oki (Graduate School of Medical Sciences, Kyushu University)

Y-15: The mitochondrial toxin, 3-nitropropionic acid induces MUTYH-dependent striatal neurodegeneration with accumulation of 8-Oxoguanine in medium spiny neurons and microglias which is effectively suppressed by OGG1 and MTH1
Zijing Sheng (Medical Institute of Bioregulation, Kyushu University)

Y-16: Human FLT3/FLK2 targets hematopoietic stem cells and granulocyte/macrophage progenitors
Yoshikane Kikushige (Kyushu University Graduate School of Medical Sciences)

Y-17: Elevated leukocyte alkaline phosphatase scores induced by the JAK2 V617F mutation
Seido Oku (Kyushu University Graduate School of Medical Sciences)

Y-18: Tbx3 controls hepatic stem cell fate
Atsushi Suzuki (Medical Institute of Bioregulation, Kyushu University)

Y-19: Adipose tissue-derived and bone marrow-derived mesenchymal cells develop into different lineage of steroidogenic cells by forced expression of SF-1
Shigeki Gondo (Department of Medicine and Bioregulatory Science, Kyushu University)

Y-20: The tumor associated role of GLTSCR2
Kohichi Kawahara (Medical Institute of Bioregulation, Kyushu University)

Y-21: Bone marrow and peripheral blood expression of *ID1* in human gastric carcinoma patients is a bona fide indicator of lymph node and peritoneal metastasis
Masaaki Iwatsuki (Medical Institute of Bioregulation, Kyushu University)

Y-22: Identification of cancer stem cells in human gastric adenosquamous carcinoma
Masahisa Ohkuma (Osaka University Graduate School of Medicine)

Y-23: Dificiency of leukotriene B4 receptors affects tumor metastasis
Hiroshi Okazaki (Graduate School of Medical Sciences, Kyushu University)

Y-24: Blocking LTB4 signaling confers the long-term antitumor effects induced by GM-CSF –transduced tumor in BLT-1 deficient mice
Hiroyuki Inoue (Medical Institute of Bioregulation, Kyushu University)

Y-25: T helper type 2 differentiation and intracellular trafficking of the interleukin 4 receptor-α subunit controlled by the Rac activator DOCK2

Yoshihiko Tanaka (Medical Institute of Bioregulation, Kyushu University)

Y-26: A critical role of CD30 ligand/CD30 in controlling inflammatory bowel diseases in mice

Xun Sun (Medical Institute of Bioregulation, Kyushu University)

18:00-20:00

Young Investigators Forum (Poster Presentation) with Buffet

Venue: JAL Resort Sea Hawk Hotel Fukuoka

18:00
Welcome Speech **Yukio Fujiki**
(Leader of Global COE Program, Kyushu University)
(Buffet will be served during the session)

18:05-18:30
Free discussion

18:30-19:30
Poster Discussion
Individual presentation time will not be provided, however all presenters in the poster session should be in front of each poster and handle questions during the following time.

18:30-19:00: Odd-numbered posters

19:00-19:30: Even-numbered posters

19:30-20:00
Free discussion to end

November 10, 2008

Venue: National Kyushu Medical Center

9:00-12:00
Session 3: Basic Aspect of Stem Cells II
Chair: Yusaku Nakabeppu, Kenzaburo Tani

S3-1 (9:00-9:40)

Genetically tagged reporter lines facilitate the monitoring of key steps in the generation of pancreatic beta cells during the in vitro differentiation mouse embryonic stem cells

Edouard G. Stanley (Monash University, Australia)

S3-2 (9:40-10:20)

Signalling pathways controlling cell fate specification of pluripotent stem cells

Ludovic Vallier (University of Cambridge, UK)

10:20-10:40 Coffee break

S3-3 (10:40-11:20)

The molecular regulation of muscle stem cell function

Michael A. Rudnicki (Ottawa Health Research Institute, Canada)

S3-4 (11:20-12:00)

Quiescence of hematopoietic stem cells

Toshio Suda (Keio University School of Medicine)

12:00-13:30 Lunch

13:30-14:50

Session 4: Clinical Aspects of Stem Cells

Chair: Koichi Akashi

S4-1 (13:30-14:10)

Therapeutic potential of human neural stem cells

Nobuko Uchida (StemCells Inc., USA)

S4-2 (14:10-14:50)

Pre-clinical development of hESC-based treatments for spinal cord injury

Hans S. Keirstead (University of California Irvine, USA)

14:50-15:40

Special Lecture

Chair: Koichi Akashi

Perspective: Stem cells and human diseases

Irving L. Weissman (Stanford University, USA)

15:40-15:50

Closing Remarks

Yasunobu Yoshikai

(Director of Medical Institute of Bioregulation, Kyushu University)

Session 1

(November 9, 2008, 9:10-12:00)

Progress in Global COE

November 9 (Sun.)
9:10-9:40

S1-1

TWO UBIQUITIN LIGASES CONTROL CELL CYCLE IN STEM, PROGENITOR, AND DIFFERENTIATED CELLS

Nakayama, K. I.[1], Onoyama, I.[1], Matsumoto, A.[1] and Nakayama, K.[2]
[1]Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan, and [2]Department of Developmental Biology, Graduate School of Medicine, Tohoku University, 2-1 Seiryo, Aoba-ku, Sendai 980-8575, Japan

Regulation of the exit of cells from the cell cycle is important in the development of multicellular organisms and is also implicated in the maintenance of stem cells. Furthermore, defects in cell cycle exit are thought to be a major cause of cancer. However, the mechanisms responsible for regulation of cell cycle exit have remained largely unknown. The F-box protein functions as a receptor subunit of the SCF-type ubiquitin ligase complex, which contribute to the ubiquitylation of many cell cycle regulators. Two F-box proteins Skp2 and Fbw7 were shown to play a significant role in cell cycle control. Skp2 targets cell cycle inhibitors such as p27 for degradation, whereas Fbw7 contributes to the proteolysis of c-Myc, cyclin E, Notch, and c-Jun, all of which appear to function as cell cycle activators and oncogenic proteins. However, the in vivo roles of these F-box proteins have been poorly understood.

We created Skp2-deficient mice and demonstrated that Skp2-p27 system is critical to regulate the cell cycle re-entry. In T cell development, for example, p27 knockout mice display thymic hyperplasia, whereas Skp2 knockout mice show marked hypoplasia. Given that the conventional Fbw7 knockout mice are early embryonic lethal, we generated a series of tissue-specific Fbw7 conditional knockout (CKO) mice. In T cell-specific Fbw7 CKO mice, T cells continued to proliferate even at $CD4^{+}CD8^{+}$ stage, in which cells normally exit from the cell cycle. These mice showed thymic hyperplasia, and finally developed T cell lymphomas. Immunoblot analysis revealed the levels of c-Myc and Notch1 were abnormally elevated in Fbw7-deficient T cells. Inactivation of c-Myc but not Notch resulted in disappearance of hyperproliferation of thymocytes. In contrast, peripheral T cells showed a defect in cell proliferation upon mitogenic stimulation with abnormal elevation of c-Myc and p53. This defect was not observed in Fbw7/p53 double knockout T cells, suggesting that c-Myc accumulation induced p53, resulting in cell cycle arrest and apoptosis. Similar results were obtained in mammary gland-specific Fbw7 CKO mice.

Analysis of bone marrow-specific Fbw7 CKO mice revealed that Fbw7 is essential for G0 maintenance. Fbw7-deficient stem cells have lost the ability to reconstitute of bone marrow by transplantation assay, suggesting that Fbw7 plays a key role in the maintenance of stem cells by suppression of excessive cell cycling. These results indicate that Skp2 and Fbw7 contribute to cell cycle regulation in vivo in a differentiation-specific manner.

November 9 (Sun.)
9:40-10:10

S1-2

PEROXISOME: BIOGENESIS, DYSFUNCTIONS, AND HOMEOSTASIS

Yukio Fujiki
Department of Biology, Faculty of Sciences, Kyushu University Graduate School of Systems Life Sciences, 6-10-1 Hakozaki, Fukuoka 812-8581; JST, CREST, Tokyo 102-0075, Japan

Peroxisome is a ubiquitous organelle that functions in essential metabolic pathways including β-oxidation of very long chain fatty acids and synthesis of ether-linked phospholipids, plasmalogens. Human peroxisome biogenesis disorders (PBDs) such as Zellweger syndrome (ZS) and neonatal adrenoleukodystrophy are autosomal recessive disease comprising 13 complementation groups (CGs). I address several issues in my talk:
1) To elucidate pathogenic genes responsible for PBDs of 13 different CGs, we have taken advantage of forward genetics using peroxisome-deficient CHO cell mutants as well as human EST database search with yeast *PEX* genes encoding peroxisome assembly factors, termed peroxins. We have isolated or identified most of 13 *PEX*s including the first ZS gene *PEX2* (*Nature* 1991; *Science* 1992) and the last *PEX26* (*NCB* 2003). Given the outstanding progress made by many groups of investigators including ours, the mission to the search for PBD pathogenic genes of 13 CGs has just been accomplished (*NCB* 2003; *AJHG*, 2003). Dysmorphogenesis of peroxisomes is also reported, apparently causing human ZS-like disease.
2) Peroxisome targeting signal 1 (PTS1) receptors, Pex5pS and Pex5pL, function in matrix protein import in mammals. Pex5pL also translocates the PTS2 receptor Pex7p-PTS2 complexes. Pex5p-cargo complexes initially dock with Pex14p of the import machinery (*MCB* 2005; *JBC* 2006). Our most recent evidence shows that Pex5p and Pex7p are imported in an ATP-independent manner whereas the import of PTS1 proteins is ATP-dependent. Pex5p requires the AAA ATPase Pex1p-Pex6p complexes docked with Pex26p and ATP-driven energy at the export step in its peroxisome-cytoplasmic shuttling.
3) Pex3p, Pex16p, and Pex19p are peroxisome membrane assembly factors. Pex19p serves as a chaperone and a transporter for peroxisomal membrane proteins (PMPs), where Pex3p functions as the membrane receptor for Pex19p-PMP complexes (*JCS* 2006). With respect to biogenesis of Pex3p, we herein demonstrate for the first time that Pex16p functions as the peroxisomal membrane receptor specific to the Pex3p-Pex19p complexes *(JCB*, in press).
4) Degradation systems of peroxisomes in mammalian cells remain unknown. Here, we show that peroxisomal proteins are degraded preferentially over cytosolic proteins in wild-type CHO-K1 cells when starved and then cultured in a normal culture medium. Peroxisome degradation is dependent on microtubule-associated protein I light chain 3 (LC3), a yeast Atg8 orthologue, and requires Pex14p known as a peroxin essential for peroxisome biogenesis (*ECR*, in press). We address two pathways for peroxisome degradation involving autophagy and ubiquitin-proteasome systems.

November 9 (Sun.)
10:10-10:40

S1-3

RECEPTORS FOR BIOACTIVE LIPID MEDIATORS

Yokomizo, T.
Department of Medical Biochemistry, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan

We have identified various G-protein coupled receptors for lipid mediators and protons, including BLT1 as a high-affinity leukotriene B4 (LTB4) receptor (1), BLT2 as a low-affinity LTB4 receptor (2), and G2A as a proton-sensing receptor (3). Studies using BLT1-KO mice revealed that LTB4 plays important roles in the development of Th1/Th2 types of immunological reactions in addition to its classical roles in inflammation (4). BLT1 serves as a good model of GPCR biochemistry, and we identified responsible domains of BLT1 in G-protein coupling and novel roles of intracellular helix 8 in receptor inactivation. Recently, we purified and identified 12-hydroxyheptadeca-5, 8, 10-trienoic acid (12-HHT) as a high-affinity ligand for BLT2 (5). 12-HHT has been recognized as a byproduct of thromboxane A2 biosynthesis without any biological function; however, our finding suggests a novel biological roles of 12-HHT-BLT2 axis *in vivo.*

(1) Yokomizo *et al*. ***Nature*** 387, p620-624 (1997)
(2) Yokomizo *et al*. ***J. Exp. Med.*** 192, p421-432 (2000)
(3) Murakami et al. ***J. Biol. Chem.*** 279, p42484-42491 (2004)
(4) Terawaki *et al*. ***J. Immunol.*** 175, p4217-4225 (2005)
(5) Okuno *et al*. ***J. Exp. Med.*** 205, p759-766 (2008)

November 9 (Sun.)
11:00-11:30

S1-4

Recent Progress of Cancer Stem Cell Research in Gastrointestinal Tumors

Hideshi Ishii[1, 2] and Masaki Mori[1,2]
[1]Department of Surgery and Molecular Oncology, Medical Institute of Bioregulation, Kyushu University, 4546 Tsurumihara, Beppu 874-0838, Japan; [2]Department of Gastroenterological Surgery, Osaka University, Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Recent discovery of rare subpopulations of cancer-initiating cells (CICs) has created a new focus in cancer research. As accepted in hematopoietic and presumably in solid tumors, CICs mimic normal adult stem cells by demonstrating the capability of self-renewal and the potential to divide and generate almost functional elements of tumors. CICs are relevant to resistance to toxic injuries and chemoradiation therapies. Although there are many uncertainties regarding the theoretical and interpretational aspects of the data supporting it, the CIC model could be used to explain the so-called biological heterogeneity of tumors. The extreme biological heterogeneity caused by the CICs could lead to a lack of consistency in clinical treatment planning, considering that similar cases under a clinico-pathological point of view may differ widely in prognosis in cancers such as gastrointestinal tract. Indeed a rare CIC population of undifferentiated gastrointestinal cancer cells remains to be explored fully. To investigate the biological and genetic characteristics of CICs in gastrointestinal cancer in vitro and in vivo analyses, we searched candidate markers of colon CICs, starting from the basis of the CD133 (PROM1, prominin-1) expression, a stem cell marker. Results indicated that CD133-positive cells were found in more than predominant fractions of colon cancer cell lines examined. Isolated marker-positive cells from the gastrointestinal cancer cell lines exhibited a higher tumorigenic potential than marker-negative cells in tumor formation assay. CD133-positive cells, apparently in a double positive to another marker CD44, were more proliferative and exhibited higher colony-forming and invasive abilities than their negative cells. By using microarray analysis, we looked into differentially expressed genes correlating with surface marker expression, which resulted in the identification of several associated molecules with relevance to the characteristics of CICs. The present study shows that gastrointestinal cancer is created and propagated by a small number of undifferentiated tumorigenic CICs, which exhibit high proliferation potential, and fastens the rationale to characterize the rare population of CICs for clinical setting. While the role of somatic mutations has been extensively documented in determining tumor phenotype, the detection and biological regulation of CICs during carcinogenesis could be undoubtedly a critical issue for the development of the effective prevention, diagnostic, and novel therapeutic approaches to gastrointestinal cancers.

Fumihiko Ishikawa

Degree

1995 October: United States Medical License Examination Step 1 (Basic Medical Science) passed

1996 November: United States Medical License Examination Step 2 (Clinical Medical Science) passed

1997 May: Japan Medical License Examination (M.D.) passed

2003 March 25th: Ph.D. (Kyushu University)

Educational and Employment History

1991: Admission to Kyushu University Medical School

1997 Mar.: Graduation from Kyushu University Medical School

1997 Apr.: Residency training at First Department of Medicine, Kyushu University

1997 Oct: Residency training at Third Department of Medicine, Kyushu University

1998 Apr-2000 Sep and 2002 Apr-2003 Mar：Kyushu University Graduate School of Medical Sciences

1998 Oct- 2002 Mar：Medical University of South Carolina, Department of Medicine, Division of Experimental Hematology (Makio Ogawa lab), Post-doctoral fellow

2003 Apr-2005 Mar：Japan Society for Promotion of Science, Post-doctoral fellow

2005 Apr-2005 Dec: Instructor, Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences

2006 Jan-present: Unit leader, Research Unit for Human Disease Model, RCAI

November 9 (Sun.)
11:30-12:00

S1-5

Reconstitution of human immunity and modeling human diseases in humanized mouse

Fumihiko Ishikawa
Research Unit for Human Disease Models, RIKEN, Research Center for Allergy and Immunology, Yokohama, JAPAN

To develop suitable *in vivo* models of human normal hematopoietic and immune systems as well as diseases involving these systems, immunosuppressed mouse strains have been constructed in the past. However, incomplete immune-suppression, the short life spans and age-dependent leakiness of murine humoral immunity have presented obstacles for achieving high levels of human cell engraftment for long-term. To overcome these problems, we have created a humanized mouse system by transplanting purified human HSCs into a novel immunodeficient strain, NOD/SCID/IL2rg^{null} mouse during the newborn period. This strain, with the life expectancy of >90 weeks, is more robust, allowing the assessment of reconstitution and immune system development of human HSCs and progenitor cells. Using NOD/SCID/IL2rg^{null} newborns, we have created the humanized mouse with a full representation of human hematopoietic and immune systems. Engrafted human T cells and B cells undergo physiological maturation process in primary and secondary lymphoid organs of the recipients. These findings suggest that the NOD/SCID/IL2rg^{null} newborn transplant model is a powerful model to study human immunohematopoietic system *in vivo*. Currently we are creating mouse models for human leukemia and investigating the biology of human leukemic stem cells using the xenotransplantation model.

Session 2

(November 9, 2008, 13:30-16:30)

Basic Aspects of Stem Cells Ⅰ

Mikiko C. Siomi

Education

1980 - 1984	Undergraduate Student in the Faculty of Agriculture Gifu University, Obtained B.Ag.
1986 - 1988	Graduate Student in the Faculty of Agriculture Kyoto University, Obtained M.Ag.
1994	Obtained PhD. in the Agricultural Chemistry Kyoto University
2003	Obtained Ph.D. in the Medical Science University of Tokushima

Research and professional experience

1994 - 1999	Post-Doctoral Fellow at the Howard Hughes Medical Institute, University of Pennsylvania School of Medicine (Dr. Gideon Dreyfuss's laboratory)
1999 - 2002	Assistant Professor at the Institute for Genome Research, University of Tokushima
2002 - 2008	Associate Professor at the Institute for Genome Research University of Tokushima
2008 - present	Associate Professor at Keio University School of Medicine

November 9 (Sun.)
13:30-14:10

S2-1

HOW SELFISH RETROTRANSPOSONS ARE SILENCED IN DROSOPHILA GERMLINE AND SOMATIC CELLS

Siomi, M.C.
Department of Molecular Biology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan

Gene silencing pathways triggered by small RNAs are generically called RNA silencing. Extensive studies on RNA silencing mechanisms revealed that members of the Argonaute family play important roles in the pathways. In *Drosophila*, the Argoanute family, defined by the presence of PAZ and PIWI domains, consists of five members, which includes AGO1, AGO2, AGO3, Piwi, and Aubergine.
Previously, we have shown that AGO1 and AGO2 in *Drosophila* function in gene silencing through specific binding with miRNA and siRNA, respectively. siRNA-loaded AGO2 functions in RNAi as Silcer, directly responsible for cleaving a target completely complementary to siRNA. miRNA-associated AGO1 is thought to repress translation of target mRNAs without cleaving them. Piwi, Aubergine, and AGO3 (the PIWI proteins) are specifically associated with a subset of endogenous small RNA, Piwi-interacting RNAs (piRNAs). piRNAs are 24-30 nt long, only expressed in gonads, and function in genome surveillance through association with the PIWI proteins by silencing mobile elements. Bioinformatics analysis of piRNAs revealed that Aub- and Piwi-piRNAs are mainly derived from antisense transcripts, whereas AGO3-piRNAs arise mostly from sense transcripts. The first 10 nt of Aub-piRNA showed complementarities to those of AGO3-piRNAs. The PIWI proteins exhibit Slicer activity as does AGO2. From these data, a model for piRNA biogenesis was proposed[1)], in which the AGO3-piRNA complex cleaves antisense retrotransposon transcripts and forms 5' ends of antisense piRNAs for Aub and Piwi. The newly made Aub- and Piwi-piRNAs lead to the formation 5' end sense piRNAs for AGO3 by cleavage of sense retrotransposon transcripts. These reciprocal reactions would occur continuously in vivo and constantly produce piRNAs. Concomitantly, retrotransposon silencing would be maintained. Deep-sequencing of small RNAs associated with AGO2 in *Drosophila* somatic cell culture has identified a novel class of ~21 nt RNAs derived from retrotransposons and other repetitive elements. We named them as endogenous siRNAs (esiRNAs)[2)]. Processing of esiRNAs was found to require Dicer2. Flies deficient in *Dicer2* or *Ago2*, and also S2 cells lacking Dicer2, show an increased expression of retrotransposons. It is concluded that *Drosophila* has two RNA silencing pathways that repress retrotransposons[3)].

Related references;
1) Gunawardane, L. S. *et al.* A Slicer-mediated mechanism for repeat-associated siRNA 5' end formation in *Drosophila*. *Science* 315, 1587-1590 (2007). 2) Kawamura, Y. *et al.* *Drosophila* endogenous small RNAs bind to Argonaute 2 in somatic cells. *Nature* 453, 793-797 (2008). 3) Siomi, M.C. *et al.* How selfish retorotransposons are silenced in *Drosophila* germline and somatic cells. *FEBS Letters* 582, 2473-2478 (2008)

Hitoshi Niwa

Education

1983-1989	Nara Medical University Award the degree of MD (1989 Medical License No.327191)
1989-1993	Kumamoto University Graduate School of Medicine Award the degree of PhD in medical physiology for a thesis entitled "An Efficient Gene-Trap Method Using Poly A Trap Vectors and Characterization of Gene-Trap Events". Work supervised by Professor Ken-ichi Yamamura in Department of Developmental Genetics, Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine.

Professional experience

1993-1994	Research Associate, Department of Developmental Genetics, Institute of Molecular Embryology and Genetics, Kumamoto University School of Medicine, working with Professor Ken-ichi Yamamura.
1994-1996	Postdoctoral Fellow, Centre for Genome Research, University of Edinburgh, working with Dr Austin Smith.
1996-2001	Research Associate, Department of Nutrition and Physiological Chemistry, Osaka University Graduate School of Medicine, working with Professor Jun-ichi Miyazaki.
2001-	Laboratory Head, Laboratory for Pluripotent Cell Studies, RIKEN Center for Developmental Biology
2002-	A guest professor, Laboratory for Developmental and Regenerative Medicine, Kobe University Graduate School of Medicine

November 9 (Sun.)
14:10-14:50

S2-2

TRANSCRIPTION FACTOR NETWORK TO MAINTAIN PLURIPOTENCY

Niwa, H
Laboratory for Pluripotent Cell Studies, RIKEN Center for Developmental Biology (CDB), 2-2-3 Minatojima-minamimachi, Chuo-ku, Kobe 650-0047, Japan

Pluripotency is defined by the combination of the transcription factors *a priori*. The function of the multiple transcription factors is the transcriptional regulation of the target genes including themselves. To determine the cellular phenotype, cross-regulation between transcription factors and co-operation of multiple transcription factors to regulate the common target genes are the important features to compose the self-organizing network. In addition, the overlapped function of multiple transcription factors with the similar molecular features contributes to complex structure of the network. The transcription factor network should achieve stability under the certain combination of the signal transduction as well as flexibility to mediate rapid and complete transition to the differentiated states. To draw up the basic scheme of the transcription factor network, its experimental modification is important to observe the dynamics of the network coupling with the biological response. If over-expression of one transcription factor can support the maintenance of the phenotype without the particular signal that is essential for it in the conventional condition but others cannot, that will indicate the functional hierarchy between them along this signal. When the subset of transcription factors are missing under the particular set of signals or genetic modifications but others are maintained, that will divide the transcription factors into two functional categories. A series of functional studies of transcription factors in mouse ES cells revealed that there are two classes of transcription factors; one mainly involves in the signal integration and processing, and other mostly works to maintain the network. One interest feature of the transcription factors in the former class is their heterogeneous expression in cell population, whereas that of the latter class distribute quite homogeneously among the population. These different classes of transcription factors look to compose sub-network structures that confer their functional hierarchy. These conceptual views will help clear understanding of the transcription factor network to maintain pluripotency.

Giulio Cossu

Current Position:

2005- Professor of Histology, Department of Biology, University of Milan

2000- Director, of the “Stem Cell Research Institute”, San Raffaele Scientific Insitute.

Previous appointments:

1994-2005: Professor of Histology II° Medical School, University of Rome "

1993-1994: Visiting Professor. Dept. of Molecular Biology, Istitut Pasteur, Paris

1986-1993: Associate Professor of Histology, University of Rome

1983-1986: Researcher, Institute Histology, University of Rome

1980-1983: USPHS Fogarty Fellow Wistar Institute, University of Pennsylvania

1978-1980: CNR Fellow, Institute Histology, University of Rome "La Sapienza"

Post-doctoral training:

1980-1983: USPHS Fogarty Fellow Wistar Institute, University of Pennsylvania

1978-1980: CNR Fellow, Institute Histology, University of Rome "La Sapienza"

Education:

1977: MD degree with honors, Faculty of Medicine, University of Rome “La Sapienza”

1972: High School Degree, Liceo Dante Alighieri, Rome

November 9 (Sun.)
15:10-15:50

S2-3

MESOANGIOBLASTS FOR THE CELL THERAPY OF MUSCULAR DYSTROPHY

Cossu, G., Messina, G., Dellavalle, A., Benedetti, S., Tedesco, S., Tonlorenzi. R.
Stem Cell Research Institute, Dibit, San Raffaele, Milan, Italy

Mesoangioblasts are recently characterized progenitor cells, associated with the vasculature and able to differentiate in different types of solid mesoderm, including skeletal muscle (Minasi et al. Development 129, 2773, 2002).

When both wild type or dystrophic, genetically corrected, mesoangioblasts were delivered intra-arterially to dystrophic muscle of α-sarcoglycan KO mice (a model for limb girdle muscular dystrophy), they resulted in a dramatic functional amelioration of the dystrophic phenotype. This was due to the widespread distribution of donor cells through the capillary network (Sampaolesi et al. Science 301, 487, 2003).

Intra-arterial delivery of wt mesoangioblasts, non DLA matched to GRMD dystrophic dogs resulted in a partial recovery of muscle morphology and function, dystrophin expression and clinical amelioration. Delivery of autologous mesoangioblasts, genetically corrected to express human micro-dystrophin, did not result in a similar amelioration, despite widespread expression of micro-dystrophin. Delivery of autologous mesoangioblasts expressing human micro-dystrophin did not cause a comparable amelioration, despite widespread micro-dystrophin expression (Sampaolesi et al. Nature 444, 574, 2006). These results show efficacy of cell therapy in a large, immuno-competent animal and set the rationale for a future clinical trial, using donor cells from an HLA-matched donor under immune suppression. Moreover, human adult mesoangioblasts were isolated and expanded in vitro from muscle biopsies: they were shown to correspond to a subset of pericytes (Dellavalle et al. Nature Cell Biol. 9, 255, 2007).

Based on these results a first clinical trial with donor mesoangioblasts is planned for the near future. Problems still facing this approach and possible strategies to overcome them will be discussed.

This work was supported by grants from the European Community, Duchenne Parent Project, MDA, AFM and the Italian Ministry of Research.

Daniel G. Tenen

Education:

1971 B.A.	University of California, Los Angeles, CA
1975 M.D.	Harvard Medical School, Boston, MA

Internship and Residencies:

1975-1976	Intern in Medicine, Peter Bent Brigham Hospital, Boston
1976-1977	Junior Resident, Internal Medicine, Peter Bent Brigham Hospital, Boston
1978-1982	Senior Assistant Resident, Peter Bent Brigham Hospital, Boston

Research Fellowships:

1977-1979	Research Fellow in Medicine, Dana-Farber Cancer Institute and Harvard Medical School, Boston
1979-1982	Cancer Research Scholar, American Cancer Society, Massachusetts Division (at Dana-Farber Cancer Institute)
1982-1983	Fellow in Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston
1983-85	James M. Faulkner Fellow of The Medical Foundation, Boston (at Dana Farber Cancer Institute)

Licensure and Certification:

1977	Massachusetts License Registration No. 39824
1980	American Board of Internal Medicine, Certificate No. 076137
1983	Diplomate, American Board of Internal Medicine, Subspecialty of Medical Oncology

Academic Appointments:

1978-1986	Instructor in Medicine, Harvard Medical School, Boston
1986-1993	Assistant Professor of Medicine, Harvard Medical School
1994-1999	Associate Professor of Medicine, Harvard Medical School
1999-	Professor of Medicine, Harvard Medical School
2008-	Saw Swee Hock Centennial Professor in Medical Sciences, National University of Singapore

Hospital Appointments:

1977-1978	Assistant in Medicine, Peter Bent Brigham Hospital, Boston
1979-1996	Clinical Associate, Dana-Farber Cancer Institute, Boston
1984-1988	Assistant in Medicine, Beth Israel Hospital, Boston
1988-	Associate Physician, Beth Israel Hospital, Boston

November 9 (Sun.)
15:50-16:30

S2-4

Transcription Factor C/EBP alpha Regulates Hematopoietic Stem Cell Proliferation and Maintenance

Daniel G. Tenen
Professor of Medicine, Harvard Medical School
Saw Swee Hock Centennial Professor in Medical Sciences, National University of Singapore

Hematopoietic stem cells (HSCs) undergo an abrupt change from an actively cycling state to largely quiescent in bone marrow 3 weeks after birth. However, little is known about how this switch is regulated. Here we report that levels of C/EBP alpha, a transcription factor that is frequently disrupted in human acute myeloid leukemia, regulate the proliferative states of HSCs. C/EBP alpha excision in adult mice results in a significant expansion of HSCs and elevated proliferation rates, indicating C/EBP alpha functions as a mitotic inhibitor in adult HSCs. Interestingly, HSCs show a rapid increase in C/EBP alpha expression 3 weeks after birth.
Consistent with levels of its expression, loss of C/EBP alpha in 4- week old mice results in a large expansion of HSCs, while only a minor change is observed in C/EBP alpha deficient newborn mice.
Furthermore, C/EBP alpha expression is diminished in adult cycling HSCs following cytotoxic cyclophosphamide treatment, suggesting that down-regulation of C/EBP alpha might contribute to the re-activation of quiescent adult HSCs. Gene profiling analysis of C/EBP alpha-/- HSCs shows up-regulation of Notch 3 and 4 and down-regulation of their inhibitors, indicating enhanced activation of Notch signaling, a signal pathway that has been implicated in promoting HSC expansion.
Finally, C/EBP alpha deficiency also causes impaired adhesion and retention of HSCs, leading to massive egress of HSCs from BM to distal organs and a repopulating failure.

Session 3

(November 10, 2008, 9:00-12:00)

Basic Aspects of Stem Cells Ⅱ

Edouard G. Stanley

POSITION TITLE: Laboratory Head Monash Immunology and Stem Cell Laboratories Monash University			
EDUCATION/TRAINING			
INSTITUTION AND LOCATION	**DEGREE**	**YEAR(s)**	**FIELD OF STUDY**
Melbourne University (Australia)	B.Sc. (Honors)	1980-83	Genetics & Biochemistry
Ludwig Institute/Melbourne University (Australia)	Ph.D.	1984-89	Molecular Biology
Ludwig Institute (Australia)		1989-92	ES cells/Haematopoiesis
National Institute of Medical research (UK)		1993-96	ES cells/Embryology
Walter & Eliza Hall Institute (Australia)		1996-200	ES cells/Embryology

Positions

1989-92 Research Officer, Molecular Biology Laboratory, Ludwig Institute for Cancer Research

1992-93 Recipient of Human Frontiers in Science Program Organization long-term fellowship (London).

1993-95 Recipient of C.J. Martin Fellowship, National Institute for Medical Research (London, UK)

1996-98 Recipient of C.J. Martin Fellowship**,** Walter & Eliza Hall Institute (Australia)

1998-2000 AMRAD Corporation funded Post Doctoral Position, Walter & Eliza Hall Institute (Australia)

2000–02 Dual Appointment: Senior Research Officer & Laboratory Head, Genetically Modified Mouse Laboratory. Walter and Eliza Hall Institute for Medical Research

2002- Laboratory Head, Embryonic Stem Cell differentiation Laboratory, Monash University.

November 10 (Mon.)
9:00-9:40

S3-1

Genetically tagged reporter lines facilitate the monitoring of key steps in the generation of pancreatic beta cells during the in vitro differentiation mouse embryonic stem cells.

Edouard G. Stanley
Embryonic Stem Cell Differentiation Laboratory, Monash University, Level 3, Building 75, Clayton, Victoria 3800, Australia
ed.Stanley@med.monash.edu.au

Embryonic Stem Cell (ESC) differentiation represents a tractable system for understanding the molecular events that govern the progressive steps of lineage restriction that occur during the transition of cells from pluripotency to fully functional end cells. We have used genetically tagged mouse ESCs to investigate processes by which undifferentiated cells sequentially pass through steps representing mesendoderm and pancreatic endoderm, as they differentiate toward Insulin^{+} beta cells. These experiments utilised a doubly tagged reporter line in which a human CD4 cDNA has been inserted into the primitive streak gene, *Mixl1*, and sequences encoding green fluorescent protein (GFP) have been inserted in the *Pdx1* locus, a gene that marks pancreatic endoderm as well as insulin^{+} beta cells. In addition, a second line harbouring a GFP reporter at the *Pdx1* locus and a red fluorescent protein (RFP) gene at the *Insulin1* locus (*Pdx1*$^{GFP/w}$*Ins1*$^{RFP/w}$) has been used to monitor the transition from pancreatic endoderm towards endocrine lineages. This analysis shows that even though it is an obligate intermediate, generation of mesendoderm per se, is a poor prognostic indicator of subsequent differentiation outcomes. Similarly, *Pdx1* expression is not sufficient to guarantee the subsequent emergence of endocrine lineages. Instead, our studies suggest that complex cell-cell interactions within cultures of differentiating cells conspire to determine eventual differentiation outcomes. These studies provide an insight in possible strategies for optimizing the differentiation of ESCs towards therapeutically useful cell types, including insulin producing beta cells for the treatment of type 1 diabetes.

Ludovic Vallier

Academic and Previous employment

September 2008-Present: MRC senior non-clinical fellowship and Junior Group Leader Cambridge Laboratory for Regenerative Medicine.
Molecular mechanisms controlling differentiation of pluripotent cells into endoderm and into pancreatic cells. Affiliated to Department of Surgery.

March 2005-2008: MRC/UK Diabetes Career Development Fellowship.
Molecular mechanisms controlling cell fate specification of human embryonic stem cells toward the endoderm germ layer.
Affiliated to Professor Roger Pedersen's group, Department of Surgery, University of Cambridge

February 2002-2005: Research Associate Stem Cell group.
Mechanisms controlling pluripotency and cell fate specification of human embryonic stem cells
In Professor Roger Pedersen's group, Department of Surgery, University of Cambridge

2001-2002: Project leader. Scientific responsibilities in genOway, a private company generating transgenic animals and in vivo models for biopharmaceutical companies.
genOway, Lyon, France.

Education

1997-2001: Ph.D at Ecole Normale Superieure of Lyon. Inducible expression system to control gene expression in mouse embryonic stem cells.
Professor Samarut's group. Ecole Normale Superieure de Lyon

1996-1997: Military Service. Officer in Charge of public relations in the " Patrouille de France" Salon de Provence.

1995-1996: DEA Différenciation, Génétique et Immunologie
Universite Claude Bernard Lyon 1

1992-1995: Magistère de biologie moléculaire et cellulaire
Ecole Normale Supérieure de Lyon.

1992: Admission to Ecole Normale Supérieure de Lyon.

1990-1992: DEUG de biologie
Université Claude Bernard Lyon 1

November 10(Mon.)
9:40-10:20

S3-2

SIGNALLING PATHWAYS CONTROLLING CELL FATE SPECIFICATION OF PLURIPOTENT STEM CELLS

Ludovic Vallier
Department of Surgery and Laboratory For Regenerative Medicine, West Forvie Building, Robinson Way, University of Cambridge, Cambridge, CB2 0SZ, United Kingdom.

The pluripotent status of human embryonic stem cells (hESCs) confers upon them unique value for regenerative medicine, as they are capable of generating a large variety of cell types. However, generating fully functional cells from hESCs and achieving this goal using clinically-compatible conditions remain major challenges owing to the presence of undefined components in standard culture media and differentiation protocols (including animal-derived serum, feeder cells and extracellular matrices). Modelling the early steps of embryonic development in vitro may provide the best approach for generating differentiated cells with native properties. The earliest cell fate decisions during mammalian embryonic development are the specification of extra-embryonic tissues starting with the trophectoderm at the late morula stage and then the primitive endoderm at the late blastocyst stage. Differentiation of definitive embryonic tissues occurs at the gastrulation stage with the specification of the ectoderm and mesendoderm. Ectoderm subdivides into neural, skin and neural crest progenitors, and mesendoderm subdivides into definitive mesoderm and endoderm from which most organs are derived. We recently developed and validated chemically defined culture conditions for achieving specification of human and mouse pluripotent stem cells into extra-embryonic tissues, neuroectoderm and mesendoderm. We also showed that these progenitors could further differentiated into pancreatic and liver cells demonstrating their capacity to produce mature cell type. Finally, these defined culture conditions are devoid of animal products, thereby eliminating factors that could obscure analysis of developmental mechanisms or render the resulting tissues incompatible with future clinical applications.

Michael A. Rudnicki

Educational Background:

1982 B.Sc.	Biology (Honours), University of Ottawa, Canada. Cell and Molecular Biology.
1988 Ph.D.	Biology, University of Ottawa, Canada. "Embryonal carcinoma derived cardiac muscle: Studies in gene expression and differentiation." Supervisor: Dr. M.W. McBurney

Current Status:

2005 - present	Scientific Director, Canadian Stem Cell Network
2001 - 2007	Senior Scientist & Director, Regenerative Medicine Program Director, Sprott Centre for Stem Cell Research Ottawa Health Research Institute
2000 - present	Professor Department of Medicine University of Ottawa

Employment History:

2000 - 2001	Senior Scientist & Head, Program in Molecular Genetics Ottawa Hospital Research Institute
2000 - 2005	Part-Time Faculty Status Department of Pathology and Molecular Medicine McMaster University
1997 - 2000	Approval granted for Tenure and Promotion to Associate Professor, McMaster University
1997 - 2000	Associate Membership Department of Biochemistry, McMaster University
1992 - present	Associate Membership Department of Biology, McMaster University
1992 - 1997	Assistant Professor, McMaster University Department of Pathology and Molecular Medicine Institute for Molecular Biology and Biotechnology
1988 - 1992	Post-doctoral Fellow Whitehead Institute, Cambridge, U.S.A. "Production and analysis of mouse developmental mutants." Supervisor: Dr. Rudolf Jaenisch.

November 10 (Mon.)
10:40-11:20

S3-3

THE MOLECULAR REGULATION OF MUSCLE STEM CELL FUNCTION

Michael A. Rudnicki
Ottawa Health Research Institute, The Sprott Centre for Stem Cell Research, Ottawa, Ontario, Canada, K1H 8L6.

Satellite cells purified from adult skeletal muscle can participate extensively in muscle regeneration and can also repopulate the satellite cell pool, suggesting that they have direct therapeutic potential for treating degenerative muscle disease1. The paired-box transcription factor Pax7 is required for satellite cells to give rise to committed myogenic progenitors. The question of whether satellite cells are stem cells, committed progenitors or de-differentiated myoblasts has remained unclear. Using Myf5-Cre and ROSA26-YFP Cre alleles, we observed that in vivo 10% of sub-laminar Pax7-expressing satellite cells have never expressed Myf5. Moreover, we found that Pax7+/Myf5- satellite cells gave rise to Pax7+/Myf5+ satellite cells through apical-basal oriented divisions that asymmetrically generated a basal Pax7+/Myf5- and an apical Pax7+/Myf5+ cells. Prospective isolation and transplantation into muscle revealed that whereas Pax7+/Myf5+ cells exhibited precocious differentiation, Pax7+/Myf5- cells extensively contributed to the satellite cell reservoir throughout the injected muscle. Therefore, we conclude that satellite cells are a heterogeneous population composed of stem cells and committed progenitors. Using comparative microarray analysis, we identified several novel and strongly regulated targets; significantly we identified Myf5 as a gene whose expression was regulated by Pax7. Using siRNA, Fluorescent Activated Cell Sorting (FACS) and Chromatin Immunoprecipitation studies we confirmed that Myf5 is directly regulated by Pax7 in satellite cell derived myoblasts. Furthermore, tandem affinity purification (TAP) and mass spectrometry were used to purify Pax7 together with its co-factors. This revealed that Pax7 associates with the Wdr5/Ash2L/MLL2 histone methyltransferase (HMT) complex that directs methylation of histone H3 lysine 4 (H3K4)4-10. Importantly, binding of the Pax7-HMT complex to Myf5 resulted in H3K4 tri-methylation of surrounding chromatin. Thus, Pax7 induces chromatin modifications that stimulate transcriptional activation of target genes to regulate entry into the myogenic developmental program.

Toshio Suda

Education and Occupation:

1974 Graduated from Yokohama City University School of Medicine

1974- Junior and Senior Resident, Kanagawa Children's Medical Center

1978- Research Associate, Division of Hematopoiesis, Institute of Hematology,
Jichi Medical School

1982- Research Associate, Department of Medicine,
Medical University of South Carolina (Dr. Makio Ogawa's Lab)

1983- Assistant Professor, Division of Hemopoiesis, Institute of Hematology,
Jichi Medical School

1991- Associate Professor, Division of Hematology, Department of Medicine,
Jichi Medical School

1992- Professor, Department of Cell Differentiation,
Institute of Molecular Embryology and Genetics,
Kumamoto University School of Medicine

2002-Present
Professor, Developmental Biology, The Sakaguchi Laboratory, School of Medicine, Keio University

2005-Present
Director, Center for Integrated Medical Reserach

November 10 (Mon.)
11:20-12:00

S3-4

Quiescence of Hematopoietic Stem Cells

Toshio Suda
Developmental Biology, The Sakaguchi Laboratory, School of Medicine, Keio University, 35 Shinano-machi, Shinjuku-ku, 160-8582, Japan

The quiescent state is thought to be a characteristic property for the maintenance of hematopoietic stem cells (HSCs). Interaction of HSCs with their particular microenvironments, known as the stem cell niches, is critical for adult hematopoiesis in the bone marrow (BM). We demonstrate that quiescent HSCs adhere to the hypoxic endosteal niche through the Tie2/ Angiopoietin-1 and/or mpl/thrombopoietin.

When the BM is ablated during BM transplantation or after treatment with myelosuppressive agents, the quiescent HSCs enter the cell cycle and proliferate to supply progenitors of committed hematopoietic cells. We found that reactive oxygen species (ROS) induce the exit of HSCs from the niche after 5-FU injection through down-regulation of N-cadherin. Similarly, ROS was elevated after serial BM transplantation, and up-regulation of MAPK p38 and INK4A was detected only in HSCs. We analyzed the metabolic characteristics of quiescent HSCs in adult BM. I will discuss the decrease in the HSC quiescence and self-renewal capacity in mutant mice with FOXO3a or HIF1a.

Session 4

(November 10, 2008, 13:30-14:50)

Clinical Aspects of Stem Cells

Nobuko Uchida

POSITION TITLE			
Vice President, Stem Cell Biology			
EDUCATION/TRAINING			
INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Wellesley College, Wellesley, MA	B.A.	1983	Molecular Biology
Stanford University, Stanford, CA	Ph.D.	1992	Cancer Biology

Positions and Employment

1981-1982	Student research assistant, Harvard Medical School
1983-1985	Research Assistant, Department of Pathology, Harvard Medical School
1985-1992	Graduate research, Stanford University School of Medicine
1992-1993	Postdoctoral fellow, Stanford University School of Medicine
1993-1994	Visiting scholar, Stanford University School of Medicine
1993-1998	Research scientist, SyStemix Inc.
1998-2003	Director, Neural Stem Cell Program, StemCells Inc. Palo Alto, CA.
2003-Present	Vice President, Stem Cell Biology, StemCells Inc. Palo Alto, CA

November 10 (Mon.)
13:30-14:10

S4-1

THERAPEUTIC POTENTIAL OF HUMAN NEURAL STEM CELLS

Nobuko Uchida
StemCells, Inc., Palo Alto, CA 94304 USA

Neural stem cell transplants have the potential to treat neurodegenerative diseases or injuries to the CNS. Prospective isolation of human central nervous system stem cells (hCNS-SC) from fetal brain tissue, with the cell surface phenotype of $CD133^{+}$, $CD34^{neg}$, $CD45^{neg}$ or $CD24^{-/lo}$, yields a highly purified stem cell population. These CD133+ central nervous system-stem cells grown as neurospheres (HuCNS-SC), have been banked and tested in pre-clinical studies for their biological properties, safety and measures of efficacy as a prelude to clinical use. Upon transplantation into the brain of NOD-scid mice, HuCNS-SC proliferate exclusively at host neurogenic sites, such as subventricular zone and maintain nestin expression, a marker for neural stem cells. Subsequently, progeny of the human cells migrate to the olfactory bulb via the rostral migratory stream as chains of neuroblasts and continue to differentiate into neurons in the olfactory bulb. The site-appropriate differentiation of HuCNS-SC can be monitored and further characterized by the combination of human specific monoclonal antibody, SC121 and lineage specific markers for neurons, astrocytes and oligodendrocytes. Engraftment has been shown to be durable, reproducible, without any evidence of tumor formation.

Neuroprotection is our current strategy where cellular transplants may be able to alter the course of disease or injury progression. We have performed preclinical studies to test whether HuCNS-SC can neuroprotect host cells through delivery of soluble proteins (such as housekeeping lysosomal enzymes, neurotrophic factors, chemokines) or through myelination of dys- or demyelinated host axons.

Among them the neuronal ceroid lipofuscinoses (NCL) is rare, fatal, neurodegenerative lysosomal storage disorder that affect infants and young children. Infantile and late infantile NCL subsets are caused by inherited genetic mutations in genes that provide cells with lysosomal enzymes Lack of these enzymes causes accumlation of storage materials leading to neuronal cell loss and manifestations of the disease. If transplanted HuCNS-SC can secrete these hous keeping enzymes, host neurons can uptake them and active donor enzymes can reduce storage materials. Therefore, our strategy was to treat the NCLs where secreted enzyme is deficient by providing the enzyme in trans via cell therapy.

In a mouse model for Infantile NCL, hCN-SCns can provide functional lysosomal enzyme that result in the reduction of lipofuscin, protection of host cortical and hippocampal neurons and extension of their life span. Based on this preclinical study, StemCells, Inc has initiated a Phase I clinical trial to investigate the safety and preliminary efficacy of HuCNS-SC as a treatment of infantile and late-infantile NCL. The open label trial studies two dose levels with the primary objective of measuring the safety of HuCNS-SC at one year post HuCNS-SC transplantation. Enrollment and treatment of the patients in this study has been completed.

A second neuroprotection strategy is to protect host neurons with axonal damage due to oligodendrocyte deficiency or death. Previously we showed that HuCNS-SC can myelinate host axons when they were transplanted into shiverer/NOD-scid mouse with a hypomyelination defect. Confocal analysis revealed that the host axons were encircled with human myelin basic protein. At electron microscope level, these cells formed myelin sheath with 16-20 dense lines .

In contusion-induced spinal cord injured immunodeficient mice, transplantation of HuCNS-SC 9 days post injury resulted in locomotion improvement (Cummings & Anderson et al. PNAS 2005). A majority of human cells differentiated into the oligodendroctye lineage and numerous myelinated host axons were detected. Synapse-forming human neurons by immunoEM staining were also observed. Selective ablation of human cells using diphtheria toxin abolished locomotor recovery suggesting integration of human cells with the mouse host is a possible mechanism for such improvements. Locomotion improvement was also observed when HuCNS-SC were transplanted at 30 days “delayed” post injury with extensive migration, increased human cell numbers and no evidence of mechanical allodynia. In contrast, transplantation of HuCNS-SC immediate after SCI is not beneficial.

We will continue to test the use of HuCNS-SC to provide neuroprotection for myelin-associated diseases, including certain LSDs, prenatal white matter injury and spinal cord injury.

Hans S. Keirstead

Education:

1986-1990	Bachelor of Science in Cell Biology University of British Columbia, Canada
1990-1994	Doctor of Philosophy in Neurobiology University of British Columbia, Canada
1995-1999	Postdoctoral Study in Neurobiology University of Cambridge, England

Academic Appointments:

2006-present	Vice-Chancellor of Academic Development, University of Community Development (UDECOM), Guinea, Africa
2007-present	Course Director, Medical Neurosciences, University of California, Irvine
2006-present	International Advisory Panel Member, Norwegian Center for Stem Cell Research, Oslo University, Norway
2007-present	Editorial Board, Journal of Stem Cells
2007-present	Editorial Board, Stem Cells and Development
2007-present	Editorial Board, Brain Research Bulletin
2005-present	Editorial Board, Neuroscience
2005-present	Editorial Board, Regenerative Medicine
2005-present	Associate Professor of Anatomy and Neurobiology Reeve-Irvine Research Center, School of Medicine University of California, Irvine
2005-present	Co-Director of the Sue and Bill Gross Stem Cell Research Center University of California, Irvine
2002-present	Elected Member, College of Medicine Representative Assembly University of California, Irvine
2000-2005	Assistant Professor of Anatomy and Neurobiology Reeve-Irvine Research Center, School of Medicine University of California, Irvine
2001-2002	Coordinator, Roman Reed Core Research Facility A state funded core facility for California spinal cord research University of California, Irvine
1999	Faculty Research Associate, Collaboration on Repair Discoveries University of British Columbia, Canada
1995-1998	Fellow, Governing Body of Downing College (lifelong Member) Member, University of Cambridge Senate University of Cambridge, England
1995-1998	Postdoctoral Fellow (1995-96), Research Associate (1996-99) MRC Cambridge Centre for Brain Repair and Department of Clinical Veterinary Medicine (Supervisor: Professor W. F. Blakemore) University of Cambridge, England
1990-1994	Graduate Student, Department of Zoology University of British Columbia, Canada

November 10 (Mon.)
14:10-14:50

S4-2

PRE-CLINICAL DEVELOPMENT OF hESC-BASED TREATMENTS FOR SPINAL CORD INJURY

Hans S. Keirstead
Co-Director of the Sue and Bill Gross Stem Cell Research Center
Associate Professor of Anatomy and Neurobiology
Reeve-Irvine Research Center
School of Medicine
University of California at Irvine

The development of research and clinical programs would benefit from a source of high purity human cell populations that are destroyed during the course of a particular injury or disease. Interest in human embryonic stem cells (hESCs) arises from their ability to provide an apparently unlimited cell supply for transplantation, and from the hope that they can be directed to desirable phenotypes in high purity. My research group has recently demonstrated that hESCs can be restricted in their differentiation potential to yield high purity oligodendrocyte cultures, demonstrating for the first time that hESCs can be manipulated to yield a high purity neural subpopulation. This cell population and its pre-clinical application to spinal cord injury forms the basis of a clinical strategy for acute spinal cord injury. Recently, we and partners have also demonstrated that hESCs can be restricted in their differentiation potential to high purity motor neuron progenitor cultures. This cell population and its pre-clinical application to spinal muscular atrophy, amyotrophic lateral sclerosis and spinal cord injury forms the basis of a clinical strategy for spinal muscular atrophy. I this lecture, I will review these data, the plans for clinical application of the cells, and review principles relevant to the clinical application of stem cell populations.

Special Lecture

(November 10, 2008, 14:50-15:40)

Irving L. Weissman

POSITION TITLE
Director, Stanford Institute of Stem Cell Biology and Regenerative Medicine, Comprehensive Cancer Center, and Stanford Ludwig Center for Stem Cell Research at Stanford; V&D Ludwig Professor of Clinical Cancer Research; Professor, Pathology, Developmental Biology, and by courtesy, Neurosurgery and Biology

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
Montana State College (Now a University)	B.Sc	1961	Pre-Med
Stanford University, Stanford, California	M.D.	1965	Medicine

1969- present Assistant Professor (1969-1974), Associate Professor (1974-1981); Professor (1981-Present), Dept of Pathology, Stanford University; 1986-2001 Chairman, Stanford University Immunology Program; 1989-present Professor, Dept. of Developmental Biology, Stanford University; 1990-present Professor, Dept. of Biology and Neurosurgery, Stanford University (by courtesy); 1990-1992 Investigator, Howard Hughes Medical Institute, Stanford University; 2002-Present Director, Institute of Stem Cell Biology and Regenerative Medicine, Comprehensive Cancer Center, and Ludwig Center for Stem Cell Research at Stanford

November 10 (Mon.)
14:50-15:40

Special Lecture

Perspective--Stem cells and human diseases

Irving L. Weissman
The Director of the Institute for Cancer and Stem Cell Biology and Medicine, and the professor in the Departments of Pathology, Developmental Biology, and Biological Sciences at Stanford University.

The recent prospective isolation of a wide variety of somatically derived stem or progenitor cells provides evidence that homeostasis of tissues and organs is maintained at least by such immature cells. This notion fuels enthusiasm for the use of these cells in strategies aimed at repairing or replacing damaged, diseased, or genetically deficient tissues and organs. It has also been shown that malignant tumors can be initiated and maintained by a population of tumor cells that share similar biologic properties to normal adult stem cells. This model, the cancer stem cell (CSC) hypothesis, is based on the observation that tumors, like adult tissues, arise from cells that exhibit the ability to self-renew as well as give rise to differentiated tissue cells. Hematopoietic stem cells (HSCs) are arguably the most well-characterized tissue-specific stem cell, with decades of basic research and clinical application providing not only a profound understanding of the principles of stem cell biology, but also of its potential pitfalls. Emerging stem cell fields can benefit greatly from an understanding of the lessons learned from the study of HSCs. In this lecture, general concepts regarding stem cell biology learned from the study of HSCs will discussed in conjunction with the recent application of these concepts to CSCs in several human malignancies.

Young Investigators Forum

(November 9, 2008, 16:30-20:00)

Y-1

Comparing 2 profiles to predict functional sites.

Oda, H.[1], Sato, T.[1] Ota, M[2], Toh, H.[1]
[1]Division of Bioinformatics, Medical Institute of Bioregulation,Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[2]Department of Complex Systems Science, Graduate School of Information Sciences, Nagoya University, Furo-cho, Chikusa-ku, Nagoya City, Aichi 464-8601, Japan

Introduction:
If we can predict protein functional sites with enough accuracy, we can reduce time and money to identify protein functional sites. So, it's important to develop methods to predict functional sites by bioinformatics. One of the simple methods is to identify conserved residues from a multiple sequence alignment. However, residue conservation means that the site is conserved due to miscellaneous constraints under which the homologous sequences have evolved during the course of molecular evolution. And miscellaneous constraints contain functional constraints and structural constraints. Recently, Chelliah *et al*. (2004, J Mol Biol,342(5):1487-504.) and Cheng *et al*. (2005, Nucleic Acids Res, 33(18):5861-7) independently developed the methods for predicting functional sites by distinguishing structural constraint from miscellaneous constraints. We also developed a new method to predict functional sites based on similar idea. In our approach, we compare 2 profiles, Position Specific Scoring Matrix (PSSM) with 3D-1Dprofile. PSSM is regarded to represent miscellaneous constraints and is used for database searching such as PSI-BLAST. 3D-1D profile is regarded to represent structural constraint. We used a program developed Ota *et al.* (2001 Protein Eng,14(8):557-64) to generate 3D-1D profile.
Materials and Method:
We collected a set of the proteins registered in Catalytic Site Atlas (http://www.ebi.ac.uk/thornton-srv/databases/CSA/). The coordinates data of the proteins were taken from PDB (http://www.rcsb.org/pdb/home/home.do). Proteins with missing atoms and/or residues were eliminated from our study. The homologous sequences of the proteins with the available coordinates were collected by BLAST searches from KEGG GENES DATABASE (http://www.genome.jp/kegg/genes.html), with the three criteria;

- e-value >0.01
- percent identity >30% to query sequences
- sequence coverage of aligned region > 80% of the full length of a query

The homologues thus obtained were aligned with the query by MAFFT (http://align.bmr.kyushu-u.ac.jp/mafft/software/). To compare PSSM with 3D-1D profile, the correlation coefficient was calculated at each corresponding site between PSSM and 3D-1D profile. The sites which show low correlation coefficient were predicted as a functional site.
Result:
We could select functional sites and sites around functional sites successfully by our method. But about some proteins, sites at interfaces were also selected. On the day, we will report detail result and discussion about our functional site prediction.

Y-2

Ageing-related telomeric change and its acceleration by disease conditions.

Toyoki Maeda, Jun-ichi Oyama, Yoshihiro Higuchi, Naoki Makino
Division of Molecular and Clinical Gerontology, Medical Institute of Bioregulation, Kyushu University

A telomere is a repetitive DNA structure capping the chromosomal ends. Telomeres stabilize the chromosome structure and prevent harmful end-to-end recombinations. Telomeres play a role in cellular aging and they may also contribute to the genetic basis of human aging and longevity. A gradual loss of the telomeric sequences has been reported in adult tissue specimens. Telomeres in somatic cells become shorter with aging and the shortening is accelerated by pathophysiological conditions including mental stress, smoking, diabetes mellitus, cardiovascular diseases, and Alzheimer's disease. The present study showed that the telomeres of peripheral blood leukocytes of healthy Japanese shortened with ageing. Furthermore, faster telomere attrition rates were identified in Parkinson's disease patients and sarcoidosis patients than in normal subjects. In addition, the ageing-related and the disease-associated telomere shortening accompanies increased subtelomeric methylation of long telomeres and decreased subtelomeric methylation of short telomeres, implying that telomeres with hypomethylated subtelomere were apt to undergo faster shortening. Thus, not only the telomere length but also the extent of subtelomeric methylation was suggested to be an indicator for ageing-related genomic changes and for the acceleration of the changes in some disease conditions.

Y-3

MOLECULAR EVOLUTIONARY STUDY OF THE IONOTROPIC GLUTAMATE-RECEPTOR GENE FAMILY AS SCHIZOPHRENIA SUSCEPTIBILITY GENES: HUMAN-SPECIFIC BALANCING SELECTION IN *GRIN2B* UPSTREAM REGION.

Shibata, H.[1], Tanaka, K.[1], Watanabe, K.[1], Goto H.[2], Mano, S.[3], Takenaka, O.[4] and Fukumaki, Y.[1]

[1]Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan
[2]Center for Comparative Genomics and Bioinformatics, Pennsylvania State University, State College, PA, USA.
[3]Graduate School of Natural Sciences, Nagoya City University, Nagoya, Aichi, Japan
[4]Primate Research Institute, Kyoto University, Inuyama, Aichi, Japan.

Schizophrenia is a common psychiatric disease with relatively strong genetic background (λ_s = 10). Typical adolescent onset characterized by loss of sociality suggests severe reduction of fitness. However, the disease prevalence is highly stable to be ~1% in any human populations. We hypothesized that the schizophrenia susceptibility alleles are maintained by non-neutral process such as balancing selection. To test this hypothesis, we resequenced the upstream region (3-5 kb) of fourteen ionotropic glutamate receptor genes: *GRIN1*, *GRIN2A*, *GRIN2B*, *GRIN2C*, *GRIN2D*, *GRIA1*, *GRIA2*, *GRIA3*, *GRIA4*, *GRIK1*, *GRIK2*, *GRIK3*, *GRIK4* and *GRIK5* using 50-72 unrelated humans and 24-50 unrelated chimpanzees as non-human controls. From the neutrality tests for the overall regions, we identified significant negative values of Tajima's *D* in *GRIA2* (-2.14) and *GRIK5* (-1.94) in humans suggesting human-specific positive selection. By window plot analyses, we identified a significant positive value of Tajima's *D* (+2.19) only in humans at the 1.5 kb upstream region of *GRIN2B*. Since population contraction is unlikely for humans, this positive Tajima's *D* is a signature of balancing selection specific to human lineage. The region harbors the common SNPs, rs1019385 of which the most significant association with schizophrenia has been shown by meta-analysis (Allen et al 2008). By coalescent simulation we dated the divergence of two common haplogroups in the region to be 1.3 MYA that is much older than the genome average TMRCA, 0.8 MYA. Therefore, we conclude that the *GRIN2B* upstream region have been subject to balancing selection associated with schizophrenia as well as human brain function.

Y-4

ARCHITECTURE OF THE DNA LIGASE-PCNA-DNA COMPLEX AND IMPLICATIONS FOR MECHANISM ASSEMBLING REPLICATION MACHINERY

Mayanagi, K.[1], Shinichi Kiyonari, S.[2], Saito, M.[3], Shirai, T.[3], Ishino, Y.[2] and Morikawa, K.[4,5]

[1]Medical Institute of Bioregulation, Kyushu University and BIRD, JST, 3-1-1 Maidashi, Fukuoka-shi, Fukuoka 812-8582, Japan. [2]Department of Genetic Resources Technology, Faculty of Agriculture, Kyushu University and BIRD, JST, 6-10-1 Hakozaki,Fukuoka-shi, Fukuoka 812-8581, Japan. [3]Nagahama Institute of Bio-Science and Technology and BIRD, JST, 1266 Tamura-cho, Nagahama, Shiga 526-0829, Japan. [4]Institute for Protein Research, Osaka University, 6-2-3 Furuedai, Suita, Osaka 565-0874, Japan. [5]CREST, JST, Japan.

DNA ligase I is an essential enzyme which joins Okazaki fragments during DNA replication. Like other important DNA-processing proteins, such as DNA polymerase and flap endonuclease 1, it accomplishes its function by forming a complex with PCNA, a DNA clamp molecule. Several crystal structures of DNA ligases, as well as its complex with DNA have been obtained, and recently the solution structure of DNA ligase-PCNA complex has been studied by SAXS. However, the structure of DNA ligase-PCNA-DNA complex still remains unknown.

We reconstituted a complex composed of eukaryotic-type DNA ligase from *Pyrococcus furiosus* (Pfu), Pfu PCNA, and nicked DNA, and investigated the 3D structure of the ternary complex by electron microscopic single particle analysis. The 3D map of the DNA ligase-PCNA-DNA complex exhibited a clear ring structure with a pseudo 6-fold symmetry covered with a density composed of three domains. The atomic structure of Pfu PCNA trimer could be fitted nicely into the ring region of the ternary complex. In addition, a rod like density, corresponding to the double-stranded DNA, was also clearly observed in the channel of the PCNA ring. The crystal structures of the three domains of the DNA ligase, i.e. DBD, AdD, OBD, could be also docked into the remaining three domain regions. However, the arrangements of the domains were quite different between our map and the known crystal structures obtained without PCNA. These results suggest that a large domain rearrangement of DNA ligase should occur by the interaction with PCNA.

Y-5

STRUCTURAL AND FUNCTIONAL ANALYSES OF AN ER-RESIDEDNT PROTEIN DISULFIDE REDUCTASE, ERdj5

Megawa, K.[1], Ushioda, R.[2], Hoseki, J.[2], Hagiwara, M.[2], Nagata, K.[2], Suzuki, M.[3], and Inaba, K.[1]

[1]Division of Protein Chemistry, Post-genome Science Center, Medical Institute of Bioregulation, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

[2]Depertment of Molecular and Cellular Biology, Institute for Frontier Medical Science, Kyoto University, Syogoin, Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

[3]Laboratory of Supramolecular Crystallography, Institute for Protein Research, Osaka University, 3-2, Yamadaoka, Suita-shi, Osaka 565-0871, Japan

In eukaryotic cells, the endoplasmic reticulum (ER) is the space where most of membrane and secretory proteins fold, assemble and form disulfide bonds. While the ER has a system that promotes correct folding of newly synthesized polypeptides, quality control mechanisms operate in this compartment to properly deal with terminally misfolded proteins. One of the ER quality control mechanisms, ER-associated degradation (ERAD), requires some ER resident proteins that recognize, reduce and retrotranslocate the terminally misfolded proteins. Recently, ERdj5 has been identified as a protein that reduces specifically incorrect disulfide bonds of misfolded proteins in conjunction with EDEM, an α-mannosidase-like lectin protein. ERdj5 is predicted to have a J-domain to bind a molecular chaperon, BiP, and more than four tandemly positioned thioredoxin-like domains. We here succeeded in solving crystal structure of a redox-active domain of ERdj5, Trx4, at 2.0 Å resolution. In addition, the redox potential, insulin reduction activity and *in vivo* ERAD activity of this domain were investigated biochemically. Now, crystal structure analysis of full-length ERdj5 is in progress. On the basis of the structural and biochemical data so far obtained, we will report on our new insights into molecular mechanisms of ERdj5.

Y-6

PHOSPHORYLATION-DEPENDENT ACTIVATION OF THE MAMMALIAN FORMIN FHOD1

Takeya, R.[1], Taniguchi, K.[1], Narumiya, S.[2], and Sumimoto, H.[1]
[1]Department of Biochemistry, School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[2]Department of Pharmacology, Faculty of Medicine, Kyoto University, Yoshida Konoe-cho, Sakyo-ku, Kyoto 606-8501, Japan

Formins nucleate unbranched actin filaments and associate processively with the barbed end of the growing filaments, thereby promoting formation of long straight actin-based structures such as stress fibers. The activity of formins on actin cytoskeleton appears to be normally inhibited by the intramolecular interaction between the C-terminal Dia-autoregulatory domain (DAD) and the N-terminal Dia-inhibitory domain. However signals for relieving the autoinhibitory interaction have remained largely unknown except the most characterized mouse formin mDia1, in which stimulus-induced RhoA binding to the N-terminal region disrupts the intramolecular interaction. Here we show that the Rho-dependent protein kinase ROCK phosphorylates the C-terminal residues Ser1131, Ser1137, and Thr1141 in the DAD region of FHOD1, a major endothelial formin. Phosphorylation of FHOD1 at the three residues fully disrupts the autoinhibitory interaction, which culminates in formation of stress fibers. We also demonstrate that, in vascular endothelial cells, thrombin, a vasoactive substance leading to Rho activation, elicits both FHOD1 phosphorylation and stress fiber formation in a ROCK-dependent manner, and that FHOD1 depletion by RNA interference impairs thrombin-induced stress fiber formation. Combined with the fact that thrombin-mediated reorganization of actin cytoskeleton is involved in regulation of endothelial permeability, this finding indicates that FHOD1 participates in endothelial function. Our present study provides a novel mechanism for activation of formin-family proteins: ROCK, activated by G protein-coupled receptor ligands such as thrombin, directly phosphorylates FHOD1 at the C-terminal region, which renders this formin in the active form, leading to stress fiber formation.

Y-7

ACTIVITY OF c-*myc* INTRON BINDING PROTEIN 1 (MIBP1) AS TRANSCRIPTION FACTOR AND ITS MODULATION BY O-GlcNAc TRANSFERASE (OGT)

Iwashita, Y., Tahira, T., Hayashi, K.
Division of Genome Analysis, Research Center for Genetic Information, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan

MIBP1 is a gigantic protein comprising of 2437 amino acids and contains two C2H2-type double zinc-fingers. It was identified as a protein that binds to the NF-kB-like motif on c-myc intron 1. Various groups identified the same protein through its DNA-binding property to other regulatory sequences, and named it differently, such as HIV-EP2, ZAS2, MBP2 and Shn2. Shn2-deficient mice were reported to show aberrant differentiation of various cell types. Abnormal behaviors are also observed for the deficient mice. Thus, MIBP1 seems to play a role in diverse phenotypes. However, the molecular mechanisms and physiological targets of MIBP1 remain elusive. We here systematically characterized MIBP1 by screening its binding proteins and the target genes. Immunoprecipitation-mass spectrometry analysis revealed that MIBP1 interacts with the *O*-GlcNAc transferase (OGT). OGT is a post-translational modification enzyme and has been shown to regulate various cellular processes including transcription. Using deletion mutants, we determined that the 154-amino acid region of MIBP1 (residues 1567-1720) is necessary and sufficient for OGT binding. Full length MIBP1 but not deletion mutants lacking the 154 a.a. region was *O*-GlcNAcylated. We also examined transcriptional profile of HEK 293 cells stably expressing MIBP1 by Affymetrix Human Gene 1.0 ST Array. Gene Set Enrichment Analysis (GSEA) revealed that MIBP1 functions mainly as a repressor. Leading edge analysis of gene sets indicated that previously proposed target genes such as myc and NF-kB-targets (including itself) are repressed. We also found that genes related to TGF-β pathway are repressed. Quantitative real-time PCR confirmed their decreased expression in cells that overexpressed MIBP1. Transient expression of MIBP1 repressed the expression of NF-kB reporter but not TGF-β reporter in the luciferase assay, suggesting that primary target of MIBP1 is NF-kB sites. The deletion mutant lacking the 154 a.a. region showed stronger repression in both reporters, indicating repression activity of MIBP1 is attenuated by OGT-binding and/or *O*-GlcNAcylation. Our results provide the evidence of involvement of MIBP1 in the NF-kB pathway through transcriptional regulation and the possible modulation by *O*-GlcNAc modification.

Y-8

Nemo-like kinase suppresses Notch signaling by interfering with formation of Notch active transcription complex

Ishitani T. [1, 2, 3], Hirao T. [2], Suzuki M. [3], Isoda M. [2], Kitagawa M. [4], Matsumoto K. [3], and Itoh M. [2]

[1]Division of Cell Regulation Systems, Department of Post-Genome Science Center, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, [2]Unit on Nervous Development Systems, [3]Group of Signal Transduction, Laboratory of Cell Regulation, Division of Biological Science, Graduate School of Science, Nagoya University, Nagoya 464-8602, [4]Department of Molecular and Tumor Pathology, Chiba University Graduate School of Medicine, Chiba 260-8670, Japan

Notch signaling pathway plays a crucial role in determining cell fates and controlling cell proliferation in multiple tissues in metazoan organisms. Upon binding to ligands, the Notch receptor is proteolyticaly cleaved to release the intracellular domain (NotchICD). The NotchICD enters the nucleus and acts cooperatively with other factors to stimulate transcription of target genes. High level of Notch mediated transcriptional activation requires ternary complex (NotchICD, CSL (CBF-1, suppressor of hairless, LAG-1), and MAML (mastermind-like family)) formation. However, how this ternary complex formation is regulated remains largely unknown. Here we show that Nemo-like kinase (NLK) negatively regulates Notch dependent transcriptional activation by inhibiting the ternary complex formation. By a biochemical screening, we identified Notch as a new substrate of NLK. NLK phosphorylates the NotchICD and the phosphorylated NotchICD shows an impaired ability to form the transcriptionally active ternary complex including MAML. Furthermore, knockdown of NLK leads to hyperactivation of Notch-signaling and consequently decreases neurogenesis in zebrafish. Our results define a new function for NLK in Notch regulation and reveal a novel mode of regulation of Notch signaling pathway.

Y-9

FUNCTIONAL REGULATION OF FATTY ACYL-CoA REDUCTASE 1 IN PLASMALOGEN BIOSYNTHESIS

Masanori Honsho and Yukio Fujiki
Department of Biology, Faculty of Sciences, Kyushu University Graduate School of Systems Life Sciences, 6-10-1 Hakozaki, Fukuoka 812-8581; JST, CREST, Tokyo, 102-0075, Japan

Plasmalogens are a major sub-class of ethanolamine- and choline-phospholipids in which *sn*-1 position has a long chain fatty alcohol attached through a vinyl ether bond. An ether-linked alkyl bond is formed in peroxisomes by replacing acyl-chain of acyl-dihydroxyacetonephosphate with fatty alcohol, which is catalyzed by alkyl-dihydroxyacetonephosphate synthase. Here, we demonstrate that fatty acyl-CoA reductase 1 (Far1) is responsible for supplying fatty alcohol in the formation of ether-linked alkyl bond. In plasmalogen-deficient cells, fatty-alcohol accumulation due to the increase in the Far activity has been reported, suggesting that activity of Far1 is regulated in a plasmalogen-dependent manner. To test this hypothesis, we attempted to address whether Far1 activity is down-regulated by restoring plasmalogen levels in a plasmalogen-defective Chinese hamster ovary cell mutant, ZPEG251 (*BBA-MCR* 2008). When Far1 was expressed in ZPEG251 cells, synthesis of fatty alcohols was elevated as compared to that in ZPEG251. In contrast, the level of fatty alcohols was lowered upon restoring the plasmalogen level in ZPEG251, indicating that Far1 activity was down-regulated in response to the intracellular plasmalogen levels. Under this condition, there was no striking difference in the transcription level of *FAR1* as assessed by semi-quantitative RT-PCR, rather the lowed level of Far1 protein was evident. Furthermore, an increased rate of Far1 degradation was also observed when the plasmalogen level was restored in ZPEG251. Taken these results together, we suggest for the first time that Far1 activity is regulated in response to the plasmalogen level by modulating Far1 stability, not at the transcriptional level.

Y-10

APPARATUS FOR PROLIFERATION AND DIVISION OF PEROXISOMES

Satoru Mukai and Yukio Fujiki
Department of Biology, Faculty of Sciences, Kyushu University Graduate School of Systems Life Sciences, 6-10-1 Hakozaki, Fukuoka 812-8581; JST, CREST, Tokyo, 102-0075, Japan

In the mid 1970s, peroxisome was found to increase in number and peroxisomal fatty-acid β-oxidation activity in the liver of rodents treated with hypolipidemic agents (peroxisome proliferators, PPs). Activation of transcription factor called PP-activated receptor (PPAR) α is responsible for peroxisome proliferation. Heterodimer of PPARα with retinoid X receptor binds to PP response element (PPRE) in its target genes and up-regulates the transcription of down-stream genes. Characterization and regulating mechanisms of PPARα activity have been investigated in many aspects including inflammations in humans. However, molecular mechanisms underlying the proliferation and division of peroxisomes remained little known. We recently showed that Fis1 and dynamin-like protein 1(Dlp1/Drp1), both identified as mitochondria fission factors, play a pivotal role in peroxisome division together with the peroxin, Pex11pβ. Indeed, aberrant morphology of both peroxisomes and mitochondria was observed in cells treated with siRNA for *FIS1* and in *DLP1*-defective Chinese hamster ovary (CHO) cell mutant ZP121 (*ECR* 2006; 2007). Given these findings, it was more likely that in the division peroxisomes partly shares the same factors with mitochondria, but utilizes machinery distinct from that of mitochondria. To further investigate molecular mechanisms of peroxisome division, we attempted to identify proteins, if any, interacting with Pex11pα and Pex11pβ, using mammalian cells stably expressing Flag-Pex11pα and Flag-Pex11pβ, respectively. Not only Fis1 but also several peroxins including Pex13p and Pex16p were co-immunoprecipitated with Flag-Pex11pα and Flag-Pex11pβ. Studies on physiological and functional significance of these interacting proteins in peroxisome division are under way.

Y-11

THE ERK-MAPK PATHWAY PHOSPHORYLATES AND TARGETS CDC25A FOR SCF$^{\text{BETA-TRCP}}$-DEPENDENT DEGRADATION FOR CELL-CYCLE ARREST

Isoda, M., Kanemori, Y. and Sagata, N.
Department of Biology, Graduate School of Sciences, Kyushu University, Hakozaki 6-10-1, Fukuoka 812-8581, Japan

The ERK-MAPK pathway is generally mitogenic, but, upon strong activation, it causes cell-cycle arrest by not-yet fully understood mechanism. Upon genotoxic stress, Chk1 phosphorylates and targets Cdc25A for degradation and elicits cell-cycle arrest. Here we show that, when strongly activated in *Xenopus* eggs, the ERK pathway induces prominent phosphorylation and SCF$^{\beta\text{-TrCP}}$-dependent degradation of Cdc25A, similar to Chk1. p90rsk, the kinase downstream of ERK, directly phosphorylates Cdc25A on multiple sites, which, interestingly, overlap with Chk1 phosphorylation sites. Furthermore, ERK itself phosphorylates Cdc25A on multiple sites, a major one of which apparently is phosphorylated by Cdk in the case of Chk1-induced degradation. Both p90rsk and ERK phosphorylations contribute to the degradation of Cdc25A, and this degradation occurs during oocyte maturation in which the endogenous ERK pathway is fully activated. Finally, ERK-induced Cdc25A degradation elicits cell-cycle arrest in early embyros. These results suggest that strong ERK activation targets Cdc25A for degradation in a manner similar to, but independent of, Chk1 for cell-cycle arrest.

Y-12

CELL CYCLE INHIBITOR p57 IS ESSENTIAL FOR CEREBELLAR DEVELOPMENT: ANALYSIS OF NEURON-SPECIFIC p57 CONDITIONAL KNOCKOUT MICE

Akinobu Matsumoto[1, 2], Etsuo Susaki[1, 2], Ichiro Onoyama[1, 2], and Keiichi I. Nakayama[1, 2]

[1]Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, The University of Kyushu, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
[2]JST CREST

Cell cycle arrest is an essential event for the development of central nervous system (CNS). p27 (Kip1) and p57 (Kip2) are members of the CDK inhibitor (CKI) family, and share conserved domain that binds to and inhibit CDKs. Analyses of p27 knockout mice revealed that p27 regulates the cell cycle in neural development. On the other hand, the neonatal mortality makes the understanding of the role of p57 in CNS difficult.

To investigate the functions of p57 in CNS, we created the nervous system-specific p57 conditional knockout (CKO) mice by crossing p57-flox mice with Nestin-Cre transgenic mice. Surprisingly, deletion of p57 in nervous system caused severe hydrocephalus and p57 CKO mice died at ~3 weeks of age. In addition, p57 CKO mice showed structural abnormality in cerebellum: Granule cells and Purkinje cells lost the polarity, and Bergmann glia and oligodendroglia exhibited structural abnormality and hypoplasia.

We next examined the expression pattern of p57 in cerebellum. Unexpectedly, p57 was expressed only in interneuron precursors, but not expressed in granule cells, Purkinje cells, Bergmann glia, or oligodendroglia. Indeed, p57 CKO mice showed a defect of interneuron precursors at P0.5, which resulted in the loss of interneurons at P15. These data suggest that p57 plays a key role in cerebellar development through the regulation of interneuron precursors.

To examine whether the function of p57 is the same of p27 function in vivo, we created p27 knock-in mice ($p57^{p27KI}$) in which *p57* were replaced by *p27*. $p57^{p27KI}$ mice did not show the hydrocephalus and any defect in cerebellar structure as seen in p57 CKO mice. Consistent with this observation, the expression patterns of p57 and p27 in wild-type mice did not overlap in cerebellar cells. These results indicate that p57 and p27 have different roles in different populations of cerebellar cells. We propose that proper spatiotemporal expression of p57 and p27 are essential for cerebellar development.

Y-13

COMPREHENSIVE IDENTIFICATION OF SUBSTRATES FOR UBIQUITIN LIGASES THAT CONTROL CELL-CYCLE USING QUANTITATIVE PROTEOMICS

Yumimoto K.[1,2], Matsumoto M.[1,2] and Nakayama K.I.[1,2]
[1]Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University, 3-3-1, Maidashi, Higashi-ku, Fukuoka-shi, Fukuoka, 812-8582, Japan
[2]JST, CREST

Selective protein degradation by the ubiquitin-proteasome pathway is a powerful regulatory mechanism in a wide variety of cellular processes. The specificity of substrates for ubiquitination is mainly controlled by ubiquitin ligases. The SCF ubiquitin ligase consists of the invariable components Skp1, Cul1 and Rbx1 as well as a variable component, known as an F-box protein, that binds to Skp1 through its F-box motif and is responsible for substrate recognition. More than 70 F-box proteins have been identified in mammals, but the roles of most F-box protein have not been elucidated because their substrates are unknown.

To identify substrates of uncharacterized F-box proteins comprehensively, we tried to explore binding proteins of F-box protein using immunoprecipitation followed by LC-MS/MS analysis. At first we optimized the condition to maximize the efficiency of binding between Skp2 (an F-box protein) and p27 (one of the substrates for Skp2) as a benchmark. To overcome the rapid ubiquitination of substrates followed by degradation, we introduced a point mutation into the F-box domain of Skp2 which stabilized the binding of p27 to Skp2. By comparing the abundance of proteins that bound to wild-type and mutant Skp2 using the quantitative proteomics method SILAC (Stable Isotope Labeling by Amino acids in Cell culture), we identified a series of substrates of Skp2 including p27. We designated this method as MFAS-system (Mutant F-box protein Affinity purification of substrate with SILAC).

We further applied this system to another F-box protein Fbxw7. c-Myc, a known substrate of Fbxw7, was detected as a substrate with MFAS-system. In addition, novel six candidates of Fbxw7 substrates which contain the conserved phospho-degron were identified. One of these candidates was a transcriptional factor which controls cell growth positively, consistent with the notion that most Fbxw7 substrates are proto-oncogenes and/or growth promoters. Now we validate the results in cultured cell lines or Fbxw7 knockout mice.

Thus we propose that MFAS-system is a powerful tool to discover a number of unidentified substrates for ubiquitin ligases. We are now applying this system to uncharacterized F-box proteins for elucidating their physiological functions.

Y-14

REVERSAL OF LEFT-RIGHT ASYMMETRY INDUCED BY ECTOPIC EXPRESSION OF *LEFTY* IN MOUSE EMBRYOS

Oki, S.[1], Kitajima, K.[1], Marques, S.[2], Belo, J. A.[2], Yokoyama, T.[3], Hamada, H.[4] and Meno, C.[1,4]

[1]Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan. [2]IBB-Institute for Biotechnology and Bioengineering, Centro de Biomedicina Molecular e Estrutural, Universidade do Algarve, Campus de Gambelas, Faro, Portugal, and Instituto Gulbenkian de Ciência, 2781-901 Oeiras, Portugal. [3]Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan. [4]Graduate School of Frontier Biosciences, Osaka University, Osaka 565-0871, Japan.

Establishment of the left-right (L-R) axis is fundamental for morphogenesis of visceral organs. The L-R axis of the mouse embryo is established by successive processes that begin with a leftward flow of fluid on the ventral surface of the node (nodal flow), which is observed at the anterior tip of the primitive streak. *Nodal*, encoding a secretory protein belonging to TGF-β superfamily, is then expressed asymmetrically in the crown cells of the node, with the level being higher on the left side. The Nodal signal is then transmitted to the left side of the lateral plate mesoderm (LPM), where it induces *Nodal* expression. Positive and negative regulatory loops amplify the small difference in the node to generate the robust expression of *Nodal* in the left LPM. Whereas this sequence of principal events in establishment of the L-R axis has been relatively well characterized, the initial molecular mechanism by which asymmetric expression develops at the node remains largely unknown. This lack of knowledge is exemplified by the lack of an explanation for the phenotype of mice with the recessive *inv* mutation. Despite the normal direction of leftward nodal flow, L-R asymmetric *Nodal* expression in the LPM is reversed in *inv/inv* mice.

Here, we will show our detailed analysis for *inv* mutant mice. We will eventually propose a mechanism that leads L-R reversal despite the normal direction of the nodal flow, especially with our novel finding that Lefty, an inhibitor for Nodal, is ectopically produced in *inv* mutant.

Y-15

THE MITOCHONDRIAL TOXIN, 3-NITROPROPIONIC ACID INDUCES MUTYH-DEPENDENT STRIATAL NEURODEGENERATION WITH ACCUMULATION OF 8-OXOGUANINE IN MEDIUM SPINY NEURONS AND MICROGLIAS WHICH IS EFFECTIVELY SUPPRESSED BY OGG1 AND MTH1

Zijing Sheng[1], Hidetaka Yamada[2] and Yusaku Nakabeppu[1]
[1]Division of Neurofunctional Genomics, Department of Immunobiology and Neuroscience, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan
[2]Cognitive and Molecular Research Institute of Brain Diseases, Kurume University School of Medicine, Kurume, Japan

8-Oxoguanine (8-oxoG) is one of the major oxidative base lesions in DNA or nucleotides, and is highly mutagenic because it can pair with adenine as well as cytosine. To minimize accumulation of 8-oxoG in mammalian DNA, MutT homolog-1 (MTH1) hydrolyzes o 8-oxo-dGTP to 8-oxo-dGMP and pyrophosphate, and 8-oxoG DNA glycosylase-1 (OGG1) excises 8-oxoG paired with cytosine in DNA, while MutY homolog (MUTYH) removes adenine misincorporated opposite 8-oxoG in template DNA. We recently demonstrated that the accumulation of 8-oxoG in nuclear DNA caused poly-ADP-ribose polymerase-dependent nuclear translocation of apoptosis-inducing factor (AIF), while that in mitochondrial DNA caused mitochondrial dysfunction and Ca^{2+} release thereby activating calpain, thus triggering two distinct cell death pathways. Both cell death were triggered by single-strand breaks (SSBs) that had accumulated in the respective DNAs and were suppressed by knockdown of MUTYH, thus indicating that excision of adenine opposite 8-oxoG lead to the accumulation of SSBs in each type of DNA (Oka *et al*., 2008).

In the present study, we examined effects of chronic administration of 3-nitropropionic acid (3-NP), a naturally occurring mycotoxin which inhibits succinate dehydrogenase, to each mutant mice. We found that double-knockout (DKO) mice lacking OGG1 and MTH1, and to a lesser extent single-KO mice lacking either one of these enzymes, significantly decreased their locomotor activity in comparison to wild-type or OGG1/MUTYH DKO mice. 3-NP administration to MTH1/OGG1 DKO mice caused a severe striatal degeneration, and early accumulation of 8-oxoG in mitochondrial DNA was observed in the striatal medium spiny neurons accompanied by calpin activation, while delayed accumulation of 8-oxoG in nuclear DNA was observed in microglias accompanied by increased poly-ADP-ribosylation and nuclear translocation of AIF. These results indicate that 3-NP induces MUTYH-dependent striatal degeneration through the two distinct death pathways for the medium spiny neuron and microglia. Our results provide new mechanistic insights into neurodegenerative diseases with medium spiny neuron loss in striatum such as Huntington’s disease.

Y-16

Human FLT3/FLK2 targets hematopoietic stem cells and granulocyte/macrophage progenitors

Yoshikane Kikushige[1, 2], Goichi Yoshimoto[1, 2], Toshihiro Miyamoto[1], Tadafumi Iino[2, 4], Yasuo Mori[1, 2], Hiromi Iwasaki[1], Hiroaki Niiro[1], Mine Harada[2], Fumihiko Ishikawa[3] and Koichi Akashi[1, 4]

[1]Center for Cellular and Molecular Medicine, and [2]Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka [3]Research Unit for Human Disease Model, RIKEN Center for Allergy and Immunology, Kanagawa [4]Department of Cancer Immunology and AIDS Dana-Farber Cancer Institute, Harvard Medical School, Boston

FLT3/FLK2, a member of the receptor tyrosine kinase family, plays a critical role in maintenance of hematopoietic homeostasis, and the constitutively active form of the FLT3 mutation is one of the most common genetic abnormalities in acute myelogenous leukemia(AML). In murine hematopoiesis, Flt3 is not expressed in self-renewing hematopoietic stem cells (HSCs), but its expression is restricted to the multipotent and the lymphoid progenitor stages at which cells are incapable of self-renewal. We extensively analyzed the expression of Flt3 in human hematopoiesis. Strikingly, in both the bone marrow (BM) and the cord blood (CB), the human HSC population capable of long-term reconstitution in xenogeneic hosts uniformly expressed Flt3. Furthermore, human Flt3 is expressed not only in early lymphoid progenitors, but also in progenitors continuously along the granulocyte/macrophage pathway, including the common myeloid progenitor(CMP) and the granulocyte/macrophage progenitor(GMP). We further found that human Flt3 signaling prevents stem and progenitors from spontaneous apoptotic cell death at least through upregulating Mcl-1, an indispensable survival factor for hematopoiesis. Thus, the distribution of Flt3 expression is totally different between human and mouse, and human FLT3 signaling plays an important role in cell survival especially at stem and progenitor stages that are critical cellular targets for AML transformation.

Y-17

ELEVATED LEUKOCYTE ALKALINE PHOSPHATASE SCORES INDUCED BY THE JAK2 V617F MUTATION

Seido Oku[1], Katsuto Takenaka[1], Takashi Kumano[1], Takuro Kuriyama[1], Shingo Urata[1], Kotaro Shide[2], Haruko Shimoda[2], Hiromi Iwasaki[3], Toshihiro Miyamoto[3], Takanori Teshima[3], Koji Nagafuji[1], Kazuya Shimoda[2], Koichi Akashi[1,3]

[1]Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, 3-1-1, Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan; [2]Department of Gastroenterology and Hematology, Faculty of Medicine, Miyazaki University, 5200, Kihara, Kiyotake, Miyazaki, 889-1692, Japan; [3]Center for Cellular and Molecular Medicine, Kyushu University Hospital, 3-1-1, Maidashi, Higashi-ku, Fukuoka, 812-8582, Japan

Leukocyte alkaline phosphatase (LAP) enzymatic activity is a marker of the last stages of myeloid differentiation. The level of LAP is quantitated as the LAP score. Estimation of the LAP score has been useful for distinguishing chronic myelogenous leukemia (CML) from BCR-ABL–negative chronic myeloproliferative disorders (MPDs) and neutrophilic reactions in severe infections. CML patients usually have a low LAP score, whereas elevated LAP scores are seen in patients with polycythemia vera (PV), primary myelofibrosis (PMF), and leukocytosis caused by infections.

An acquired Jak2 V617F mutation is seen in approximately 95% of patients with PV and in about 50% of patients with essential thrombocythemia or PMF. It has been shown that Jak2 V617F mutation induced constitutive activation of the JAK-STAT signaling pathway.

We speculated that an elevated LAP score might be caused due to activation of JAK-STAT signaling through a Jak2 V617F mutation, and conducted this study to address this question.

We analyzed the LAP scores in Jak2 V617F-positive and -negative MPD patients. Jak2 V617F-positive MPD patients had higher LAP scores than Jak2 V617F-negative patients. Moreover, patients carrying homozygous mutations had higher LAP scores than patients with heterozygous mutations.

AG490, the Jak2 inhibitor, was shown to significantly decrease the LAP expression in neutrophils of Jak2 V617F-positive patients.

We lentivirally transfected the acute promyelocytic leukemia cell line NB4 with the Jak2 V617F mutation and wild-type Jak2. The expression level of Jak2 was not significantly different between the Jak2 V617F mutation and wild-type Jak2. We then examined the LAP scores of transfected NB4 cells after these cells were differentiated by *all-trans* retinoic acid and granulocyte colony stimulating factor. It was observed that the Jak2 V617F mutation and not the wild-type Jak2 induced elevated LAP scores.

Furthermore, we showed that Jak2 followed the MAP kinase pathway and not the PI3 kinase pathway, as a downstream signaling pathway to elevate the LAP scores using MEK 1/2 (U0126) and PI3 kinase (LY294002) inhibitors.

In conclusion, we obtained direct evidence that Jak2 V617F mutation induces elevated LAP scores via the MAP kinase pathway.

Y-18

TBX3 CONTROLS HEPATIC STEM CELL FATE

Suzuki, A
Division of Organogenesis and Regeneration, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka, Fukuoka 812-8582, Japan

Hepatic stem cells are defined as clonogenic cells that can differentiate into both hepatocytes and cholangiocytes (biliary epithelial cells), renew themselves, and are essential for liver development and regeneration. Any analyses using unfractionated liver cells, however, could not provide evidence for the presence of hepatic stem cells due to the lack of a method for excluding differentiated hepatic cells and other types of cells, including hematopoietic, vascular epithelial, and mesenchymal cells, from whole liver cells and eventually isolating stem cells alone. For this reason, we have developed a strategy for prospective isolation of hepatic stem cells by combining flow-cytometry and fluorescence-conjugated antibodies. Cells separated into various cell subpopulations were severally sorted out and clonally cultured (1 cell/well) to examine the potential for proliferation and differentiation of each cell precisely. This clonal analysis allowed us to identify and isolate cells capable of self-renewing and differentiating into both hepatocytes and cholangiocytes *in vitro*. Moreover, upon cell transplantation, the progeny of an isolated single stem cell could reconstitute liver tissues *in vivo*. Prospective identification and isolation of hepatic stem cells achieved a much higher enrichment of these cells, and thus this method could facilitate identification of a discrete set of transcription factors that are activated in this specific cell population. By using this strategy, we examined the developmental role of the T-box family of transcription factors in proliferation and differentiation of hepatic stem cells, and found that Tbx3 plays a crucial role in controlling proliferation and cell-fate determination of hepatic stem cells by suppressing $p19^{ARF}$ expression for promoting liver development. Our method for prospectively isolating hepatic stem cells could be used efficiently to identify new genes, like *Tbx3*, that are fundamentally required for their activities, and to improve our understanding of the molecular nature of liver development, regeneration, and carcinogenesis.

Y-19

Adipose tissue-derived and bone marrow-derived mesenchymal cells develop into different lineage of steroidogenic cells by forced expression of SF-1

Shigeki Gondo[1 3], Tomoko Tanaka[1], Hisaki Gondo[3], Taijiro Okabe[1], Hidetaka Morinaga[1], Masatoshi Nomura[1], Ryoichi Takayanagi[1], Hajime Nawata[2], Toshihiko Yanase[1]

[1]Department of Medicine and Bioregulatory Science, [2]Graduate School of Medical Science, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka-city, Fukuoka, 812-8582, Japan

[3]Gondo Clinic, yayoigaoka 2-1-1, Tosu-city, Saga, 841-0005, Japan

SF-1/Ad4BP is an essential nuclear receptor for steroidogenesis as well as for adrenal and gonadal gland development. We have previously clarified that adenovirus-mediated forced expression of SF-1 can transform long-term cultured mouse bone marrow mesenchymal cells (BMCs) into ACTH-responsive steroidogenic cells. In the present study, we extended this work to adipose tissue-derived mesenchymal cells (AMCs) and compared its steroidogenic capacity with those of BMCs prepared from the identical mouse. Several cell surface markers, including potential mesenchymal cell markers, were identical in both cell types and, as expected, forced expression of SF-1 caused AMCs to be transformed into ACTH-responsive steroidogenic cells. However, more elaborate studies revealed that the steroidogenic property of AMCs was rather different from that of BMCs, especially in steroidogenic lineage. In response to increased SF-1 expression and/or treatment with retinoic acid, AMCs were much more prone to produce adrenal steroid, corticosterone (B) rather than gonadal steroid, testosterone (T), whereas the contrary was evident in BMCs. Such marked differences in steroidogenic profiles between AMCs and BMCs were also evident by the changes of steroidogenic enzymes. These novel results suggest a promising utility of AMCs for autologous cell regeneration therapy for patients with steroid insufficiency and also a necessity for appropriate tissue selection in preparing mesenchymal stem cells according to the aim. The different steroidogenic potency of AMCs or BMCs might provide a good model for the clarification of the mechanism of tissue- or cell-specific adrenal and gonadal steroidogenic cell differentiation.

Y-20

The tumor associated role of GLTSCR2

Kawahara, K.[1], Sasaki, M.[2] Hamada, K[1] and Suzuki, A.[1]
[1]Division of Embryonic and Genetic Engineering, Medical Institute of Bioregulation, Kyushu University, Maidashi 3-1-1, Higashiku, fukuoka 812-8582 Japan
[2]Campbell Family Institute for Breast Cancer Research, University of Toronto, 620 University Avenue, Suite 706, Toronto, Ontario,
Canada;

Glioma tumor suppressor candidate region gene 2 (GLTSCR2/PICT-1) is localized within the well-known 1.4-Mb tumor suppressive region of chromosome 19q, which is frequently altered in various human tumors, including diffuse gliomas. Several lines of evidence suggested that GLTSCR2 associated with and stabilized tumor suppressor PTEN which regulates diverse cellular functions including apoptosis, proliferation by negatively regulating PI3K/Akt signaling pathway. In addition, *in vitro* knockdown experiments revealed that reduction of GLTCR2 expression in tumor cells induced cell growth and transformation associated with reduction of PTEN stability and function. These observations suggested that GLTSCR2 might be involved in suppression of tumor growth and development by regulating PTEN stability. However, contrary to these results, there are data showing oligodedrogliomas patients with loss of heterozygosity (LOH) in GLTSCR2 region displayed higher survival rates and had longer survival period than those without LOH. This implicated that loss of GLTSCR2 might cause increase of tumor suppression. Thus it is unclear whether GLTSCR2 might have function as tumor suppressor or tumor progressive factor *in vivo*. To demonstrate function of GLTSCR2 associated with tumor *in vivo,* we generated GLTSCR2 deficient mice. Here we would like to present biological function of GLTSCR2 and relevance of GLTSCR2 to tumorigenesis *in vivo*.

Y-21

Bone Marrow and Peripheral Blood Expression of *ID1* in Human Gastric Carcinoma Patients Is a Bona Fide Indicator of Lymph Node and Peritoneal Metastasis

Masaaki Iwatsuki[1、2], Koshi Mimori[1], Hideshi Ishii[1], Hideo Baba[2], Masaki Mori[1].

1. Department of Surgical Oncology, Medical Institute of Bioregulation, Kyushu University, Beppu, Japan
2. Department of Gastroenterological Surgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan

Purpose: Recent studies have revealed that bone marrow-derived endothelial progenitor cells (EPC) play a critical role in metastasis and that transcription factor Id1 is required in metastasis as a critical regulator of angiogenesis. Therefore, we investigated the clinical significance of *ID1* mRNA expression in bone marrow, peripheral blood and peritoneal wash samples in patients with gastric cancer.
Experimental Design: Both bone marrow and peripheral blood samples from 289 gastric cancer patients were collected at the Central Hospital, National Cancer Center, Tokyo, Japan, and peritoneal wash samples were obtained from 98 gastric cancer cases in Aichi Cancer Center. The samples were analyzed by quantitative real-time RT-PCR for *ID1*. ID1 protein expression in bone marrow, metastatic lymph nodes and peritoneal disseminated tumors was examined by immunohistochemical methods.
Results: In both bone marrow and peripheral blood samples, *ID1* mRNA expression in the metastatic group was significantly higher than in the non-metastatic group ($p = 0.0032$, $p = 0.0001$, respectively) and significantly associated with lymph node metastasis and peritoneal dissemination. The cells in bone marrow from patients with metastatic cancer stained strongly with ID1 antibody compared to those of a healthy volunteer. There was no detectable ID1 expression in cancer cells in metastatic lymph nodes or peritoneal dissemination.
Conclusions: Expression of *ID1* mRNA in bone marrow and peripheral blood was significantly associated with lymph node metastasis and peritoneal dissemination, and therefore constitutes a predictable marker for lymph node metastasis and peritoneal dissemination.

Y-22

IDENTIFICATION OF CANCER STEM CELLS IN HUMAN GASTRIC ADENOSQUAMOUS CARCINOMA

Ohkuma, M.[1], Haraguchi, N.[1], Ishii, H. [1], Yanaga, K. [2], and Mori, M. [1]
[1]Department of Gastroenterological Surgery, Osaka University Graduate School of Medicine, 2-2, Yamadaoka, Suita, Osaka 565-0871, Japan
[2]Department of Surgery, Jikei University School of Medicine, 3-25-8, Nishi-Shinbashi, Minato-ku, Tokyo 105-8461, Japan

(Background) Recent studies indicate that cancer stem cells (CSCs) are involved in carcinogenesis from early to advanced stages of hematopoietic and solid tumors, as well as recurrence and metastasis after the treatment of diseases. We report here the identification and characterization of a small population of MKN-1 gastric adenosquamous cancer cells, which presumably are eligible to be CSCs.
(Materials and Methods) The MKN-1 cells were cultured in the medium with 5FU and were subjected to the expression study of several cell surface markers, compared with control parent cells. Utilizing surface markers, which show the differential expression before and after the exposure to 5FU in culture, cells were sorted and studied for the biological characterization. The immunohistochemical (IHC) study with specific antibodies was performed to assess the localization of malignant cells in tumor tissue, which suggest the relevance to CSCs of gastric adenosquamous carcinoma.
(Results) After MKN-1 cells were treated with 5FU, the population of surface marker CD71(-) cells were increased than that of parental control cells. Colony formation and proliferation assay showed that the capacity of CD71(-) cells were higher than those of CD71(+) cells. Apparently, three-dimensional culture of colonies formed by CD71(-) cells indicated that the cells have more morphologic variety than those formed by CD71(+) cells. Subcutaneous injection of CD71(-) cells in NOD/SCID mice demonstrated that the occurrence of tumors and their sizes are much frequent and larger compared with CD71(+) cells. Furthermore, IHC analysis fastened our conclusion that fraction of CD71(-) cells harbor multi-potentiality for differentiation, which characterizes self-renewal of CSCs and distinct from CD71(+) cells. In gastric adenosquamous carcinoma, CD71(-) cells likely locate predominantly in leading parts of tumor.
(Conclusion) In gastric adenosquamous carcinoma cell line MKN-1, the absence of surface marker CD71, but not CD71(+) cells have high potentiality for self-renewal and multi-differentiation, which is critical character of CSCs. We conclude that CD71 has the candidacy for CSC marker in gastric adenosquamous carcinoma.

Y-23

DIFICIENCY OF LEUKOTRIENE B4 RECEPTORS AFFECTS TUMOR METASTASIS

Okazaki, H. Okuno, T. and Yokomizo, T
Department of Medical Biochemistry, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-3-1, Higashi-ku, Fukuoka 812-8582, Japan

Malignant tumor, including cancer originated from epithelial cells, is a major cause of death in the world, and its incidence is still increasing. Most of malignancy death is caused by metastases from tumors in distant organs. Tumor metastasis consists of a series of complex steps, and the process of metastasis is entirely regulated by interactions among the tumor cells, stromal cells, extracellular matrix, and molecular mechanism of this interactions remains poorly understood.

Leukotriene B4 (LTB4; 5(S), 12(R)-dihydroxy-6, 14-cis-8, 10-trans-eicosatetraenoic acid), a potent chemotactic lipid mediator, plays important roles in controlling inflammatory responses via binding to its specific G-protein coupled receptors. Two types of receptors, LTB4 receptor 1 (BLT1), a high-affinity LTB4 receptor and LTB4 receptor 2 (BLT2), a low-affinity one are known. BLT1 is mainly involved in inflammatory responses.

On the other hand, 12-HHT (12(S)-hydroxyheptadeca-5Z, 8E, 10E-trienoic acid) is also a metabolite of arachidonic acid as LTB4. Recently we reported that 12-HHT functions as a natural lipid agonist for BLT2 both in vitro and in vivo. However, the function of BLT2 activation by 12-HHT remains poorly understood.

To investigate the effect of BLT1 or BLT2 in tumor metastasis, we are trying to evaluate the effects of abrogation of BLT1 and 2 in mice model of cancer metastasis. Lewis lung carcinoma (LLC) cells were injected through the tail vein of wild type, BLT1-dificient, and BLT2-dificient mice. To quantify the metastasis, EGFP expression vector was transfected into LLC (LLC-EGFP) to express EGFP as a marker protein. Flowcytometric analysis of whole lung cells and western blotting of lung homogenate were performed after 14 days of EGFP-LLC transfer.

Survival analysis showed that BLT1-deficient mice died earlier than wild type mice. Macroscopic and microscopic examination suggested that BLT1-deficient mice were more susceptible to metastasis of LLC, which was confirmed by flowcytometric and the western blotting analyses.

Y-24

Blocking LTB4 Signaling Confers the Long-Term Antitumor Effects Induced by GM-CSF –Transduced Tumor in BLT-1 deficient Mice

Hiroyuki Inoue[1,2], Yosuke Yokota[1], Haruka Nabeta[1], Mutsunori Iga[1], Chika Sakamoto[1], Fumiyuki Sasaki[3], Yoichi Nakanishi[2], Takehiko Yokomizo[3] and Kenzaburo Tani[1].

[1]Department of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka-shi Fukuoka, 812-8582, Japan

[2] Research Institute of Diseases of the Chest, Kyushu University

[3] Department of Medical Biochemistry, Graduate School of Medical Sciences, Kyushu University.

It has been reported that the gene transduction of GM-CSF (granulocyte-colony stimulating-factor) gene into murine leukemia cell vaccine is promising approach to enhance patients' specific antitumor immune response in leukemia patients. The enhancement of this specific antitumor immunity is considered to be clinically beneficial to maintain complete remission for long time after chemotherapy in leukemia patients without stem cell transplant donors.

Leukotriene B4 (LTB4) is an extremely potent lipid inflammatory mediator derived from membrane phospholipids by the sequential actions of cytosolic phospholipase A2, 5-lipoxygenase (5-LO) and LTA4 hydrolase. The major activities of LTB4 include the recruitment and activation of leukocytes including neutrophils. Though structurally completely different, the lipid LTB4 and the peptide chemokines mediate their function through the same class of receptors, the G protein-coupled seven transmembrane domain receptor (GPCR) superfamily, BLT1 and BLT2. By mediating the activities of LTB4, these receptors participate both in the recruitment and activation of leukocytes as part of host immune responses to invading pathogens, as well as in the pathogenesis of inflammatory diseases. However, the role of LTB4 in tumor immunology is not well known. Previously we demonstrated the GM-CSF gene transduction into murine monocytic leukemia cell line of WEHI3B (W/GM) eliminated the tumorigenicity in subcutaneous challenge model using wild type (WT) BALB/c mice. The same implanted tumor rejection was also reproducible in $BLT1^{-/-}$ mice (n=12). And 50 days after the challenge, all $BLT1^{-/-}$ mice rejected the rechallenge of WEHI3B cells and survived, but none of wt mice rejected and survived. To explore the mechanism underlying the different outcome from rechallenge test, we next compared several inflammatory cytokine levels produced from splenocytes of WT or BLT-$1^{-/-}$ mice challenged with either WEHI3B or W/GM cells by Cytometric Beads Array method. The results demonstrated that higher production of IL-2, IL-4, TNF-α, and IFN-γ from splenocytes of BLT-$1^{-/-}$ mice treated with W/GM cells (KO/GM) was observed compared with WT mice treated with W/GM cells (WT/GM). Furthermore, increased numbers of several immune cells of mature DCs ($CD80^+CD11c^+$, $CD86^+CD11c^+$, and $CD80^+CD86^+CD11c^+$), lymphoid DCs ($CD11c^+B220^-CD8^+CD11b^-$), $CD4^+$ T_{EM} cells ($CD4^+CD44^+CD62L^-$) and $CD8^+$ T_{CM} cells ($CD8^+CD44^+CD62L^+$) were observed in draining lymph nodes harvested from KO/GM mice compared with WT/GM mice.

These observations suggested that the absence of LTB4 signaling in vivo could alter the immune cell distribution and result in the long term antitumor effects observed in the above rechallenge test.

These results suggested the blocking of LTB4 signaling may be useful strategy to maintain the antitumor effects of GM-CSF-transduced tumor vaccines over long period.

Y-25

T HELPER TYPE 2 DIFFERENTIATION AND INTRACELLULAR TRAFFICKING OF THE INTERLEUKIN 4 RECEPTOR-α SUBUNIT CONTROLLED BY THE RAC ACTIVATOR DOCK2

Tanaka, Y.[1] and Fukui, Y.[1]
[1]Division of Immunogenetics, Medical Institute of Bioregulation, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan

During immune response, antigen-stimulated naive $CD4^+$ T cells differentiate into functionally distinct types of T helper (Th) cells with different cytokine profiles. Th1 cells play an important role in cell-mediated immunity through secretion of interferon-γ (IFN-γ), whereas Th2 cells producing cytokines such as interleukin 4 (IL-4), IL-5 and IL-13 are involved in humoral immunity and allergic response. It is well established that the Th1/Th2 balance critically affects the disease outcome in a variety of immune-related and infectious diseases.

Polarization of naive $CD4^+$ T cells toward Th1 or Th2 cells is coordinately regulated by the signals through TCR and cytokine receptors, yet how these signals are integrated remains unknown. DOCK2, a mammalian homolog of *Caenorhabditis elegans* CED-5 and *Drosophila melanogaster* Myobast City, critically regulates Rac activation by functioning downstream of TCR. We found that DOCK2-deficiency renders Th1-prone C57BL/6 mice susceptible to *Leishmania major* infection and causes allergic disease in Th2-prone BALB/c mice. In DOCK2-deficient $CD4^+$ T cells, antigen-driven downregulation of IL-4Rα chain was impaired, resulting in sustained IL-4R signals and excessive Th2-type response. Although DOCK2 was not required for internalization of IL-4Rα chain, DOCK2 controlled lysosomal trafficking and degradation of the internalized IL-4Rα chain through Rac activation and microtubule dynamics. These results indicate that DOCK2 links TCR signals to IL-4 receptor downregulation to control lineage commitment of $CD4^+$ T cells at an early stage of differentiation.

Y-26

A critical role of CD30 ligand/CD30 in controlling inflammatory bowel diseases in mice

Xun Sun[1], Hisakata Yamada[1], Kensuke Shibata[1], Hiromi Muta[2], Kenzaburo Tani[2], Eckhard R. Podack[4], and Yasunobu Yoshikai[1]
[1]Division of Host Defense, and [2]Division of Molecular Genetics, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan. [4]Department of Microbiology and Immunology, University of Miami, Miami, FL 33101, USA.

Background & Aims: A CD30-ligand (CD30L) is a 40-kDa type II membrane associated glycoprotein belonging to the TNF family. Serum levels of soluble CD30 increased in inflammatory bowel diseases (IBD), suggesting that CD30L/CD30 signaling is involved in the pathogenesis of IBD. In this study, we investigated the role of CD30L in oxazolone (OXA)- and trinitrobenzene sulfonic acid (TNBS) induced colitis in CD30L knock out (KO) mice. **Methods:** Colitis was induced by OXA or TNBS in CD30LKO mice with BALB/c or C57BL/6 background, respectively, and diverse clinical signs of the disease were evaluated. Cytokines production form lamina propria (LP) T cells of the colon were assessed by enzyme-linked immunosorbent assay (ELISA). Anti-interleukin(IL)-4 monoclonal antibody (mAb) or agonistic anti-CD30 mAb was inoculated in mice with colitis induced by OXA or TNBS. **Results:** CD30LKO mice were susceptible to OXA-induced colitis, but resistant to TNBS-induced acute colitis. The levels of Th2 type cytokines such as IL-4 and IL-13 in the LP T cells were significantly higher, but the levels of IFN-γ were lower in OXA- or TNBS-treated CD30LKO mice than in wild type (WT) mice. In vivo administration of agonistic anti-CD30 mAb ameliorated OXA-induced colitis but aggravated TNBS-induced colitis in CD30LKO mice. **Conclusions:** These results suggest that CD30L/CD30 signaling is involved in development of both OXA- and TNBS-induced colitis. Modulation of CD30L/CD30 signaling by mAb could be a novel biological therapy for IBD.