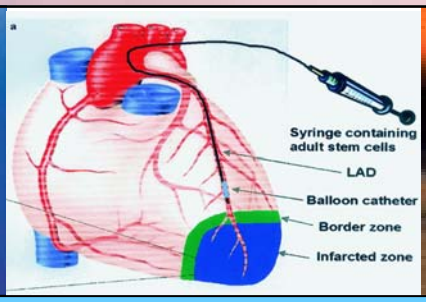
The 11th US-Japan Cellular and Gene Therapy Conference on

Tissue Engineering



Program and Abstract Book

Natcher Conference Center
National Institutes of Health
Bethesda, Maryland

February 28, 2008

US Food and Drug Administration,
Center for Biologics Evaluation and Research and
Ministry of Education, Culture, Sports, Science, and Technology, Japan

The Eleventh US-Japan Cellular and Gene Therapy Conference on Tissue Engineering

Natcher Conference Center
Building 45, Room E1/E2, National Institutes of Health
Bethesda, Maryland 20892

Thursday, February 28, 2008

The conference is jointly supported by the Center for Biologics Evaluation and Research (CBER), US Food and Drug Administration (FDA) and the Ministry of Education, Culture, Sports, Science and Technology, Japan under the US-Japan Cooperative Research Program. The goal of this conference is to exchange ideas on cutting edge areas of biomedical research and to enhance opportunities for collaborations among scientists from the US and Japan. This year is our eleventh annual meeting and the scientific focus for this year is **Tissue Engineering**. Six speakers each from the US and Japan are invited to discuss the advances in this multidisciplinary field and highlight FDA's activities in this cutting edge area of biomedical research.
Seating is limited to 150 attendees and is on a first-come-first-served basis. No registration is required. For more information, please contact Dr. Syed R. Husain by e-mailing syed.husain@fda.hhs.gov or by phone at (301) 827-0475.

Program

8:00 a.m. – 8:30 a.m.	**Registration** **Coffee and Breakfast**
Moderator:	**Dr. Syed R. Husain,** Senior Staff Scientist, Division of Cellular and Gene Therapies, CBER, US Food and Drug Administration, Bethesda, Maryland
8:30 a.m. – 8:35 a.m.	**Opening Remarks** **Dr. Celia Witten,** Director, Office of Cellular, Tissue and Gene Therapies, CBER, US Food and Drug Administration, Rockville, Maryland
8:35 a.m. – 8:40 a.m.	**Dr. Yoshikazu Ohya,** Professor, Department of Integrated Biosciences Graduate School of Frontier Sciences, University of Tokyo, Japan

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AM Session

Moderators:	**Dr. Yoshikazu Ohya,** Professor, Department of Integrated Biosciences Graduate School of Frontier Sciences, University of Tokyo, Japan **Dr. Toru Nakano,** Professor, Department of Pathology, Medical School/Graduate School of Frontier Biosciences, Osaka University and Department of Molecular Biology, Akita University School of Medicine, Japan
8:40 a.m. – 9:10 a.m.	**Regulation of Regenerative Medicine and Tissue Engineering: An FDA Perspective**

	Dr. Richard McFarland, Associate Director of Policy, Office of Cellular, Tissue and Gene Therapies, CBER, US Food and Drug Administration, Rockville, Maryland
9:10 a.m. – 9:40 a.m.	**Cellular Wound Dressings, Regulation of Such Combination Products under the Medical Device Authorities** **Dr. Charles Durfor,** Division of General, Restorative, and Neurological Devices, Center of Devices and Radiological Health, US Food and Drug Administration, Rockville, Maryland
9:40 a.m. – 10:10 a.m.	**Clinical Applications of Tissue Engineering Technology in Maxillofacial Region** **Dr. Minoru Ueda,** Professor and Chairman, Department of Oral and Maxillofacial Surgery, Nagoya University, School of Medicine, Japan

10:10 a.m. – 10:30 a.m. **Coffee Break**

10:30 a.m. – 11:00 a.m.	**Regeneration in the Musculoskeletal System** **Dr. Jennifer Elisseeff,** Associate Professor, Department of Biomedical Engineering and Orthopedic Surgery, Johns Hopkins University, Baltimore, Maryland
11:00 a.m. **–** 11:30 a.m.	**Cell Sheet Tissue Engineering for Regenerative Medicine** **Dr. Teruo Okano,** Professor and Director, Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Japan
11:30 a.m. – 12:00 p.m.	**Application of Nanomaterials and Adult Stem Cells in Skeletal Tissue Engineering and Regeneration** **Dr. Rocky S. Tuan,** Chief, Cartilage Biology and Orthopedics Branch, National Institute of Arthritis, and Musculoskeletal and Skin Diseases, NIH, Bethesda, Maryland
12:00 p.m. – 1:00 p.m.	**Lunch**

PM Session

***Moderators**:*	**Dr. Raj Puri,** Director, Division of Cellular and Gene Therapies, Center for Biologics Evaluation and Research, Bethesda, FDA **Dr. Shigeo Koyasu**, Professor, Department of Microbiology and Immunology, Keio University School of Medicine, Japan **Dr. Rocky S. Tuan,** Chief, Cartilage Biology and Orthopedics Branch, National Institute of Arthritis, and Musculoskeletal & Skin Diseases, NIH, Bethesda, Maryland
1:00 p.m. – 1:30 p.m.	**Biomaterials-Based Tissue Engineering to Realize Tissue Regenerative Therapy** **Dr. Yasuhiko Tabata**, Professor, Department of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University, Japan
1:30 p.m. – 2:00 p.m.	**Isolation and Clinical Application of Human Corneal Epithelial and Endothelial Adult Stem Cells/Precursors** **Dr. Satoru Yamagami,** Professor, Department of Corneal Tissue

Regeneration, Tokyo University Graduate School of Medicine, Japan

2:00 p.m. – 2:30 p.m.	**TGF-beta Signaling Pathway in Stem Cells and Cancer** **Dr. Lopa Mishra**, Vice Chair Department of Surgery, Professor of Medicine and Surgery, Georgetown University, Washington DC
2:30 p.m. – 2:50 p.m.	**Coffee Break**
2:50 p.m. – 3:20 p.m.	**Notch Signaling Controls Cardiac Smooth Muscle Cell Proliferation** **Dr. Brenton McCright,** Senior Staff Fellow, Division of Cellular and Gene Therapies, Office of Cell, Tissue and Gene Therapies, CBER, Bethesda, Maryland
3:20 p.m. – 3:50 p.m.	**Role of PI3K in the Development of Gastrointestinal Mast Cells** **Dr. Shigeo Koyasu**, Professor, Akiko Minowa and Satoshi Matsuda Department of Microbiology and Immunology, Keio University School of Medicine
3:50 p.m. – 4:20 p.m.	**Regulation of Stem Cell Systems by PI3K/Akt Signaling** **Dr. Toru Nakano,** Professor, Medical School/Graduate School of Frontier Biosciences, Osaka University and Department of Molecular Biology, Akita University School of Medicine, Japan
4:20 p.m. – 4:30 p.m.	**Closing Remarks** **Dr. Raj K Puri**, Director, Division of Cellular and Gene Therapies, Center for Biologics Evaluation and Research, US Food and Drug Administration, Bethesda, Maryland

Conference Organizers:	**Syed R. Husain, Ph.D.** **Raj K Puri, M.D., Ph.D.** Tumor Vaccines and Biotechnology Branch Division of Cellular and Gene Therapies Center for Biologics Evaluation and Research, FDA NIH Bldg. 29B, Room 2G07, HFM-735 Bethesda, Maryland, 20892 Tel. 301-827-0475; Fax 301-827-0449 syed.husain@fda.hhs.gov raj.puri@fda.hhs.gov

AM Session

Moderators: Yoshikazu Ohya Toru Nakano

8:40 am:

Regulation of Regenerative Medicine and Tissue Engineering: An FDA Perspective

Richard McFarland, Associate Director of Policy, Office of Cellular, Tissue and Gene Therapies, CBER, US Food and Drug Administration, Rockville, Maryland

This presentation will present an overview of the current FDA regulatory scheme for regenerative medicine and tissue engineering products in the United States.

9:10 am

Cellular Wound Dressings, Regulation of these Combination Products Under the Medical Device Authorities

Charles N. Durfor, Ph.D., Plastics and Reconstructive Surgery Devices Branch
Division of General, Restorative and Neurological Devices, Office of Device Evaluation, CDRH, FDA

Advancements in both biotechnology and biomaterials fabrication have made the promise of tissue engineering a reality. Depending on product composition and the mechanism of clinical action, products containing cell and "scaffold" components may be reviewed as a biologic or medical device by the U.S. Food and Drug Administration.

This presentation will provide information concerning methods for delineating how product jurisdiction is performed within the FDA. In addition, the presentation will discuss the medical device review process and guidance documents employed by FDA during the review of cellular wound dressing devices. Such information will also identify critical scientific and medical issues involved in initiating investigational studies and subsequently, bringing a cellular medical device into commercial distribution.

9:40 am

Clinical Applications of Tissue Engineering Technology in Maxillofacial Region

Minoru Ueda, Professor and Chairman, Department of Oral and Maxillofacial Surgery, Nagoya University, School of Medicine, Nagoya, Japan

INTRODUCTION: We have established the mucosal fibroblast and osteogenic cell injection system which can improve the face wrinkles and alveolar bone formation. In this presentation, I will introduce the new method for the cosmetic treatment and dental implant for skin and bone using living cell and its 3-years clinical data.

METHODS: These procedures were developed by our team and prospective clinical study was begun in 2004. The cell culture process was started with a 3-mm gingival punch biopsy or bone marrow aspiration. The specimen was shipped to the cell processing center where the cells were expanded by proprietary tissue culture techniques. Six weeks after the biopsy, the cells were injected with a 30-gauge needle into the superficial dermis or alveolar bone defect. All patients were followed on monthly basis. Results were evaluated on CT scan, photographic analysis and patient outcome surveys.

RESULTS: A total of 89 patients were treated in our clinic (face 37, papilla 2, alveolus 50).
Skin: In case of fibroblast injection, the patients received a total of 111 injections from 2005 through 2007. Improvements of skin wrinkle were evaluated with pre-and post-injection photographs at 3 months and 6-month intervals thereafter. A subjective patient satisfaction survey was also utilized to evaluate results. The patients were asked to grade correction on a 5-point scale. From our study group, only 1 patient reported mild erythema at the injection site, which resolved in 2 to 3 days. At 12 months the patients' average grading of their degree of correction was 4.8 points. There were no reports of infection, rejection, granuloma formation, keloid formation, or

overcorrection of the treated areas.
Bone: In case of alveolar bone regeneration, osteogenic cell injection was performed with implant installation. The alveolar bone regeneration has been evaluated by CT scan images at the 3 months intervals after surgery. As results the average bone regeneration was 7.0mm in thickness.
The ideal material for tissue regeneration would be an autologous injectable material that provides long-term results with minimal invasiveness. Autologous cultured cell satisfied all these criteria.

CONCLUSION: Mucosal cultured autologous fibroblasts are the first attempt at cellular therapy in the field of maxillofacial surgery. Continual correction of face wrinkles provides a high patient satisfaction. Also osteogenic cells derived from bone marrow can successfully create the alveolar bone. By using this technology the success rate of dental implant remarkably improved. Tissue engineering technology will open a new window in the field of maxillofacial surgery.

10:30 am
Regeneration in the Musculoskeletal System

Jennifer Elisseeff, Department of Biomedical Engineering and Orthopedic Surgery, Johns Hopkins University, Baltimore, MD 21218

Regenerative medicine aims to provide biological tissue substitutes as an alternative to synthetic implants. In some cases, biomaterials alone can be applied to direct and stimulate endogenous cells to generate new tissue. However, to achieve regeneration in large defects or diseased/aged environments, exogenous cells may be required. In our application of cartilage regeneration there are a number of cell types that can be utilized to make new tissue. To evaluate cell types for cartilage repair, we compared the differentiation and tissue forming capabilities of cells derived from adult marrow, embryonic germ and stem cells, in addition to primary cells (chondrocytes). The different cell types, particularly the embryonic-derived, required unique biological signals to be incorporated in the biomaterial scaffold to direct the cell behavior and achieve functional tissue formation. Understanding the biology of cell response and tissue development was critical for proper biomaterial design consideration. To translate cell and tissue engineering strategies to the challenging joint environment, novel chemistry to integrate synthetic materials and surrounding tissue was developed. We both chemically modified the tissue surface and a cartilage-derived polysaccharide to covalently bond the lubricous cartilage surface with a synthetic biomaterial. Biomaterial integration was required to retain biomaterials in vivo and was the basis for a one year large animal study to demonstrate efficacy in cartilage repair. Modification of tissue surfaces to improve biomaterial implantation is an important general technique that may be applied to other fields and both short and long term implantation of synthetic materials.

11:00 am
Cell Sheet Tissue Engineering for Regenerative Medicine

Teruo Okano, Professor and Director, Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, 8-1kawada-cho Shinjuku-ku Tokyo Japan 162-8666, Tel:+81-3-3353-8112ext 30233, Fax:+81-3-3359-6046
Email:tokano@abmes.twmu.ac.jp

Ideal tissue construction in vitro and in vivo should be achieved by cell and cell sheet manipulation engineering that would be established with the following three core technologies: first, the noninvasive harvest of cultured cells and cell sheets are realized with our developed temperature-responsive culture dishes. The surfaces are hydrophobic at 37° C, but change to hydrophilic below 32° C. Various cell lines adhere, spread and proliferate on the surfaces similarly to those on commercial tissue culture dishes. Only by reducing temperature, cells spontaneously detached from the surfaces without the need for trypsin. Confluent cells are also recovered as a single contiguous monolayer sheet with intact cell-cell junctions and deposited extracellular matrix. Second, the harvested viable cell sheets can be transferred to other surfaces of culture dishes or devices (2D cell sheet manipulation) because the extracellular matrix associated with the basal side of cell sheets shows adhesion. Thus, tissue regeneration with cell sheet tissue engineering can be accomplished either by transplantation of single cell sheets, as with skin, cornea and periodontal ligaments. Finally, the recovered cell sheets can be stratified to reconstruct complex stratified tissue architectures such as liver lobule, kidney glomeruli, and cardiac patches (3D cell sheet manipulation). For example, layered cardiomyocyte sheets harvested from temperature responsive dishes pulsate simultaneously and show diffuse gap junction formation. When transplanted into the subcutaneous tissues of nude rats, spontaneous beatings could be macroscopically observed after 3 weeks and maintained for over 1 year. We believe that these 2D and 3D cell manipulations, cell sheet tissue engineering, will become new revolutionary tools for tissue engineering.

11:30 am

Application of Nanomaterials and Adult Stem Cells in Skeletal Tissue Engineering and Regeneration

Rocky S. Tuan, Ph.D., Chief, Cartilage Biology and Orthopaedics Branch, National Institute of Arthritis, and Musculoskeletal & Skin Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, MD 20892, U.S.A.
(Email: tuanr@mail.nih.gov)

Nanoscale materials are the fundamental building blocks and functional subunits of cells, including subcellular organelles and extracellular matrix components. Currently, there is growing recognition of the importance of understanding and incorporating nanobiology into biomedical applications. This issue is of particular importance in the emerging field of regenerative medicine, the goal of which is to develop methods to repair, replace, and regenerate diseased, injured, or non-functional tissues. Towards this goal, stem or progenitor cells have been considered a highly desirable candidate cell type, because of their expandability and potential to be induced toward specific cell differentiation lineages. A key requirement in tissue engineering and regenerative medicine is that ultimately the “regenerate tissue” needs to be a three-dimensional structure. In weight-bearing musculoskeletal tissues, this requirement is particularly critical. Musculoskeletal disorders affect one out of seven Americans. This severe disease burden underscores the need to develop novel and effective treatment protocols. This lecture will present the promises as well as the challenges in the field of skeletal tissue engineering and regeneration, specifically the application of adult stem cells and nanomaterial scaffolds. The biology of human adult mesenchymal stem cells, particularly the mechanisms regulating their proliferation *versus* differentiation into specific lineages, is intricately regulated by cell-cell interactions, signaling by extracellular bioactive factors, and transcriptional and

epigenetic activities. More importantly, the extracellular matrix milieu provides critical cues, both architectural and structure-dependent, to guide cell-based tissue morphogenesis. We have developed biomimetic and biodegradable nanofibrous biomaterials to serve as scaffolds for cell-based tissue engineering. Information on the fabrication and biological basis of the scale-dependent bioactivities of the nanofibrous scaffold will be presented. Cell-nanofibrous constructs are currently being developed for the engineering of cartilaginous tissues, including articular cartilage and intervertebral disc. In conclusion, tissue engineering represents a unique, emerging inter-disciplinary research field that is a natural platform for life scientists, engineers, and clinicians working together to advance regenerative medicine.

Relevant Publications:

1. Chen, F.H., Rousche, K.T., and Tuan, R.S. (2006) Technology insight: Adult stem cells in cartilage regeneration and tissue engineering. *Nature Clin. Pract.*, 2: 373-382.

2. Kolf, C.M., Cho-Fertikh, E., and Tuan, R.S. (2007) Biology of adult mesenchymal stem cells: Regulation of niche, self-renewal, and differentiation. *Arthr. Res. Therapy.* 9: 204-213.

3. Song, L., Webb, N.E., Song, Y.J. and Tuan, R.S. (2006) Identification and functional analysis of genes regulating mesenchymal stem cell self-renewal and multipotency. *Stem Cells.* 24: 1707-1718.

4. Li, W.-j., Tuli, R., Huang, X., Laquerriere, P., and Tuan, R.S. (2005) Multilineage differentiation of human mesenchymal stem cells in a three-dimensional nanofibrous scaffold. *Biomaterials.* 26: 599-609.

5. Li, W.j., Mauck, R.L., Cooper, J., Yuan, X., and Tuan, R.S. (2007) Engineering controllable anisotropy in electrospun biodegradable nanofibrous scaffolds for musculoskeletal tissue engineering. *J. Biomech.* 40: 1686-1693.

PM Session

Moderators: Raj K Puri Shigeo Koyasu Rocky S Tuan

1:00 pm

Biomaterials-Based Tissue Engineering to Realize Tissue Regenerative Therapy

Yasuhiko Tabata, Ph.D., D.Med.Sci., D.Pharm., Department of Biomaterials, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

Following reconstruction surgery and organ transplantation, a new therapeutic trial based on the natural potential of body to induce tissues and organs regeneration, has been recently expected. To realize this tissue regenerative therapy, there are two practical approaches; cell transplantation therapy and tissue engineering. Tissue engineering is a biomedical technology or methodology to artificially create a local environment which enables cells to enhance their proliferation and differentiation, resulting in cell-induced tissue regeneration. If cells around a target site to be regenerated have an inherent ability to induce tissue regeneration, tissue regeneration will be induced only by surgically supplying a temporary cell scaffold of biomaterials to the target site. However, for the site of poor regeneration potential, it is necessary to combine cells or growth factors and genes with the scaffold. If a key growth factor is supplied to the right place at the right time period and concentration, the body system will initiate to physiologically function for natural induction of tissue regeneration. However, the biological activities of un-stable growth factor cannot always be expected only by the direct injection in the solution form. One

practically possible way to enhance the in vivo efficacy of growth factors is to make use of drug delivery system (DDS) technology. We have designed biodegradable hydrogels for the controlled release of growth factors and succeeded in repairing various tissues based on the inherent potential of living body to induce tissue regeneration. Some clinical experiments of growth factor-induced regeneration therapy for blood vessels, bone, periodontal tissue, and skin dermis have been started to demonstrate several superior therapeutic efficacy. This release system can be also combined with stem cells to significantly enhance the efficacy of cell therapy in tissue regeneration.

This hydrogel system is also applicable to the controlled release of gene, such as plasmid DNA and small interference RNA (siRNA). The controlled release enabled a plasmid DNA to enhance the level of in vivo gene expression and prolong the expression time period to a significantly great extent compared with the plasmid DNA solution. Hydrogel microspheres, after internalizing into stem cells, could release the plasmid DNA inside the cells. By this intracellular release of plasmid DNA or a new non-viral gene carrier of polysaccharide, stem cells were genetically engineered to activate the biological functions. The cells genetically engineered enhanced the therapeutic efficacy in cell therapy-based tissue regeneration (cell-gene hybrid therapy) to a significantly great extent compared with the original cells.

The basic idea of tissue regenerative therapy is also applicable to internal medicine. For chronic fibrotic diseases, the disease site is occupied by fibrous tissue of excessive collagen. Therefore, if such a fibrotic tissue can be digested by any method to loosen or disappear, it is highly expected that the disease site is regenerated and repaired based on the natural healing potential of the surrounding healthy tissue. This is defined as physical tissue engineering of internal medicine, because in this therapy, tissue repairing is achieved through the natural induction of tissue regeneration accompanied with the drug treatment strategy of internal medicine.

In this paper, several concrete experimental data on promoted regeneration of various tissues by the DDS technology of growth factors and genes are presented to emphasize scientific and clinical significance of biomaterials-based tissue engineering to realize tissue regenerative therapy.

References: Tissue Engineering, 9(1), 5 (2003); Drug Delivery Today, 23/24, 1639 (2005); Advanced Drug Delivery Reviews, 58, 535 (2006); Reproductive BioMedice Online, 16(1), 70 (2007); Tissue Engineering, 13(2), 245 (2007)

1:30 pm

Isolation and clinical application of human corneal epithelial and endothelial adult stem cells/precursors

Satoru Yamagami, Department of Corneal Tissue Regeneration, Tokyo University Graduate School of Medicine, Tokyo, Japan

Cornea is a transparent tissue, composed of epithelium, stroma, and endothelium. The corneal epithelium, self-renewing squamous cells, covers the surface of the eye and represents the outer barrier against microbes and harmful substances. This epithelium is maintained by a population of adult stem cells residing in part of the cornea called the “limbus.” Cells from the corneal limbus have been amplified *ex vivo* to repair injuries or genetic defects of the corneal surface with transparent epithelium, thus avoiding corneal keratinization and vascularization with a poor visual outcome. Corneal epithelium can be reconstituted successfully by using autologous or allogenic limbal tissue under circumstances where adult stems are deficient, such as in Stevens-Johnson syndrome, ocular pemphigoid, and chemical burns. However, isolation technique to enrich the adult stem cells has not been established in the human corneal epithelium.

The corneal endothelium is a single layer of flat hexagonal cells that lies on a basement membrane, Descemet’s membrane, and forms a pure cell sheet without any other cell types. The corneal endothelium is essential for maintaining corneal transparency. This function is dependent

on endothelial regulation of stromal hydration, including the barrier and pump functions of the aqueous humor. Damage to the human corneal endothelium caused by intraocular surgery, glaucoma, trauma, or congenital corneal disease results in irreversible corneal edema, because there is no or extremely low mitotic activity in the human corneal endothelium after birth, which leads to a gradual decrease in the cell population with age. Recently, we isolated human corneal endothelium precursors by sphere-forming assay and developed a new technique for precursor transplantation experimentally.

In this presentation, I will introduce the isolation technique of adult stem cells/ precursors in the human corneal epithelium and endothelium, and current and future clinical application using these cells.

2:00 pm

TGF-β signaling pathway in Stem Cells and Cancer

Lopa Mishra MD, Vice Chair, Department of Surgery, Professor of Medicine and Surgery, Georgetown University, Washington DC 20007

Inactivation of the TGF-β signaling pathway occurs in nearly all gastrointestinal cancers: mouse and human studies. However, to date the role of the TGF-β pathway at specific stages in gastrointestinal (GI) tumor development such as metaplasia, dysplasia and carcinoma remains poorly delineated, particularly in conjunction with activation of oncogenic pathways. A clear delineation of the role of this important tumor suppressor pathway could lead to powerful new therapeutics targeted at difficult to treat cancers such as pancreatic, gastric and hepatocellular that are often stem cell derived.

For instance, Smad4 is deleted in up to 60% pancreatic cancers, mutated in hereditary juvenile polyposis coli, TBR2 is mutated in up to 30% colon cancers, TBR1 is mutated in 15 % biliary cancers. Loss of ELF is observed in human hepatocellular cancers and results in spontaneous development of hepatocellular cancers in mice. ELF is also lost in human Beckwith-Wiedemann syndrome (BWS), a hereditary human cancer syndrome. ELF/Smad3 mutant mice are a mouse model for human BWS. We previously found that deletion of ELF results in a dramatic and spontaneous formation of liver and gastrointestinal (GI) cancers, with a splice site mutation in elf exon 15 in 11% of human GI cancer cell lines tested so far. Thus the Smad3/4 adaptor protein ELF is emerging as a potent regulator of tumorigenesis by its ability to effect TGF-β tumor suppressor function (Science, 2003, 299:574-577, Science, 2005, 310:68-71).

These observations are supported by mouse and human studies, that include our surprising and serendipitous recent discovery that elf$^{+/-}$ and elf$^{+/-}$/Smad3$^{+/-}$ mice develop visceromegaly and multiple GI cancers (70% of mice), including metastatic pancreatic, hepatocellular, intestinal adenocarcinomas and others spontaneously, providing compelling evidence as a mouse model of Beckwith-Wiedemann syndrome (BWS), a hereditary human cancer syndrome. In addition, 90% of elf$^{+/-}$/Smad4$^{+/-}$ mice develop gastric cancer, 20% develop colonic adenomas, and ELF expression is lost in human gastric cancer as well as Dukes B1 adenomas indicating a role for ELF in suppression of early human gastrointestinal cancer. Molecular profiling of the tumors in these mice and human GI cancers demonstrate markedly high levels of cell cycle regulators that include CDK4, cyclin D1, c-Myc, TERT, Beta-catenin and PRAJA an ELF/Smad3 specific E3 ligase. Interestingly, Smad3 has recently been shown to be a CDK4 substrate, yet Smad3 mutant mice do not develop cancers. Thus, activated CDK4 and

PRAJA associate with ELF with or without Smad3 and most likely exert their oncogenic activity through suppression of ELF/Smad3. Most importantly ELF is lost in human BWS, providing new insights into this hereditary cancer syndrome, as well as sporadic human tumors and cancer stem cells from tissues such as pancreatic, gastric and hepatocellular.

2:50 pm

Notch signaling Controls Cardiac Smooth Muscle Cell proliferation

Brenton McCright, Division of Cellular and Gene Therapies, Office of Cell, Tissue and Gene Therapies, CBER, Bethesda, Maryland

Cellular and tissue engineering have tremendous potential for creating new therapeutic products for the treatment of degenerative disease but currently no study has been able to demonstrate the productive integration and formation of new heart tissue from cells grown ex vivo. In order to manufacture a product that can contribute to the regeneration of damaged hearts it will be necessary to understand the signaling and cell interactions that control the formation of the embryonic heart. Mutations in Notch receptors and their ligands have previously been identified as the cause of human congenital heart diseases indicating the importance of the Notch signaling pathway during heart development. In our study we use Cre-Lox technology to inactivate, or activate Notch2 in several cardiac cell lineages to determine the functional requirements for Notch2 during murine heart development. Inactivation of Notch2 in cardiac neural crest cells resulted in abnormally narrow aortas and pulmonary arteries due to a decrease in smooth muscle tissue. The reduction in smooth muscle tissue was not due to cell migration defects but instead was found to be caused by less proliferating smooth muscle cells during mid to late gestation. In contrast, over-expression of Notch in the heart results in the hyper-proliferation of smooth muscle tissue. Our findings demonstrate that Notch2 is required cell autonomously for proper formation of the heart outflow tract and provides insights into the role of Notch2 in vascular smooth muscle development and the cardiovascular defects associated with Alagille syndrome.

3:20 pm

Role of PI3K in the Development of Gastrointestinal Mast Cells

Shigeo Koyasu, Akiko Minowa and Satoshi Matsuda, Department of Microbiology and Immunology, Keio University School of Medicine, Tokyo, Japan

Phosphoinositide 3-kinases (PI3Ks) are important for diverse physiological reactions including immune reactions (1). We have previously shown that mice lacking the p85a regulatory subunit of class IA PI3K ($PI3K^{-/-}$ mice) lack gastrointestinal mast cells and are more susceptible to the infection by intestinal nematode *Strongyloides venezuelensis* than wild type mice (2). We examined the role of PI3K in the development of gastrointestinal mast cells in a steady state and that in the recruitment of mast cells (mastocytosis) upon nematode infection. Limiting dilution analysis showed that the frequency of mast cell precursors in the intestine of $PI3K^{-/-}$ mice was greatly reduced compared to wild type mice, suggesting that either the migration or proliferation of precursors is impaired. The $c\text{-}Kit^{+}a4b7^{+}$ population in the bone marrow contained a high number of precursors. IL-3-induced $c\text{-}Kit^{+}a4b7^{+}$ bone marrow-derived mast cells (BMMCs)

from PI3K$^{-/-}$ mice were impaired for the migration to the intestine compared to those from wild type mice. The *in vivo* proliferation of PI3K$^{-/-}$ c-Kit^{+}a4b7^{+} BMMCs in the intestine after transfer and their *in vitro* proliferation in response to SCF were significantly impaired. Furthermore, SCF-induced haptotaxis of c-Kit^{+}a4b7^{+} BMMCs on MAdCAM1-coated surface was impaired in the absence of PI3K. These results indicate that the migration of precursor cells to the intestine and the proliferation of precursor cells are impaired in the absence of PI3K in a steady state condition. It has been reported that IL-3 plays an important role in nematode-induced mastocytosis (3). PI3K/IL-3 double deficient mice were significantly more sensitive to the infection by *S. venezuelensis* than each single deficient mouse line. Mastocytosis was also defective in a food allergy induction model. Finally, transfer of wild type but not IL-3-deficient T cells restored mastocytosis, indicating the critical role of IL-3 in the induction of mastocytosis. Our results collectively indicate that PI3K and IL-3-mediated signaling differentially regulate mast cell differentiation in the gastrointestinal tract.

References:

(1) Koyasu, S. Nat. Immunol. 4:313-319 (2003).

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(3) Lantz, C. S. *et al*., Nature 392:90-93 (1998).

3:50 pm

Regulation of Stem Cell Systems by PI3K/Akt Signaling

Tohru Kimura, Akira Suzuki, and **Toru Nakano,** Department of Pathology, Medical School/Graduate School of Frontier Biosciences, Osaka University and Department of Molecular Biology, Akita University School of Medicine, Japan

Stem cells can replenish their own population while supplying the cells necessary to maintain tissue homeostasis. Pluripotent stem cells, which have broader developmental potency than tissue stem cells, are derived from the same source in mice and humans. We have been analyzing the functions of phosophoinositide-3 kinase (PI3K) and its downstream serine/threonine kinase Akt in a variety of stem cell systems.

Primordial germ cells (PGCs), which are embryonic germ cell precursors, are unique in that they acquire pluripotency under cultural and pathological conditions. PGCs lacking *Pten*, which encodes a phosphatase that antagonizes PI3K signaling, give rise to early-onset testicular teratomas *in vivo* and augment the derivation of pluripotent embryonic germ (EG) cells *in vitro*. Transient activation of Akt sufficiently recapitulates the effects of *Pten* deficiency on EG cell derivation. Enhanced EG cell derivation is brought about by the Akt-mediated inhibition of the tumor suppressor p53. In embryonic stem (ES) cells, PI3K/Akt signaling plays a pivotal role in maintaining pluripotency in part via transcriptional activation of the pluripotent transcription factor Nanog. In turn, the expression of Tcl1, a cofactor of Akt, is activated by pluripotent transcription factors, including Oct-3/4. Therefore, PI3K/Akt signaling and the transcription factor network constitute the positive feedback circuitry necessary to maintain pluripotency in ES cells.

In tissue stem cells, such as hair follicular, intestinal, and hematopoietic stem cells, PI3K/Akt signaling activates quiescent stem cells, leading to the generation of committed progenitors and cancer stem cells. These findings underscore the idea that PI3K/Akt signaling regulates "stemness" in many stem cell systems.

References:

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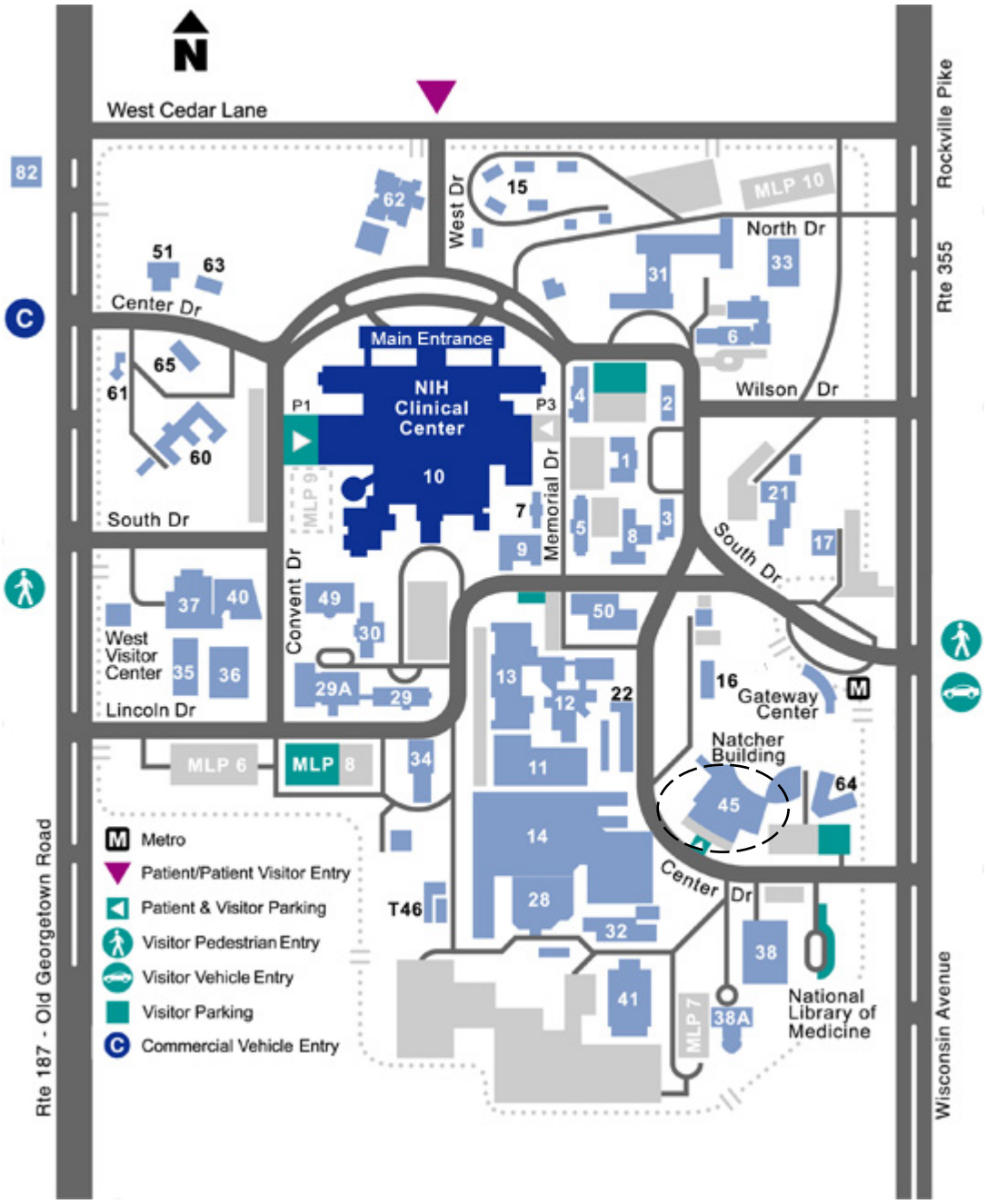
Thank you for your participation!

NIH Visitor's Map



To view an online version of this map or to learn more about visitor and security issues at the NIH, visit: http://www.nih.gov/about/visitor/index.htm

For questions about campus access, please contact the ORS Information Line at orsinfo@mail.nih.gov or 301-594-6677, TTY - 301-435-1908.