

StemConn 2011

SPEAKER ABSTRACTS

March 22, 2011
Hartford Marriott Farmington
Farmington, Connecticut

Stem Cells and Their Niche in the Adult Mammalian Brain

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Stem cells persist in specialized niches in the adult mammalian brain where they continuously generate large numbers of neurons that become functionally integrated into neural circuits. The subventricular zone (SVZ) is an extensive germinal layer adjacent to the lateral ventricles. A subset of SVZ astrocytes are stem cells in this region and generate rapidly dividing transit-amplifying cells, which in turn produce neuroblasts that migrate to the olfactory bulb. A major limitation in the neural stem cell field has been the ability to prospectively purify stem cell astrocytes from other astrocytes in the SVZ. We have recently developed a simple method to simultaneously purify each SVZ stem cell type by means of fluorescence activated cell sorting. Defining the transcriptional and post-transcriptional regulatory networks that mediate adult neural stem cell self-renewal and differentiation are key to understanding the biology of neural stem cells. I will present our findings about the post-transcriptional regulation of adult neural stem cells by microRNAs, as well as the specialized niche that supports adult neurogenesis.

dsRNA and Noncoding RNAs in Human Embryonic Stem Cells

Gordon G. Carmichael, Ph.D., University of Connecticut Health Center

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We are interested in how human embryonic stem cells express and use RNA molecules and we are especially interested in cellular responses to double stranded RNA (dsRNA). Cells respond quite differently to dsRNA, depending on whether these molecules are in the nucleus or in the cytoplasm. In the nucleus, RNAs that contain dsRNA structures are susceptible to RNA editing and are inefficiently exported to the cytoplasm. These molecules accumulate in nuclear subcompartments called paraspeckles. Paraspeckles not only contain nuclear-retained mRNAs, but are also associated with an abundant nuclear-retained noncoding RNA called NEAT1. We have found that NEAT1 RNA plays an essential role in both the assembly and function of paraspeckles and have also discovered that this pathway may play a role in human embryonic stem cell (hESC) biology, because it is lacking in hESCs but is induced upon differentiation. In the cytoplasm, dsRNAs generally act as strong inducers of the interferon pathway, but this pathway is silenced in hESCs. Thus, the major nuclear and cytoplasmic dsRNA response pathways are both altered in stem cells. I will discuss this work as well as ongoing studies aimed at identifying and characterizing a number of other interesting long nuclear noncoding RNAs, some of which are specifically expressed in hESCs, or specifically blocked from expression in these cells.

Substantial Mouse Calvarial Bone Defect Healing by Human Embryonic Stem Cells

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Human embryonic stem cells (hESCs) may one day provide an unlimited source of progenitor cells for orthopaedic repair applications. Previous attempts at in vivo bone repair using osteogenic progenitor cells derived from hESCs were complicated and not successful due to a lack of homogeneity of the cells within the cultures, sparse new bone formation, and the formation of teratomas after in vivo implantation. Our objective was to identify a simple, highly efficient method for deriving a large population of osteogenic precursor cells from hESCs and demonstrate successful in vitro and in vivo osteogenesis by these cells. Based on the more narrow potentiality of osteo-chondrogenic progenitors (OCP) derived from hESCs using a simple protocol as described in [1] we evaluated their capability for in vitro and in vivo osteogenesis. While the cells demonstrated a delayed and incomplete ability to differentiate into osteoblasts within in vitro cultures, substantial new bone via endochondral ossification was observed 6 weeks after in vivo implantation in mouse calvarial bone defects. ALU in situ hybridization revealed that human cells were present within the new bone as osteocytes and within a thick cell-rich layer above the new bone, but not in the original mouse bone. Adult mesenchymal stem cells from marrow had limited inconsistent healing. This is the first demonstration of substantial new in vivo bone formation by hESC-derived cells with no evidence of teratoma formation. The high efficiency and simplicity of the process make the method a suitable approach for future attempts at deriving a large population of progenitors for bone tissue engineering from iPSCs.

[1] Boyd NL et al. Tissue Eng Part A. 2009;15:1897-907.

Skeletal muscle stem cells in regeneration and disease

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Associate Professor of Molecular and Cell Biology, Director of the Center for Regenerative Biology, Associate Director of the UConn Stem Cell Institute

Heterotopic ossification is a debilitating condition that can result from traumatic injury, surgery, or genetic disease. We investigated the cellular origins of heterotopic bone using Cre/loxP lineage analysis and mouse models of heterotopic ossification. Despite their osteogenic potential under some experimental settings in culture, muscle stem cells (satellite cells) are not a significant contributor to heterotopic skeletogenesis in vivo. In contrast, progenitors in skeletal muscle that express the receptor tyrosine kinase Tie2, but are not of endothelial origin, exhibit robust BMP-dependent osteogenic activity. This progenitor population, which resides in the skeletal muscle interstitium, was prospectively isolated by FACS and shown to possess bipotentiality in culture and following intramuscular cell transplantation. Identifying the cells-of-origin responsible for heterotopic ossification provides a potential therapeutic target to treat, mitigate or prevent this disabling condition.

Regulation of the organogenesis by the vascular niche cells

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Interaction of stem cells with their niche cells is essential for self-renewal and differentiation of stem and progenitor cells. We have found that endothelial cells (ECs) are not just passive conduits to deliver oxygen and nutrients, but also establish an instructive vascular niche, which by elaboration of paracrine trophogens, known as angiocrine factors, directly promote organogenesis. Activation of Akt-mTOR pathway in the ECs stimulates expression of angiocrine factors, including Notch-ligands, IGFBPs, FGFs and TGF-modulators, that induce expansion of hematopoietic and hepatic stem and progenitor cells. Specifically, angiocrine expression of Notch ligands and IGFBP2 promoted expansion of authentic long-term repopulating hematopoietic stem cells, while angiocrine expression of Wnt2 and hepatocyte growth factor (HGF) induce liver regeneration. Modulation of specific angiocrine factors in ECs will provide for a therapeutically effective means to stimulate and sustain organogenesis.

Vascular endothelial growth factor receptor 3 directly regulates murine neurogenesis

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Neural stem cells (NSCs) are slowly dividing astrocytes that are intimately associated with capillary endothelial cells in the subventricular zone (SVZ) of the brain. Functionally, members of the vascular endothelial growth factor (VEGF) family can stimulate neurogenesis as well as angiogenesis, but it has been unclear whether they act directly via VEGF receptors expressed by neural cells, or indirectly via the release of growth factors from angiogenic capillaries. Here, we show that VEGFR-3, a receptor required for lymphangiogenesis, is expressed by NSCs and is directly implicated in the control of neurogenesis. Vegfr3::YFP reporter mice show VEGFR-3 expression in multipotent NSCs which are capable of self-renewal and are directly activated by VEGFR-3 ligand VEGF-C, in vitro. Overexpression of VEGF-C stimulates VEGFR-3-expressing NSCs and neurogenesis in the SVZ, without angiogenic effect. Conversely, targeted deletions of Vegfr-3 in neural cells and subventricular astrocytes, as well as blocking VEGFR-3 signaling with antibodies reduce SVZ neurogenesis. Therefore, VEGF-C/VEGFR-3 signaling directly regulates NSCs and promotes adult neurogenesis, opening potential approaches for neurodegenerative disease.

Strategies for lung regeneration

Laura Niklason, M.D., Ph.D., Yale University

Professor of Anesthesiology and Biomedical Engineering

Because adult lung tissue has limited regenerative capacity, lung transplantation is the primary therapy for severely damaged lungs. To explore whether lung tissue can be regenerated in vitro, we treated lungs from adult rats using a procedure that removes cellular components but leaves behind a scaffold of extracellular matrix that retains the hierarchical branching structures of airways and vasculature. We then used a bioreactor to culture pulmonary epithelium and vascular endothelium on the acellular lung matrix. The seeded epithelium displayed remarkable hierarchical organization within the matrix and the seeded endothelial cells efficiently repopulated the vascular compartment. In vitro, the mechanical characteristics of the engineered lungs were similar to those of native lung tissue, and when implanted into rats in vivo for short time intervals (45-120 min), the engineered lungs participated in gas exchange. Although representing only an initial step toward the ultimate goal of generating fully functional lungs in vitro, these results suggest that further investigation of this approach is warranted.

Defining the niche for stem cells in the skin

Valerie Horsley, Ph.D., Yale University

Assistant Professor of Molecular, Cellular, and Developmental Biology and 2010 Pew Scholar in the BioMedical Sciences

The skin epithelium maintains tissue homeostasis and regeneration through dynamic interactions with multiple cell types in the underlying dermis, including fibroblasts, blood vessels, and adipocytes. However, despite the importance of these cellular interactions in the skin, the mechanisms that mediate cellular crosstalk during skin homeostasis and regeneration are not well understood. To define how cells within the skin epithelium interact with cells in the dermis, we have analyzed the biology and function of subdermal adipocytes during skin tissue homeostasis. Subdermal adipocytes compose a unique white adipose tissue depot that underlies the dermal fibroblasts and surrounds the hair follicle during hair growth. Our data show that a critical interplay exists between hair follicle stem cells and subdermal adipocytes. During regeneration and stem cell activation in the murine hair follicle, adipogenesis characterizes changes in the skin tissue microenvironment through de novo formation of subdermal adipocytes. We find that defects in subdermal adipocytes modulate the activity of murine adult follicular stem cells. These data implicate adipocytes as niche cells for epithelial stem cells and define a vital crosstalk between adipocytes and adult epithelial stem cells in murine skin.

Embryonic Stem Cell-Derived Thymic Epithelial Cell Progenitors Enhance T Cell Reconstitution after Bone Marrow Transplantation

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Assistant Professor, Department of Immunology

Laijun Lai, Jingjun Jin.

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T-lymphocytes (T-cells) play a central role in the adaptive immune system by protecting against infections and neoplasia. T cell deficiencies can occur in a number of physiological and pathological situations. For examples, thymic involution during aging represents the most important cause of thymic atrophy resulting in decreased number and functional activities of T-cells in the elderly. Various genetic and infectious diseases (such as AIDS) are associated with T-cell deficiencies, as is intensive chemotherapy or radiotherapy of cancer. In addition, the recovery of T cells after bone marrow transplantation (BMT) is often slow and incomplete.

T cell development in the thymus depends on the thymic microenvironment, of which thymic epithelial cells (TECs) are the major components. We have demonstrated that embryonic stem cells (ESCs) can be selectively induced in vitro to differentiate into cells that have the phenotype and genotype of thymic epithelial progenitors (TEPs). When placed in vivo, these ESC-derived TEPs self-renew, develop into all types of TECs, and reconstitute the normal thymic architecture. Functionally, these ESC-derived TEPs enhanced T cell regeneration after BMT in animals. In addition to providing a model to study the molecular events underlying TEP and TEC development, the ability to selectively induce the development of TEPs in vitro from ESCs has important implications regarding the prevention and/or treatment of T-cell immunodeficiencies.

Leveraging Stem Cells and Reprogramming for a greater understanding of neural degeneration

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It has been proposed that human embryonic stem cells could be used to provide an inexhaustible supply of differentiated cell types for the study of disease processes. Although methods for differentiating embryonic stem cells into specific cell types have become increasingly sophisticated, the utility of the resulting cells for modeling disease has not been determined. We have asked whether specific neuronal subtypes produced from human embryonic stem cells and induced pluripotent stem cells can be used to investigate the mechanisms leading to neural degeneration in amyotrophic lateral sclerosis (ALS). We show that human spinal motor neurons, but not interneurons, are selectively sensitive to the toxic effect of glial cells carrying an ALS-causing mutation in the SOD1 gene. Our findings demonstrate the relevance of these non-cell-autonomous effects to human motor neurons and more broadly demonstrate the utility of human embryonic stem cells for studying disease and identifying potential therapeutics.