BioPharmaceutical Technology Center
I N S T I T U T E

11th Annual Wisconsin Stem Cell Symposium

Stem Cells in the 4th Dimension: Mechanisms of Stem Cell Aging and Maturation

SPEAKER ABSTRACTS

Hormonal Modulation of Neural Stem Cell Dynamics during Development and Disease
Craig Atwood, Ph.D

What is aging and how do we age? Does 'aging' lead to the development of age-related diseases? And if so, what mechanisms are involved? Is development an aging process? I will describe our basic, epidemiological and clinical research in the fields of neurodegeneration and embryology that address these questions and led to the development of the 'Reproductive-Cell Cycle Theory of Aging'. The basic premise of this theory is that the hormones that regulate reproduction act in an antagonistic pleiotropic manner to control aging via cell cycle signaling; promoting growth and development early in life in order to achieve reproduction, but later in life, in a futile attempt to maintain reproduction, become dysregulated and drive senescence. With respect to neurodegeneration, I will present data that the dysregulation of the hypothalamic-pituitary-gonadal (reproductive) axis with menopause and andropause drives the aberrant re-entry of post-mitotic pyramidal neurons into the cell cycle. This mechanism involves key mitogenic (luteinizing hormone; LH) and differentiation (TGFb family members) factors that regulate cell cycle dynamics and the related biochemical and neuropathological changes associated with Alzheimer's disease (polyploidy, neuronal hypertrophy, mitochondrial biogenesis, tau phosphorylation, amyloid deposition, etc.). This research led to an examination of the pregnancy-associated LH homolog - human chorionic gonadotropin (hCG) and its central role in the proliferation and differentiation of human embryonic stem cells (hESC) required for blastulation and neurulation in early embryogenesis. This hormonal regulation of the cell cycle appears to be partly mediated by the amyloid-b precursor protein (AbPP), a ubiquitously expressed transmembrane protein whose cleavage product, the amyloid-b (Ab) protein, deposits in amyloid plaques in aging and neurodegenerative conditions such as AD. The differential processing of AbPP towards the amyloidogenic pathway increases hESC and neural stem cell proliferation, while non-amyloidogenic processing promotes the differentiation of pluripotent hESC into neural precursor cells. Taken together, these findings suggest that the balance between mitogenic and differentiation factors throughout life regulates development, adult tissue maintenance and cell senescence and disease.

Differentiation, De-differentiation and Re-differentiation of Pancreatic Beta Cells
Barak Blum, Ph.D.

How does a newly formed stem or progenitor cell "know" it has reached its full differentiation capacity, and should assume its mature function? What part of the signal towards terminal differentiation comes from the cell's interactions with its surrounding environment, and what is encoded in the blueprint of its intrinsic developmental program? And how is this functionally mature state, once achieved, sustained throughout adult life, or tip off balance and erode in degenerative disease? I will discuss our lab's efforts to discover the regulatory circuits controlling the development, maintenance, collapse and recovery of the fully differentiated, functionally mature pancreatic beta cell state in mice and humans. Efforts to identify early markers of beta cell

de-differentiation in type-2 diabetes, and the role of the beta cells in the initiation of type-1 diabetes will also be discussed.

Metabolic Maturation-based Cardiac Disease Modeling with Patient-specific iPSCs: Present and Future
Vincent Chen, M.D., Ph.D.

Cardiovascular diseases remain the major cause of death in the US. Recent advances in reprogramming somatic cells from cardiac patients into induced pluripotent stem cells (iPSCs) enables *in-vitro* modeling of human cardiac diseases for pathogenic studies and therapeutic screens. However, most published iPSC-based cardiac models used immature cardiomyocytes (CMs) derived from patient-specific iPSCs (iPSC-CMs) and cultured them in a non-physiological milieu. As a result, exaggerated cardiac pathologies and arrhythmia frequently appeared within 30 days in these non-physiological models, which are not consistent with the clinical courses of these diseases. Further improvement of the current iPSC-CM based disease model is needed. Arrhythmogenic Right Ventricular Dysplasia (ARVD) is an inherited cardiomyopathy with most identified mutations in genes encoding cardiac desmosomes, especially in plakophilin-2 (*PKP2*). Pathological hallmarks of ARVD are progressive fibro-fatty replacement of CMs with increased CM apoptosis primarily in the RV, leading to sudden death in the young. We developed a method to induce adult-like, fatty acid dominant metabolism of primitive iPSC-CMs and established the first metabolic maturation-based *in-vitro* cardiac disease model for human ARVD. We showed that ARVD iPSC-CMs manifested pathologies only after inducing peroxisome proliferator-activated receptor-alpha (PPARα)-dependent, adult-like metabolism. More importantly, abnormal PPARγ activation after achieving PPARα-dependent metabolism resulted in pathological lipogenesis, apoptosis and defective Ca^{2+} handling in ARVD CMs, recapitulating the pathological signatures of ARVD (*Nature*, 2013). PPARγ antagonists rescued all ARVD pathologies and reactive oxygen species scavengers could reduce CM apoptosis. We further find that ARVD mesenchymal stromal cells (MSCs) are abnormally adipogenic and interact with pathological ARVD CMs to recapitulate the pathological fatty infiltration of ARVD hearts (ARVD Model 2.0). We have used our improved ARVD model 2.0 to decipher clinically relevant pathogenic pathways and develop novel therapeutic strategies.

Stem Cell-Derived Extracellular Vesicles as Therapeutics
Paul Robbins, Ph.D.

With aging, there is an inevitable and progressive loss of the ability of tissues to recover from stress, in part through loss of stem cell function. As a consequence, the incidence of chronic degenerative diseases increases exponentially starting at the age of 65. This includes neurodegeneration, cardiovascular disease, diabetes, osteoarthritis, cancers, and osteoporosis. More than 90% of people over 65 years of age have at least one chronic disease, while 75% have at least two. Thus, it is imperative to find a way to target therapeutically the process of aging to compress the period of functional decline in old age. Such a therapeutic approach would simultaneously prevent, delay or alleviate multiple diseases of old age. We are using both naturally aged mice and the ERCC1-deficient mouse model of accelerated aging mice as a model of accelerated aging to identify therapeutic strategies for extending healthy aging. Previously we demonstrated that intraperitoneal (IP) administration muscle-derived stem/progenitor cells (MDSPCs) isolated from young wild-type mice into ERCC1-deficient mice conferred significant lifespan and healthspan extension through a paracrine/endocrine mechanism. More recently, we demonstrated that BM-MSCs from young, but not old mice, also prolonged lifespan and healthspan in Ercc1-deficient mice, similar to MDSPCs. Taken together, these results suggest that at least two

types of adult stem cell populations, BM-MSCs and MDSPCs, isolated from young mice extend lifespan and healthspan following IP injection in a mouse model of accelerated aging. We also have been characterizing and identifying the factors released by young, functional stem cells important for this extension of lifespan and healthspan. Conditioned media (CM) from young, but not old MDSPCs and BM-MSCs, rescued the function of aged, dysfunctional stem cells as well as senescent fibroblasts in culture. Moreover, this activity in the CM co-purifies with extracellular vesicles released by young, but not old stem cells. Progress towards developing clinically relevant approaches using stem cell derived extracellular vesicles to treat autoimmune and age-related pathologies will be presented.

Metabolic Remodeling of Stem Cells and Cardiomyocyte Maturation
Hannele Ruohola-Baker, Ph.D.

Aberrations in metabolism contribute to a large number of diseases, such as diabetes, obesity, cancer, and cardiovascular diseases. However, the mechanisms leading to these changes in metabolic state is not well understood. Changes in metabolism similar to those seen in pathological conditions are observed during normal development in a number of different cell types, providing hope that understanding the mechanism of these metabolic switches in normal development may provide useful insight in correcting them in pathological cases. We have analyzed regulation of metabolic remodeling and its function in epigenetic control both in early stage embryonic stem cells and during the maturation of cardiomyocytes. Change in cardiomyocyte metabolism is a vital component in the regulation of both cardiomyocyte differentiation and maturation. We showed that overexpressing one microRNA (miR), Let7, can lead to a robust metabolic maturation of cardiomyocytes. We have now identified additional maturation-miRNAs and are generating a novel cocktail to further accelerate maturation and analyze the molecular mechanism of each microRNA in cardiomyocyte maturation. We have shown that metabolites and metabolic remodeling play key roles in regulating other cell types as well, the newly derived naïve hESC and primed hESC. The laboratory has identified metabolic differences that regulate the stem cell epigenetic state. We showed that metabolic enzyme NNMT regulates PRC2 dependent H3K27me3 marks in naïve and primed stages, however, it was not shown if this modification was essential for the human pluripotent stages. We applied computational protein design to engineer a synthetic, novel protein, EED-binder that incorporated the EZH2 N-terminal helical peptide so as to achieve 300-fold tighter binding to EED compared to endogenous EZH2. Importantly, we show that the inducible expression from the AAVS1 site of the active, but not the control, EED-binder abolished the stem cell morphology, significantly reduced the level of H3K27me3 marks, EZH2 protein and stem cell markers, measured by Western, ChIP-seq and FACS analysis. These data show that in contrast to mouse, in human PRC2 has an essential function in naïve, as well as in primed hESC. The challenge now is to understand how the metabolic switches are regulated just at the right time to initiate the correct gene expression required for cell fate. Interestingly environmental factors may play an important role in this process, giving hope for future easy manipulation on the metabolic state both in normal and pathological states.

Directing Fate and Timing in Human Pluripotent Stem Cell-derived Neural Lineages
Lorenz Studer, M.D., Ph.D.
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Adult Stem Cells and Biomimetic Matrices for Tissue Engineering and Modeling: Repair, Restore and Re-create
Rocky S. Truan, Ph.D.

Degenerative joint diseases, the most prevalent cause of physical disabilities, affect up to 15% of the population, particularly the elderly. In osteoarthritis, the low, intrinsic reparative capacity of cartilage is a clinical challenge to effective treatment. Current treatments, such as anti-inflammatory drugs, are only able to provide short-term pain relief. Total joint arthroplasty remains the only effective procedure, but is ultimately limited by the finite life expectancy of the implant. Tissue engineering and regenerative medicine, an emerging scientific discipline encompassing translational application of cells, scaffolds, and biological signals, is a potentially promising approach to repair damaged/diseased tissues to restore joint function and mobility. Adult mesenchymal stem cells (MSCs), from tissue sources such as bone marrow, adipose, and skeletal muscle, exhibit multi-lineage mesenchymal differentiation potential, including chondrogenesis, and are considered a promising candidate cell type for cartilage repair. A critical component to successful cell-based cartilage tissue engineering is a biocompatible biomaterial cell-carrier scaffold that ideally also enhances proliferation and differentiation of the seeded cells. We have previously shown that electrospun biomimetic scaffolds that simulate the structure of native extracellular matrix, e.g., the nanoscalar fibrous nature of collagen, are effective in MSC-based skeletal tissue engineering, both *in vitro* and *in vivo*. We have recently custom-designed photocrosslinked hydrogel scaffolds derived from natural and synthetic polymers, to achieve live cell encapsulation during fabrication, with high fidelity tissue infrastructure reproduction and excellent cell retention, viability, and differentiation, and generated robust cartilage and bone tissue constructs. Bioactivation of these biomimetic scaffolds by incorporating biologically targeted gene constructs results in transduction of both exogenous and endogenous host cells. These constructs may also be formed in situ, serving to both deliver cells and create custom-designed shapes for joint cartilage re-surfacing, potentially amenable to minimally invasive, arthroscopic procedures. Most recently, we have applied 3D-printing approach and a custom-designed microbioreactor to fabricate an MSC-derived microtissue analogue of the biophasic osteochondral junction of the articular joint, demonstrating functional biological crosstalk between the chondral/osseous components. This osteochondral microphysiological system is currently being used to model the pathogenesis of osteoarthritis, e.g., exposure to pro-inflammatory agents, and to study biological, hormonal, pharmacological, and mechanical incluences on osteochondral health. Adult stem cells, with their multi-differentiation potential and their recently discovered trophic activities, in combination with biomimetic scaffolds, present a powerful platform for regenerative, therapeutic, and disease modeling applications in biomedicine. [Support: Pennsylvania Department of Health, NIH, Department of Defense, EPA]

Blood-borne Factors as Modulators of Neural Stem Cell Activity and Brain Function
Tony Wyss-Coray, Ph.D.

Age is the main risk factor for dementia and major neurodegenerative diseases and aging of peripheral organs may contribute to the demise of the brain. How the systemic environment affects brain health is largely unknown but we observed that blood-borne signaling proteins can modulate brain cell function including adult neural stem cell activity. Our findings, and those of others, show that factors in old blood can accelerate brain aging while factors in young blood can activate neurogenesis and rejuvenate the brain. We use different physiological methods to manipulate the systemic environment and proteomic tools and bioinformatics to try to identify critical factors that age or potentially rejuvenate the brain. Our findings may be relevant for the understanding of age-related neurodegeneration.