

KEYNOTE SPEAKERS

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*2nd Annual
Wisconsin
Stem Cell Symposium:*

Heart & Blood

April 18, 2007 Madison, WI

Presented by:

*BioPharmaceutical Technology Center Institute
UW-Madison NIH Stem Cell Training Program
UW Stem Cell and Regenerative Center*

OVERVIEW

Focusing on heart and blood stem cells: This symposium brings together some of the leading researchers in the world who are investigating how stem cells can form blood and heart cells, as well as pioneering clinical applications using these stem cells to treat blood and heart diseases.

Highlighted issues are:

- Embryonic stem cells differentiation to blood and cardiac lineages
- Development and regulation of hematopoietic stem cells
- Plasticity of bone marrow cells
- Cellular therapy for heart disease

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Gabriela Cezar, D.V.M., Ph.D. (*Asst. Prof., Animal Sciences, UW-Madison*)
Peiman Hematti, M.D. (*Asst. Prof., Medicine, UW-Madison*)
Timothy J. Kamp, M.D., Ph.D. (*Assoc. Prof., Medicine, UW-Madison*)
Youngsook Lee, Ph.D. (*Assoc. Prof., Anatomy, UW-Madison*)
Gary E. Lyons, Ph.D. (*Assoc. Prof., Anatomy, UW-Madison*)
Aiman Shaaban, Ph.D. (*Asst. Prof., Surgery, UW-Madison*)
Igor I. Slukvin, M.D., Ph.D. (*Asst. Prof., Path. and Lab. Med., UW-Madison*)
Clive Svendsen, Ph.D. (*Prof., Waisman Center, UW-Madison*)

PROGRAM SCHEDULE

8:00am Registration and Continental Breakfast
Breakfast Sponsors: *Cellular Dynamics International and Stem Cell Products, Inc.*

8:30am **Welcome**
Timothy J. Kamp (Moderator)
William Linton
Carl E. Gulbrandsen

8:45am **Session I: Blood**
Chair: *Emery Bresnick*
Lineage Specific Differentiation of Embryonic Stem Cells
Gordon M. Keller

Developmentally Determined Heterogeneity in Hematopoietic Stem Cell Properties
Connie J. Eaves

10:15am Break

10:30am **Session II: Heart**
Chair: *Gary E. Lyons*

Cardiomyocytes from Human Embryonic Stem Cells: Properties and Use
Christine L. Mummery

Embryonic Stem Cells: Tools for Cardiac Repair and Regeneration
Timothy J. Kamp

12:00pm Lunch and Posters/Exhibits

1:20pm **Welcome:** *Robert N. Golden*
Session III: Blood
Chair: *Peiman Hematti*

Regulation of Hematopoietic Stem Cells
Stuart H. Orkin

Hematopoietic Development from Human Embryonic Stem (ES) Cells
Igor I. Slukvin

3:00pm Break

3:15pm **Session IV: Heart**
Chair: *Youngsook Lee*

Plasticity in Bone Marrow Cells
Piero Anversa

Autologous CD34+ Cell Therapy for Ischemic Tissue Repair
Douglas W. Losordo

4:45-6:30pm Reception and Posters/Exhibits
Reception Sponsor: *Quarles & Brady, LLP*

Lineage Specific Differentiation of Embryonic Stem Cells

Gordon M. Keller

The hematopoietic and cardiac lineages develop from mesoderm, one of the germ layers formed from epiblast cells as they migrate through a structure known as the primitive streak (PS). Lineage tracing studies indicate that subsets of mesoderm are induced in a defined temporal pattern, with development of hematopoietic mesoderm preceding the formation of cardiac mesoderm. To model mesoderm induction and specification in embryonic stem (ES) cell differentiation cultures, we used ES cells carrying the green fluorescent protein cDNA targeted to the locus of the PS gene brachyury (GFP-Bry cells). Analysis of early stage serum-induced embryoid bodies (EBs) generated from the GFP-Bry cells revealed the presence of three distinct populations based on expression of GFP and Flk-1: GFP-Bryneg/Flk-1neg GFP-Bry+/Flk-1neg and GFP-Bry+/Flk-1+. Evaluation of the developmental potential of these populations demonstrated that hemangioblasts, representing the earliest stage of hematopoietic commitment, were found in the GFP-Bry+/Flk-1+ fraction whereas cells with cardiac potential segregated to the GFP-Bry+/Flk-1neg population. When allowed to aggregate for 24 hours the GFP-Bry+/Flk-1neg population generated a second Flk-1+ population (20 Flk-1) that contained all cardiomyocyte potential. To determine the lineage potential of the progenitors that generated cardiomyocytes, cells from the 20 Flk-1 population were cultured in methylcellulose in the presence of factors known to function in the heart primordium. Following 4 to 6 days of culture, distinct colonies of cells developed that displayed cardiac, endothelial and vascular smooth muscle potential. Single cell deposition experiments revealed that the colonies were clonal indicating that they arise from a cardiovascular colony-forming cell (CV-CFC). Analysis of head-fold stage embryos demonstrated the presence of a similar Flk-1+ CV-CFC. Together, these findings demonstrate the existence of a Flk-1+ multipotential cardiovascular progenitor that develops following the establishment of the hematopoietic lineage.

Developmentally Determined Heterogeneity in Hematopoietic Stem Cell Properties

Connie J. Eaves

The conventional model of hematopoietic stem cell generation and maintenance assumes the creation of cells primed to differentiate into multiple lineages but able to proliferate without activating this process in either one or both progeny, thereby allowing either asymmetric or symmetric self-renewal divisions to occur. Such a model is widely supported by the demonstration that hematopoietic populations can be subdivided into phenotypically distinct populations whose growth and differentiation activities reconstruct a hierarchical process of stepwise lineage restriction and loss of proliferative potential from a common stem cell pool. However, it has also been appreciated for many years that hematopoietic stem cells and their differentiating progeny evolve different properties during development, although the molecular regulation of these differences and the cell types in which they originate remain poorly understood. To examine these issues, we first analyzed the progeny regenerated from a large number of transplanted single adult bone marrow lin-CD45midRho-SP cells with long-term repopulating ability. The results showed that these cells had one of 4 distinct differentiation programs, two of which were sustained and faithfully propagated, in some cases through 3 serial transplants, although incubation in vitro rapidly altered the programs subsequently obtained in vivo. In addition, we have identified a number of differences in the biological properties of fetal and adult hematopoietic stem cells that change in an abrupt and co-ordinated fashion between 3 and 4 weeks after birth. Although the molecular basis for this switch has yet to be determined, preliminary evidence indicates that it is autonomously acquired and affects a downstream target of c-kit activation. These findings indicate new complexity in the regulation of hematopoietic stem cell properties that will be important to understand in attempting to recapitulate their generation in vitro from embryonic stem cells.

Cardiomyocytes from Human Embryonic Stem Cells: Properties and Use

Christine Mummery

Derivation of heart cells from human embryonic stem cells (HESCs) and understanding the underlying developmental mechanisms is the main focus of the research. Culture conditions have now been sufficiently refined that cardiomyocyte differentiation is an efficient and reproducible process. Genetically marked HESCs have been produced in which expression of the green fluorescent protein marker is retained after differentiation. This has permitted unambiguous tracing of cardiomyocytes following transplantation into a mouse heart. Long term survival of the cells and integration into the host heart has been observed and the ability of these cells to restore cardiac function in mice that have undergone myocardial infarction is being investigated. Microarray analysis of modulations in gene expression during differentiation has shown that the major known cardiac genes are upregulated but that novel genes are also expressed. In situ hybridization in beating HESC cultures, mouse and zebrafish embryos confirmed the importance of several of these genes for heart development. Resequencing in patients with congenital heart defects is leading to new insights into the molecular basis of these defects.

Embryonic Stem Cells: Tools for Cardiac Repair and Regeneration

Timothy J. Kamp

The potential ability of human embryonic stem cells (ESCs) and their derivatives to repair or regenerate diseased hearts has captured the imagination of researchers and the public. Initial studies using mouse ESCs in animal models of myocardial infarction (MI) have demonstrated that transplantation of mouse ESCs can result in reduced adverse remodeling of the left ventricle and an improvement in cardiac function. Ongoing investigations are defining the mechanisms underlying the benefit of ESC transplantation post-MI focusing on the fate of transplanted ESCs and paracrine effects from the transplanted cells. Studies are being expanded to rhesus ESCs and nonhuman primate models of MI as well as investigations using human ESCs. Substantial challenges remain before this cell source is ready for widespread clinical application in heart disease, but the potential abundance, pluripotency, ability to genetically manipulate, and availability of cell banks warrant ongoing investigation of ESCs. Insights obtained from these basic research studies will ultimately provide for a broad variety of new therapeutic approaches to promote cardiac repair.

Regulation of Hematopoietic Stem Cells

Stuart H. Orkin

Hematopoietic stem cells (HSCs) are the best characterized of adult type stem cells. HSCs arise from specialized mesoderm during development and then sustain blood cell formation throughout adult life. In the past several years many transcriptional regulators involved in HSC formation and/or function have been studied. Combinatorial mechanisms, including direct antagonism between critical factors, underlie and refine lineage decision-making. Yet, much remained to be learned regarding the molecular control of the choice between quiescence and self-renewal or differentiation, pathways required for their maintenance, interactions between HSCs and the microenvironment in the bone marrow, and the stepwise processes involved in leukemogenesis. Assisted by targeted mutation of genes in the mouse, we have dissected the roles of several transcription factors in HSC function, and also investigated how HSCs respond to initiating somatic events observed in leukemias. In this presentation, specific examples will be discussed to illustrate the dynamic control of HSCs and previously unsuspected pathways that regulate their maintenance in vivo.

Hematopoietic Development from Human Embryonic Stem (ES) Cells

Igor I. Slukvin

Directed hematopoietic differentiation of ES cells reproduces many aspects of embryonic hematopoiesis, and provides a unique opportunity to study molecular and cellular pathways of hematopoietic development in humans. In our laboratory, we established a system for efficient hematopoietic differentiation of hES cells through coculture with OP9 bone marrow stromal cells. Using this system we were able to directly differentiate hES cells into cells of all major blood lineages (erythroid, myeloid and lymphoid), as well as identify different stages of hematopoietic

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commitment. We found that the earliest hematopoietic progenitors (HPs) in humans arise within CD34+ population and could be ultimately defined by surface expression of leukosialin (CD43). In addition, within CD43+ population, we identified lin-CD34+CD43+CD45- hematopoietic progenitors capable of differentiating toward all blood lineages including lymphoid cells, suggesting their hierarchical proximity to hematopoietic stem cells. However, molecular profiling of hES lin-CD34+CD43+CD45- cells revealed altered expression of genes associated with hematopoietic stem cell self-renewal and survival, reflecting limited engraftment potential of ES cell-derived hematopoietic progenitors. With increasing interest in potential therapeutic application of hES cell derivatives, identification of genes essential for hematopoietic stem cell development and diversification is of particular importance. The described experimental system sets a solid platform to advance in this direction.

Plasticity in Bone Marrow Cells

Piero Anversa

The possibility that adult bone marrow cells retain a remarkable degree of developmental plasticity and acquire the cardiomyocyte lineage after infarction has been challenged and the notion of bone marrow cell transdifferentiation has been questioned. The center of the controversy is the lack of unequivocal evidence in favor of myocardial regeneration by the injection of bone marrow cells in the infarcted heart. Because of the great interest in cell-based therapy for heart failure, several approaches including gene reporter assay, genetic tagging, cell genotyping, PCR-based detection of donor genes, and direct immunofluorescence with quantum dots were employed to document or disprove bone marrow cell transdifferentiation into functionally competent myocardium. Together, our studies indicate that locally delivered bone marrow cells generate de novo myocardium composed of integrated cardiomyocytes and coronary vessels. Importantly, this process occurs independently of cell fusion and ameliorates structurally and functionally the outcome of the post-infarcted heart.

Autologous CD34+ Cell Therapy for Ischemic Tissue Repair

Douglas W. Losordo

Background: A growing population of patients with coronary artery disease experiences angina that is not amenable to revascularization and is refractory to medical therapy. Pre-clinical studies have indicated that human CD34+ stem cells induce neovascularization in ischemic myocardium, enhancing perfusion and function. Methods: 24 patients (19M; 5F; age 48-84) with CCS class 3 or 4 angina on optimal medical Rx and who were not candidates for mechanical revascularization were enrolled in a double-blind, randomized (3:1) placebo controlled dose escalating study. The primary goals of this study were to assess safety and feasibility and to make observations regarding bioactivity. Pts received GCSF 5ug/kg/d for 5d with leukoapheresis on the 5th day. Selection of CD34+ cells was performed with an FDA approved device. Electromechanical mapping was performed to identify ischemic but viable regions of myocardium for injection of cells (vs. saline). The total dose of cells was distributed in 10 intramyocardial injections performed with the Myostar catheter. Pts were required to have an ICD or to temporarily wear a LifeVest wearable defibrillator. Results: Mobilization of CD34+ cells with GCSF resulted in a transient increase in angina frequency. There was no incidence of MI induced by mobilization or intramyocardial injection. The intramyocardial injection of cells/saline did not result in cardiac enzyme elevation, perforation or pericardial effusion. ICD/LifeVest recordings revealed no incidence of VT/VF during the administration of GCSF or intramyocardial injections. One patient with a history of SCD/VT/VF had catheter induced VT during mapping requiring cardioversion. The procedure was completed safely in this patient and no further arrhythmias occurred during injection or follow-up. In the entire study population serious adverse events(SAE's) were evenly distributed. Efficacy parameters including angina frequency, nitroglycerine usage, exercise time, and CCS class showed trends favoring CD34+ cell treated pts vs. placebo controls. Conclusion: A randomized trial of intramyocardial injection of autologous CD34+ cells in patients with intractable angina was completed providing evidence for feasibility, safety and bioactivity. A larger phase IIb study which is currently underway to further evaluate this technology.

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