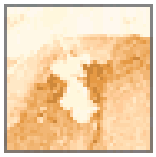
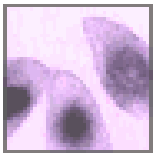
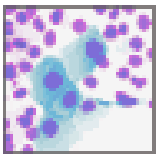
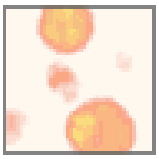
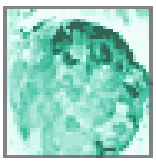
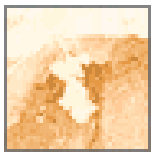
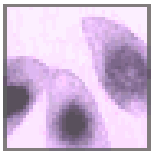
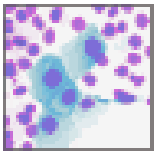
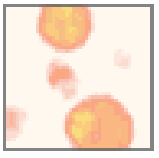
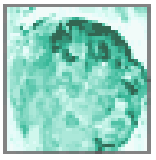











2nd *Annual Stem Cell Symposium* at UNSW

Friday 28 November 2008
Lecture Theatre & Function Room
Edmund Blacket Building
Prince of Wales Hospital
Randwick, NSW

2nd *Annual Stem Cell Symposium* at UNSW

Program

8:15 - 9:00 - REGISTRATION -

8:30 - 8:50 **Breakfast / Coffee Seminar: Flow Cytometry on Demand**
Gloria Santander, Business Development Manager, Life Science Research, Millipore

9:00 - 9:10 **Official Welcome**
Prof Bruce Brew, Head of Neurology & Neurosciences, St Vincent's Hospital

9:10 - 9:40 **Keynote Address**
Global politics of human embryonic stem cell research
A/Prof Catherine Waldby, Department of Sociology & Social Policy, University of Sydney

Morning Session: Blood *Chaired by Dr Tracy O'Brien*

9:40 - 10:00 **Gene regulatory networks in blood stem cell development**
Dr John Pimanda, Prince of Wales Hospital

10:00 - 10:20 **Preclinical development of cord blood stem cells for transplantation**
Dr Alla Dolnikov, Cord and Marrow Transplant Facility, Sydney Children's Hospital

10:20 - 10:40 **Differentiation and mobilisation of cardiac side population stem cells**
Prof Beng Chong, St George Hospital

10:40 - 11:00 **CD7+ hemopoietic stem cells**
Dr Stephen Carlin, St Vincent's Hospital

- MORNING TEA -

Lunch Session: Ocular and Neuroscience *Chaired by A/Prof Kuldip Sidhu*

11:20 - 11:40 **A contact lens-based technique for ocular surface reconstruction**
Dr Nick Di Girolamo, School of Medical Sciences, Department of Pathology UNSW

11:40 - 12:00 **The role of neuropeptide Y in the differentiation of adult olfactory precursor cells**
Dr Kharen Doyle, Adult Stem Cell Group, Garvan Institute of Medical Research

12:00 - 12:20 **Kynurenine pathyway and stem cell differentiation**
Dr Juliana Lamoury, Neuro Stem Cell Group, St. Vincent's Centre for Applied Medical Research

12:20 - 12:50 **An anti-inflammatory response by neurons is essential for regeneration after brain injury**
Dr Bryce Vissel, Neuroscience Research Program, Garvan Institute of Medical Research

- LUNCH -

Afternoon Session: Pancreatic, Cardiac and More Blood *Chaired by Prof Bernie Tuch*

1:40 - 2:00 **Exploring using 3D scaffolds for hESC differentiation towards the pancreatic lineage**
Steven Gao, Diabetes Transplant Unit, Prince of Wales Hospital

2:00 - 2:20 **Human pancreatic progenitors for cell replacement therapy in diabetes**
Dr Anand Hardikar, National Center for Cell Science, Pune, India

2:20 - 2:40 **Characterisation of a novel cardiac cell-type with stem and progenitor cell properties**
Dr Vashe Chandrakanthan, Developmental Biology Unit , Victor Chang Cardiac Research Institute

2:40 - 3:00 **Hematopoietic stem cells as a therapeutic platform**
Dr Geoff Symonds, Johnson & Johnson Research

- AFTERNOON TEA -

BREAKFAST / COFFEE SEMINAR

FLOW CYTOMETRY ON DEMAND

Presented by:

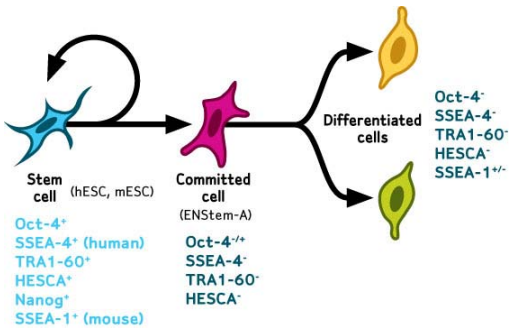
Gloria Santander
Business Development Manager, Millipore Australia Pty Ltd

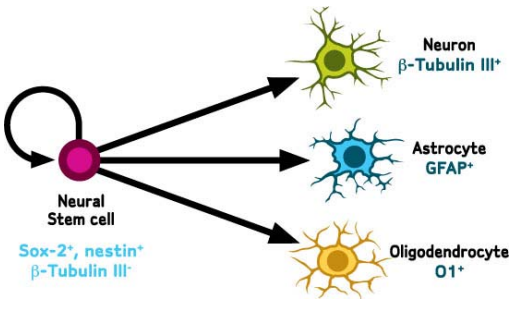
Millipore is proud to announce the partnership agreement signed with Guava Instruments. This partnership will provide Millipore with a Flow Cytometry platform to advance in the manufacturing of validated kits in the areas of stem cells, neuroscience, signalling and more.

The Guava EasyCyte Flow Cytometry systems offer all the capabilities of a floor standing Instrument in a bench top-size. * User friendly software * patented microcapillary flow cell technology that eliminates the requirement for sheath fluid * side scatter * 4 colours.

To coincide with the launching of the Instruments Millipore is pleased to announce the release of the first of a series of FlowCellect kits developed and optimised using the Guava technology.

The Stem Cell Characterisation kits are designed to provide rapid and sensitive assessments of embryonic and neural stem cell phenotypes at various stage of differentiation. The antibodies used in these kits have been carefully screened for high specificity in both Western blots and Immuno-histochemistry before using them in the FlowCellect kits. Antibody concentrations are then optimized for Flow Cytometry to give the greatest shift when negative and positive cells are compared.





OFFICIAL WELCOME

Professor Bruce Brew

Head of Neurology & Neurosciences, St. Vincent's Hospital

KEYNOTE ADDRESS

THE GLOBAL POLITICS OF HUMAN EMBRYONIC STEM CELL RESEARCH

Catherine Waldby

University of Sydney

This talk will present some of the key themes and findings from the speaker's forthcoming book *The Global Politics of Human Embryonic Stem Cell Research*, co-authored with Herbert Gottweis and Brian Salter. Stem cell science is a field characterized by a global struggle for scientific, economic and national advantage. Drawing on a wide range of interviews, primary and secondary sources, the book investigates the dynamic interactions between national regulatory formation and the global biopolitics of regenerative medicine and human embryonic stem cell science. Today governments are under intense competitive pressure to fund and develop attractive national environments for embryonic stem cell science, which promises both to improve the health and productivity of aging populations and to develop therapies for global health markets. The book traces the development of internationally circulating arguments for and against stem cell research, and the various transnational bioethical spaces that have opened up to try and steer these arguments towards compromise and implementation. It investigates the place of transnational regulatory bodies like the EU and the UN in organizing and modifying the international and national debates around stem cell science, and ways in which national debates and policies influence each other. The presentation will in particular look at the rise of peak bioethical bodies and the social role of scientific research networks and standardization initiatives as examples of 'soft' governance in the field of stem cell regulation.

Associate Professor Catherine Waldby is International Research Fellow in the Department of Sociology and Social Policy, Sydney University, and Adjunct Professor, the Centre for Biomedicine and Society, King's College, London. She researches and publishes in social studies of biomedicine and the life sciences. Her books include *AIDS and the Body Politic: Biomedicine and Sexual Difference* (1996 Routledge), *The Visible Human Project: Informatic Bodies and Posthuman Medicine* (2000 Routledge) and with Robert Mitchell *Tissue Economies: Blood, Organs and Cell Lines in Late Capitalism* (Duke University Press 2006). She is a foundation member of the global biopolitics research group, an international consortium of scholars who investigate the effects of cultural, political and economic globalization on the social relations of biomedicine. She has received national and international research grants for her work on embryonic stem cells, blood donation and biobanking.

GENE REGULATORY NETWORKS IN BLOOD STEM CELL DEVELOPMENT

John Pimanda

The Lowy UNSW Cancer Research Centre

Development can be viewed as a dynamic progression through regulatory states that characterise the various cell types within a given differentiation cascade. To understand the progression of regulatory states that define the origin and subsequent development of haematopoietic stem cells, the first imperative is to understand the ontogeny of haematopoiesis. We are fortunate that the ontogeny of blood development is one of the best characterized mammalian developmental systems. However, the field is still in its infancy with regard to the reconstruction of gene regulatory networks and their interactions with cell signalling cascades that drive a mesodermal progenitor to adopt the identity of a haematopoietic stem cell and beyond. Nevertheless, a framework to dissect these networks and comprehend the logic of its circuitry does exist and although they may not as yet be available, a sense for the tools that will be required to achieve this aim is also emerging. In this overview, I shall cover methods used in our laboratory to reconstruct haematopoietic transcriptional networks, current challenges and future outlook.

PRECLINICAL DEVELOPMENT OF CORD BLOOD STEM CELLS FOR TRANSPLANTATION

Alla Dolnikov, Kap-hyoun Ko, Emma Song, Tiffany Holmes and Tracey A O'Brien

Sydney Cord & Marrow Research Facility, Centre for Children's Cancer & Blood Disorders, Sydney Children's Hospital

Wingless (Wnt) signaling appears to play an integral role in expansion and quiescence of stem and progenitor hematopoietic cell populations. To better understand this dichotomous role and aid in the translation of therapeutic goals including cellular therapies, greater understanding of the molecular mechanisms that regulate Wnt/β-catenin in the bone marrow niche are needed. We explored the impact of Wnt signalling in the context of the human HSC niche by using co-culture of human cord blood or bone marrow derived HSC with mesenchymal stem cells (MSCs) together with pharmacological inhibition of GSK-3β (iGSK-3β). We have previously shown that activation of the Wnt/β-catenin pathway using pharmacological inhibition of GSK-3β (iGSK-3β) in human cord blood derived HSCs acts to preserve their primitive status, often lost during the ex-vivo expansion process (1). Here we show using cell division tracking that iGSK-3β delays stem cell divisions induced by cytokines in co-culture with MSCs. Functional analysis of iGSK-3β-treated cells revealed an increased proportion of primitive cells producing mixed Colony Forming Units (CFU-GEMMs) formed in the methylcellulose cultures and Cobblestone Area Forming Cells identified in long-term co-cultures with the bone marrow stroma MS5 cells. In addition, iGSK-3β-treated cells exhibited a higher frequency of SCID Repopulating Cells. We also show that the iGSK-3β effect, at least in part, is mediated through VLA4 integrin engagement by fibronectin. Importantly, iGSK-3β also induced niche-independent delay in stem cell divisions seen in cytokine supported suspension cultures. Up-regulation of HoxB4 and p21Waf1 and down-regulation of cyclin D1 was observed in HSCs treated with iGSK-3β. These findings confirm that iGSK-3β–regulated Wnt/β-catenin activity is crucial for the maintenance of HSC quiescence, both in the MSC niche and in a niche-independent environment and that iGSK-3β can circumvent the requirement of exogenous Wnt ligand for the induction of Wnt/β-catenin signalling in MSC niche. Our results are consistent recent data showing that blocking of canonical Wnt signaling, by over-expression of dickkopf1 (Dkk1) increases the number of proliferating HSCs in the murine marrow microenvironment but reduces the ability to reconstitute the hematopoietic system of irradiated recipient transgenic mice (2). These findings contribute to a better understanding of the mechanisms regulating the balance between self renewal and quiescence and help to facilitate progress towards clinical application of Wnts as mediators of ex-vivo stem cell expansion or interruption of the Wnt pathway as potential anti-leukemic therapeutic strategy.

(1) Holmes et al. Glycogen synthase kinase-3beta inhibition preserves hematopoietic stem cell activity and inhibits leukemic cell growth. Stem Cells, 2008;26;1288-97.

(2) Flemming et al. Wnt signalling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal in vivo. Cell Stem Cell, 2, 274-283.

DIFFERENTIATION AND MOBILISATION OF Sca1+/CD31- CARDIAC SIDE POPULATION CELLS IN A MOUSE MYCARDIAL ISCHAEMIC MODEL

Terence Tan, Simon Liang and **Beng Chong**

St George Hospital

Background: Myocardial infarction (MI) is the leading cause of heart failure and death in developed countries. Current treatments do not regenerate cardiac muscles damaged by MI. Cardiac regeneration through the differentiation of stem or progenitor cells is being investigated and it has been proposed that 'side population' (SP) cells (with stem cell characteristics) isolated from adult tissues may be able to regenerate the heart after an infarction.

Aim: To investigate the role of cardiac side population cells in the recovery of damaged cardiomyocytes after an MI in a mouse model.

Method: Sca1+/CD31- cardiac SP cells labeled with red fluorescent dye were injected intramyocardially into infarcted mouse heart. After 2 weeks hearts were harvested, sectioned and stained with cardiac and endothelial markers. Labeled Sca1+/CD31- cardiac SP cells were also injected into non-damaged areas of the heart which was subsequently harvested and sectioned after 3 days.

Results and Conclusions: Sca1+/CD31- cardiac SP cells which did not express cardiac or endothelial markers pre-implantation, expressed them post-implantation suggesting differentiation into cardiomyocytes and endothelial cells in the infarcted heart in vivo. Sca1+/CD31- cardiac SP cells were observed to mobilize from a non-damaged area of the heart to the infarcted area (demonstrated by inflammatory cell infiltrate and the disruption of cardiac fibres) in vivo. A demonstrated upregulation of CXCR4 expression in Sca1+/CD31- cardiac SP cells suggests the SDF-1/CXCR4 pathway may play a role in this mobilization.

CD7+ HAEMOPOIETIC STEM CELLS

Stephen Carlin, David Ma, John Moore

St Vincent's Hospital

We have characterized a CD7+ subset of CD34+ haemopoietic stem cells (HSCs) with T- lymphocyte potential. Although CD7 is a mature T cell lineage marker, CD7+ HSCs were present in Cord Blood (CB), Bone Marrow, GCSF-mobilized peripheral blood (HSC transplant tissue), and unmobilized peripheral blood. In a standard thymic culture (OP9D stromal cells), CB CD7+ cells differentiated with higher expression of CD4 and CD3, compared with CB CD7-. Adult CD7+ cells had higher expression potential for CD3 only. To test the role of CD7 in precursor cells, we used anti-CD7 (stimulating) antibodies, and lactose as a blocker of the CD7 ligand galectin-1. CD7 inhibition blocked development in earliest co-culture, while later in development, CD7 stimulation promoted cell death, particularly in immature cells expressing CD4. These results show that a subset of HSCs express the mature T cell marker CD7, and these cells have enhanced thymic potential, particularly in CB. CD7 also participates in stage-dependent pro-differentation and pro-apoptotic signalling.

A CONTACT LENS-BASED TECHNIQUE FOR EXPANSION AND TRANSPLANTATION OF AUTOLOGOUS EPITHELIAL PROGENITORS FOR OCULAR SURFACE RECONSTRUCTION

Nick Di Girolamo

School of Medical Sciences, Department of Pathology, UNSW

Background: A healthy cornea is reliant on a distinct population of stem cells (SC) that replace damaged or aging epithelium throughout life. Depletion of the SC pool or damage to the niche can result in a blinding and painful condition known as limbal stem cell deficiency (LSCD). While current treatment strategies for reconstituting the ocular surface for patients suffering LSCD are promising, they are complicated by transferring autologous or allogeneic progenitors in the presence of animal, human and synthetic products. We report on the safely and efficacy of a unique autologous SC transfer technique that utilizes an FDA-approved contact lens as the SC substrate and carrier for patients with LSCD.

Methods: Three patients with LSCD, due to aniridia (n=1) and post-treatment for recurrent ocular surface melanoma (n=2) were included. Limbal (n=2) or conjunctival biopsies (n=1) were harvested and progenitors expanded ex vivo on therapeutic contact lenses in the presence of autologous serum. Cell-laden contact lenses were transferred to the patient's corneal surface and clinical outcome measures recorded (follow up range, 6-10 months).

Results: A stable transparent corneal epithelium was restored in each patient. There was no recurrence of conjunctivalisation or corneal vascularization and a significant improvement in symptom score occurred in all patients. Best-corrected visual acuity was increased in all eyes after the procedure.

Conclusion: Ex vivo expansion of ocular surface epithelium in the presence of autologous serum and transplantation with the aid of a soft contact lens is a promising new technique capable of achieving ocular surface rehabilitation.

THE ROLE OF NEURPEPTIDE Y IN THE DIFFERENTIATION OF ADULT OLFACTORY PRECURSOR CELLS

Kharen L Doyle, Yvonne Hort, Christian Leeb and John Shine

Adult Stem Cell Group, Neuroscience Research Program, Garvan Institute of Medical Research

Neurogenesis of the olfactory neuroepithelium (ON) involves the proliferation and differentiation of multipotent olfactory precursors which are located near the base of the ON. The process of replacing olfactory receptor neurons (ORNs) occurs in a very anatomically defined manner throughout adulthood, which makes this part of the nervous system ideal for the analysis of neurogenesis. The regulation of the differentiation and maturation of the ON involves a range of transcription factors (TFs) working in concert. The development of the olfactory neuron, like other specific cell types, involves the sequential activation and inactivation of a hierarchy of transcriptional regulators. To date, different classes of TFs have been shown to specify olfactory cell fates: patterning proteins, such as Pax-6; progenitor protein, such as Lhx2; proneural proteins, such as Mash1, Ngn1; neuronal differentiation protein, such as NeuroD; inhibitory protein, such as Hes1. We have previously shown that neuropeptide Y (NPY) regulates the neuroproliferation of olfactory precursor cells via the Y1 receptor. Neurospheres derived from NPY-/- and Y1-/- mice have a reduced capacity to proliferate. Hence we studied the differentiation capacity of NPY-/-, Y1-/-, NPYPYY-/- and PYY-/- mice and have shown a significant increase in the number of both immature and mature ORNs in vivo in NPYPYY-/- and PYY-/- mice. The number of mature olfactory neurons in NPY-/- mice is significantly less than WT controls. We have also shown that there is a significant increase in the number of olfactory receptor neurons that label with the TFs; Mash1, Musashi1 and Wnt3a in NPYPYY-/- mice. We therefore hypothesise that NPY is a survival factor for olfactory receptor neurons.

KYNURENINE PATHYWAY AND STEM CELL DIFFERENTIATION

Juliana Lamoury[1], Francois MJ Lamoury[1], John J. Zaunders[1], Kazuo Suzuki[1], Nabila Seddiki[1], Michael Caristo[1], David Walker[3], George Smythe[4], Osamu Takikawa[5] & Bruce J Brew[1,2]

1 St Vincent's Centre for Applied Medical Research
2 Department of Neurology, St Vincent's Hospital
3 Department of Physiology, Monash University,
4 Biomedical Mass Spectrometry Facility, Faculty of Medicine, University of New South Wales
5 National Institute for Longevity Sciences, National Center for Geriatrics and Gerontology, Aichi, Japan.

In most tissues, including the brain, the essential amino acid tryptophan (Trp) is degraded primarily by the kynurenine pathway (KP), which generates neuroactive metabolites including the neurotoxin quinolinic acid (QUIN) and kynurenic acid (KYNA), a neuroprotectant. Activation of the KP also leads to immune tolerance through Trp depletion and the direct effect of KP metabolites. The KP has been implicated in diverse aspects of physiology and pathophysiology, such as Multiple Sclerosis (MS) and AIDS dementia complex. However, the role of Trp metabolism and its functional consequences in stem cell biology such as mesenchymal stem cells (MSCs) and neural stem cells (NSCs) have been poorly explored.
We therefore investigated the expression and regulation of the KP in both mouse and human MSCs and NSCs (SCs) by real-time RT-PCR, western-blot, immunocytochemistry and HPLC.

We demonstrate for the first time that mouse and human SCs express all KP enzymes including indoleamine 2,3 dioxygenase (IDO), the first and rate-limiting KP enzyme. Moreover, we show that specific induction factors i.e. IFN-γ, or current MS therapies such as IFN-β1b, significantly alter the KP gene expression in SC cultures, together with KYN and KYNA production.

Finally, our preliminary results also demonstrate that the selective inhibition of the KP expression in human MSCs is feasible and can lead to an altered differentiation capacity of MSCs.

These results suggest that a) SCs possess a complete, functional expression of the KP, b) Trp depletion through KP induction in SCs could compromise cell division and lead to death of surrounding NMDA expressing cells, and lastly c) KP induction in SCs could compromise differentiation of endogenous and/or transplanted SCs. Furthermore, the results generate the hypothesis that the selective inhibition of the KP in SCs could minimize neuronal death and optimise stem cell differentiation, which might lead to novel therapeutic strategies.

AN ANTI-INFLAMMATORY RESPONSE BY NEURONS IS ESSENTIAL FOR REGENERATION AFTER BRAIN INJURY

Bryce Vissel

Neuroscience Research Program, Garvan Institute of Medical Research

Activin A, a member of the transforming growth factor beta-like (TGFβ like) superfamily of growth factors, is expressed by neurons following excitotoxic injury to the hippocampus, but its role in central nervous system function is not well understood. We found that intraventricular infusion of activin A increased the number of newly born neurons in the CA1, CA3 layers and in the dentate gyrus of the normal adult hippocampus and, following lipopolysacharide administration, had a potent inhibitory effect both on gliosis in vivo and on proliferation of microglia in vivo and in vitro. Consistent with a role of Activin A in regulating CNS inflammation and neurogenesis, intraventricular infusion of follistatin, a high affinity antagonist of activin A, profoundly impaired neurogenesis and increased the number of microglia and reactive astrocytes following onset of kainic acid induced neurodegeneration in vivo. Follistatin's inhibitory effect of on neurogenesis results, at least in part, from inhibiting Activin's effect on inflammation, because co-administration of non-steroidal anti-inflammatory drugs reversed follistatin's inhibitory effects on neurogenesis. Our data supports the notion that activin A has a potent anti-inflammatory role in the central nervous system, and this, possibly in concert with the action of other TGF-beta superfamily molecules, is permissive for neurogenesis following an acute excitotoxic stimulus. Recent studies have shown that inflammation is a critical negative regulator of neurogenesis. Ours is the first study demonstrating a neural response that regulates neurogenesis by contributing to a potent overall anti-inflammatory response in the central nervous system, a finding with implications for understanding the pathology of neurodegenerative diseases.

EXPLORING THE POSSIBILITY OF USING 3D SCAFFOLDS FOR HUMAN EMBRYONIC STEM CELL DIFFERENTIATION TOWARDS THE PANCREATIC LINEAGE

Steven Gao

Diabetes Transplant Unit, Prince of Wales Hospital

Human embryonic stem (ES) cells have been differentiated in conventional 2D monolayer culture systems in vitro, into specialised cell types of the endoderm lineage. However, fully functional cells have yet to be generated from this system. One possible reason for this is that 2D cultures cannot re-create the 3-D in vivo environment which has been shown to have a profound effect on cellular development. In comparison, 3D in vitro culture through the use of biodegradable scaffolds has been demonstrated to have the ability to more closely mimic the in vivo environment, and therefore may have a positive effect on cellular development.

The first step of culturing human ES cells is to attach the cells on to a surface coated with appropriate extra cellular matrix (ECM) proteins. Until now Matrigel is the most commonly used ECM in ES cell differentiation, however a more defined matrix is desirable. In this on-going study, we initially developed a 2D system that imitates the surface properties of the 3D poly(lactic-co-glycolic acid) (PLGA) scaffolds to model human ES cell adhesion in the 3D environment. In the 2D model, single cell preparations of pluripotent human ES cells adhered efficiently and predominantly to PLGA surfaces coated with laminin when compared to collagen I, collagen IV or fibronectin coated surfaces. Flow cytometry analysis revealed that almost all the pluripotent single cells expressed the integrin α6, which facilitates adhesion to laminin. This data was then translated into the 3-D environment, with the efficient binding of single pluripotent hESCs to PLGA scaffolds coated with laminin.

Human ES cells seeded on laminin coated scaffolds were then differentiated towards definitive endoderm, the first and most important step in pancreatic differentiation. Compared to cells differentiated on conventional 2D monolayers, the cells cultured on the scaffolds exhibited a different gene expression profile of the important endoderm markers, SOX17, FOXA2, CXCR4 and CER. The difference may indicate that the endoderm formed on the scaffolds was possibly patterned in a less anterior fashion. The same trend was also observed with cells cultured on matrigel, indicating the difference was not matrix dependent. This demonstrated the profound effect of 3D culturing on early human ES cell differentiation and thus illustrated the importance of studying ES cell differentiation in a 3D environment.

HUMAN PANCREATIC PROGENITORS FOR CELL REPLACEMENT THERAPY IN DIABETES

Anand Hardikar

National Center for Cell Science, Pune, India

Several studies on differentiation of islet progenitors and stem cells have demonstrated that each of these cell types differ in their potential to commit to endocrine pancreatic lineage. Since mesenchymal cells derived from human pancreatic islets show epigenetic marks of active insulin promoter conformation, we believe that these cells are better progenitors for directed differentiation to insulin-producing cells. I will discuss the process by which cells within human fetal pancreatic islets undergo epithelial-to-mesenchymal transition (EMT) to form mesenchymal-like islet progenitors. We demonstrate that a specific microRNA seed sequence is essential for maintenance of epithelial cell phenotype in human pancreatic islets. Over-expression of microRNAs with anti-sense seed sequence leads to rapid depletion of these microRNAs and concurrent transition of pancreatic epithelial cells to mesenchymal cell type. Our data demonstrate a seed sequence that underlies the regulatory signaling events involved in cellular response of epithelial cells during mesenchymal transition. I will also discuss the potential of such islet-derived progenitors for cell replacement therapy in diabetes.

CHARACTERISATION OF NOVEL CARDIAC CELL-TYPE WITH STEM AND PROGENITOR CELL PROPERTIES

Vashe Chandrakanthan, Owen Prall, Ishtiaq Ahmed, Munira Xaymardan, Joan Li, James Chong, Chris Blair, Somayeh Nasr-esfahani and Richard P Harvey

Developmental Biology Unit, Victor Chang Cardiac Research Institute

An ongoing and latent mitogenic potential in the adult mammalian heart is now well established. Numerous stem cell-like populations have been described within side-population, or using cell surface markers of the haemopoietic stem cell lineage (ckit and Sca1) or a transcription factor marker of cardiac progenitor cells in the embryo (Isl1). The relationship between populations defined by these markers is unknown, and few studies have formally surveyed cardiac intestitial cell populations using multiple markers or relative to a stem cell characteristic other than marker expression. We have explored non-diseased, infracted and aged murine adult cardiomyocyte interstitium for colony-forming ability in a colony-forming unit-fibroblast (CFU-F) assay and for lineage potentiality in vitro. A single fraction defined as Sca1+/Pdgfra+/ Pecam1-/CD90+ was enriched for CFU-F activity. CFU-F were distributed throughout the ventricles and atria, but also found abundantly in the aortic root and cardiac valves. Analysis using GFP expression from a Pdgfra-GFP knockin allele suggest that CFU-F in the aortic root and coronary arterioles includes cells located within the tunica adventitia, previously described to have smooth muscle and endothelial potential. Embryonic studies suggest an epicardial origin for these cells in development. Single cell replating assay gave secondary colonies at ~15% efficiency. These CFU-F cells can expand more than 30 passages and differentiate into cardiomyocytes, endothelial, smooth muscle, adipocytes, and osteocytes, but not haemopoietic cells, with high efficiency in vitro. Nkx2-5 is a cardiac homeodomain transcription factor normally expressed in cardiac progenitor cells in the embryo and mature cardiomyocytes. We found abundant Nkx2-5-GFP expression in a subset of all interstitial populations surveyed, including endothelial cells, although only the Sca1+/Pdgfra+/ Pecam1-/Nkx2-5-GFP- fraction had CFU-F activity in non-diseased heart. In the infarcted heart, CFU-F activity was seen in both Sca1+/Pdgfra+/ Pecam1-/Nkx2-5-GFP- and Sca1+/Pdgfra+/ Pecam1-/Nkx2-5-GFP+ populations, suggesting functional implications for Nkx2-5 expression. Our study defines one class of endogenous cardiac lineage precursor that is clonogenic, self-renewing and mulitpotent for a subset of mesodermal lineages. These cells may represent perivascular precursor cells with pro-vasculogenic potential during homeostasis and ischaemic repair. Their behaviour in disease and pro-regenerative models, and their ability to form cardiomyocytes in vivo are under investigation.

HEMATOPOIETIC STEM CELLS AS A THERAPEUTIC PLATFORM

Geoff Symonds

Johnson & Johnson Research

Hematopoietic Stem Cells are multipotential and able to contribute to the various blood cell lineages for the life of an individual. It is possible to isolate Hematopoietic Stem Cells and insert a gene into their genome ex vivo. These autologous gene-modified cells can then be re-introduced to the individual. The talk focuses on the use of this strategy using HIV as a specific example but also considers other diseases amenable to this approach such as the Severe Combined Immunodeficiency Diseases SCID-XI and SCID-ADA as well as Chronic Granulomatous Disease. Autologous cell therapy can be viewed as personalized medicine and Hematopoietic Stem Cells as a major therapeutic platform.

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