NSW Stem Cell Network

8th Stem Cell Workshop

"Mesenchymal Stem Cells"

Tuesday 6, June 2006
12:30 pm to 5:30 pm
Lecture Theatre
Sydney Children's Hospital
Randwick, NSW

Welcome to the 8th Stem Cell Workshop *supporting stem cell research and the emerging stem cell industry in Australia.*

The enormous plasticity of Mesenchymal Stem Cell (MSC) to differentiate into many cell types makes them an exciting area of biomedical research. Not only have MSC been converted into cells of different lineages such as bone, cartilage and muscle cells but they have a lower immunogenicity and being adult, they do not present the ethical issues posed by pluripotent embryonic stem cells.

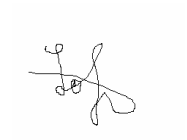
Some of the best Australian scientist working on MSC will be speaking today on cutting edge issues in MSC research and presenting exciting initial results of clinical trials. We are grateful to all speakers and in particular to interstate travellers for sharing their knowledge and providing a sense of where mesenchymal stem cell research is at as well as a sense of excitement about the therapeutic potential of these cells in the medical revolution.

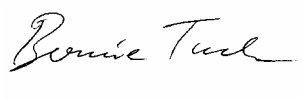
Thanks must also go to each session chair, Dr Tracey O'Brien from Sydney Children's Hospital and Professor Richard Harvey from the Victor Chang Institute for giving their time and expertise.

We would also like to thank our major sponsor Chemicon, and other sponsors Invitrogen, BD Bioscience, Integrated Science and Cygenics for their commitment to supporting the NSW Stem Cell Network.

Enjoy this Workshop and we look forward to keeping in touch through the Network.

Kind regards,





Sophie Diller ***Prof Bernie Tuch***
Manager ***Director***

NSW Stem Cell Network

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8th Stem Cell Workshop

Mesenchymal Stem Cells

Presented by
NSW Stem Cell Network

12:30pm	Registration
1:00pm	Welcome **Michael O'Sullivan, Department of State & Regional Development**
Session 1: BIOLOGY OF MESENCHYMAL STEM CELLS Chair: Dr Tracey O'Brien, Sydney Children's Hospital	
1:10pm	Approaches to prospective isolation of MSC: use of surface markers **Prof Paul Simmons, Peter MacCallum Cancer Centre**
1:35pm	Immune suppressive potential of MSC in preclinical models **Dr Alison Rice, Mater Medical Research Institute**
2:00pm	Novel methods for the control of proliferation and differentiation of MSCs **Prof Victor Nurcombe, Institute of Molecular and Cell Biology, Singapore**
2:25pm	Differentiation of BM MSC into cells of osteochondrocytic lineage in preclinical models. **Prof David Ma, St Vincent's Hospital, Sydney**
2:50pm	Afternoon Tea
Session 2: CLINICAL APPLICATIONS OF MESENCHYMAL STEM CELLS Chair: Prof Richard Harvey, Victor Chang Cardiac Research Institute	
3:30pm	MPC for vascular network formation and diseases of bone and heart muscle **Prof Silviu Itescu, Mesoblast Ltd**
3:55pm	The role of MSC in the treatment of acute graft verses host disease **Dr Ian Lewis, Institute of Medical & Veterinary Science Adelaide**
4.25pm	MSC Studies in Non-human Primates **Prof John Rasko, Centenary Institute of Cancer Medicine**
4:50pm	Announcements and Refreshments

APPROACHES TO PROSPECTIVE ISOLATION OF MSC: USE OF SURFACE MARKERS, Paul J Simmons, Nathalie Brouard, Brenton Short, Daniel Blashki, Makoto Tanaka and Naoki Nakayama

Adult mammalian bone marrow contains not one but two ostensibly discrete populations of adult stem cells. The first and by far the most fully characterized are the hematopoietic stem cells (HSC) responsible for maintaining lifelong production of blood cells. The biological characteristics and properties of the second marrow resident population of stem cells, variously termed bone marrow stromal cells or mesenchymal stem cells *(MSC*) are, in contrast, much less well understood. MSC are defined largely by their in vitro properties as multipotent clonogenic cells with the capacity for differentiation into bone, cartilage and adipose tissue. The fact that relatively little is known about the precise phenotypic characteristics of the primary clonogenic stromal precursors in the bone marrow responsible for initiating MSC growth *in vitro* coupled with their low incidence in the marrow has meant that much of our current knowledge of MSC has been gained through in vitro assays and culture manipulations. However, in defining MSC by their *in vitro* properties much remains unknown about their cellular identity, anatomical location, ontogeny and physiological role. Seeking to address these issues we have sought to develop methodologies to prospectively isolate MSC and their immediate progeny in highly enriched form from mouse hematopoietic tissues in order to explore the biological properties of these cells in an unmanipulated state, unaltered by culture epiphenomena.

These studies have culminated in the establishment of robust methodologies to prospectively isolate MSC not only from hematopoietic tissues of the adult mouse but also from a range of other adult mouse tissues such as dermis, lung and heart. A comprehensive analysis of the transcriptome of these prospectively isolated populations is providing important new insights into the identity of MSC and suggests the existence of a common molecular signature associated with MSC properties. Seeking to probe the origin of MSC during development we have also developed a methodology to induce the differentiation of pluripotent mouse ES cells to paraxial mesoderm. A minor subpopulation of cells identified by their co-expression of PGFRα and N-Cadherin exhibit hallmark features of paraxial mesoderm as shown by transcriptome and real-time PCR analysis and exhibit a robust capacity for both osteogenic and chondrogenic differentiation in vitro. A brief review of these studies will be presented.

ASSOCIATE PROFESSOR PAUL J. SIMMONS

Program Head, Stem Cell Laboratory, Peter MacCallum

A/Prof Paul Simmons is currently head of the Stem Cell Program at the Peter MacCallum Cancer Research Centre. Studies currently underway in his laboratory encompass the characterisation of skeletal stem cells and the mechanisms responsible for stromal cell regulation of haemopoiesis. In his additional role as director of the Adult Stem Cell Platform of the Australian Stem Cell Centre, a broader research program encompassing the study of stem cell niches in various adult tissues is in progress.

A/Prof Simmons has recently accepted the position of Director of the Stem Cell Centre at the Brown Foundation Institute of Molecular Medicine for the Prevention of Human Diseases (IMM) in Houston, Texas, USA.

IMMUNE SUPPRESSIVE POTENTIAL OF MSC IN PRECLINICAL MODELS

Alison Rice, Steven McTaggart, Kerry Atkinson.

The MSC is an adult stem cell located within the stroma of the bone marrow and other organs including placenta. When isolated by plastic adherence and expanded *ex vivo*, these cells differentiate into cell types of mesenchymal origin including chondrocytes, adipocytes and osteocytes. In the bone marrow they provide support for haematopoiesis. MSC can differentiate into mesodermal and non-mesodermal lineages, they have a high expansion potential and are good candidates for the repair and regeneration of a large variety of tissues. Importantly, MSC appear to have a major advantage over many other cell types for cellular therapy, in that they are immunologically privileged and even in large outbred animals can be transplanted across MHC barriers without the need for immune suppression. MSC evade the alloimmune response via a number of mechanisms, but one of the major mechanisms is a reduced expression of major histocompatability complex (MHC) class I and II antigens - MSC do not express MHC class II and have only intermediate expression of class I antigens. Immunosuppressive mechanisms of MSC are thought to include; (1) production of suppressive cytokines; (2) induction of suppressive APC and regulatory T-cells; and, (3) production of suppressive enzymes. This has important implications for the therapeutic application of MSC, because MSC derived from healthy unrelated volunteer donors can be cryopreserved, thus making them available in a timely manner for patients in a variety of acute and chronic clinical settings. MHC-haploidentical MSC have been used successfully in the clinic. MSC have been induced to differentiate into cardiomyocytes in mice, and have improved myocardial function after myocardial ischaemia in rodents and pigs. This, in combination with their tropism for inflamed tissue, indicates their potential for treatment of damage caused by ischemic myocardial infarction. Additionally, their immunosuppressive ability indicates their potential to prevent or treat immune-mediated diseases such as autoimmune disorders including inflammatory bowel disease, solid organ transplant rejection and GVHD.

Dr Alison Rice

Team Leader Biotherapy Program, Mater Medical Research Institute

Dr Rice completed a B.Sc. Honours degree at the University of Adelaide and undertook Ph.D. studies at the University of Bordeaux II in France. Her Ph.D. studies focused on the characterisation of blood haematopietic stem cells for transplantation. Dr Rice then returned to Australia in 1993 to take up a position at Children's Cancer Institute Australia for Medical Research where she established the Stem Cell Biology Program. Research efforts concentrated on means of increasing the rate of recovery post stem cell transplantation with particular focus on cord blood transplant recipients. Dr Rice was recruited to take up the position of Team Leader in the Cancer Biotherapy laboratory at the Mater Medical Research Institute in December 2001. Dr Rice's research focuses on ways to improve the therapeutic options and long term survival of patients requiring stem cell transplantation as treatment for cancer or leukaemia.

NOVEL METHODS FOR THE CONTROL OF PROLIFERATION AND DIFFERENTIATION OF MSC V. Nurcombe & S.M. Cool

Stem cells, whether adult or embryonic, will only become clinically useful when we can reliably control their differentiation. These cells can be encouraged to differentiate, albeit inefficiently, after exposure to several species of soluble or adhesive factor (including SHH, FGFs, BMPs, Wnts), almost all of which use a glycosaminoglycan sugar, heparan sulfate (HS), as an essential co-factor. Such sugars, secreted onto the membranes of differentiating stem cells, are distinct, and characteristic. They are capable of activating a large number of extracellular mitogenic and adhesion factors, and it appears that tissue-specific lineages encode on their membranes HS binding sites for the configuration of extracellular factors they need to adopt, and then maintain, phenotype. Adding purified HS back onto stem cells in culture has been shown to accelerate the growth and differentiation of many cell types, and purification of particular mitogen-binding domains from parent HS chains allows the possibility of significant levels of control over the fate of precursor cells, a schema that is likely to hold true for all tissue stem cells. Identification of the HS cells produced during their phenotypic transitions, and the mitogenic factors they couple and activate, is thus likely to be of biomedical importance. We will discuss the various phenotypes we have been able to induce in embryonic, adult neural and mesenchymal stem cells with different sugar combinations, and particular mitogen-activating sequences derived from them.

Prof Victor Nurcombe

Institute of Molecular and Cell Biology, Biopolis Drive Singapore

Victor Nurcombe obtained his PhD in Developmental Neurobiology from the University of Sydney in 1984. He then took both CJ Martin and Humboldt Fellowships to initiate his postdoctoral training at the Max-Planck Institute for Biochemistry in Munich for a period of 5 years, and then worked in Oxford, Paris and New York, before returning to Australia in 1990 first to the Walter & Eliza Hall Institute, and thence in 1992 to the University of Melbourne as a Senior Lecturer. In 1998 he became a Reader and Associate Professor at the University of Queensland as Head of the Developmental Biology Program, before being headhunted to the IMCB in Singapore as a Principal Investigator in Stem Cell Biology in 2003. He is also an Adjunct Professor to the National University of Singapore and to the University of Lille in France.

DIFFERENTIATION OF BM MSC INTO CELLS OF OSTEOCHONDROCYTIC LINEAGE IN PRECLINICAL MODELS

David D Ma[1], Aiqun Wei[2], Helen Tao[1], Bojiang Shen[1], Jean Hsu[1], Helena Brisby[3], Sylvia.Chung[2], and Ashish D Diwan[2]

[1]Department of Haematology and BM Stem cell Transplant Unit, St. Vincent's Hospital Sydney and University of New South Wales, [2]Orthopaedic Research Institute and Department of Orthopaedic Surgery, St George Hospital, Sydney, Australia, [3]Department of Orthopaedics, Sahlgrenska University Hospital, Göteborg University, Sweden

Adult bone marrow mesenchymal stem cells are capable of differentiating into cells of different lineages in vitro when stimulated with appropriate differentiation signals. We demonstrated BMMSC could be induced to differentiate into osteochondrocytic cells in culture using different growth factors and extra-cellular scaffolds. A rodent intervertebral disc (IVD) model was developed to evaluate the feasibility of syngeneic and xenogeneic bone marrow stem cells to differentiate into cells of chondrocytic lineages. In the first study, we showed syngeneic bone marrow mononuclear cell transplanted into intervertebral discs survived and differentiate towards chondrocytic disc-like cells. In the second study, human BM CD34- but not CD34+ cells survived in rat IVDs without requiring immunosuppressant. Furthermore, the surviving cells expressed collagen II and Sox-9, providing evidence of differentiation of transplanted human cells in rodent IVDs. These findings suggest that human BMMSCs have the ability to develop into chondrocytic disc-like cells. Secondly, intervertebral disc has the properties of a relative immune privileged site and BMMSC may play a role in immunosuppression.

Prof David Ma

Department of Haematology and BM Stem Cell Transplant Unit, St. Vincent's Hospital Sydney and University of New South Wales

Professor David Ma is the Director of Research at the Department of Haematology and BM Stem Cell Transplant Unit, St Vincent's Hospital Sydney and a Professor of Medicine, UNSW. His main research interest is biology of adult BM stem cell and its clinical relevance. He is the co-convenor of the Australasian QAP programme for haemopoietic stem cell quantification for clinical transplantation. He is a member of the St Vincent's BM Stem cell transplant team and has conducted clinical trials of PB stem cell transplant for the treatment of autoimmune and cardiac diseases.

MESENCHYMAL PRECURSOR CELLS FOR VASCULAR NETWORK FORMATION, AND DISEASES OF BONE AND HEART MUSCLE

Prospective immunoselection of mesenchymal precursor cells enhances reproducible functional outgrowth of cells capable of differentiating to multiple mesenchymal lineage tissue types *in vitro*. Mesenchymal precursor cells are characterized by a CA12+/STRO-1bright phenotype, can be immunoselected by mAbs reactive with these markers, and *in vivo* are located in perivascular niches in the bone marrow and throughout the body. Consistent with their likely role as perivascular pericytes, cumulative data show that mesenchymal precursors regulate adult vasculature formation. Since neovascularization is a pre-requisite for both bone and cardiac tissue growth, mesenchymal lineage precursors appear ideal candidates for clinical use in conditions of bone and heart muscle injury. Data indicating that mesenchymal lineage precursors evade immune recognition raises the exciting prospect that allogeneic use of these cells may be feasible. We have established GMP protocols for immunoselection and large-scale culture expansion of mesenchymal precursor cells, and have recently shown that such allogeneic cells are safe and effective for both bone regeneration and for treatment of heart failure in large preclinical animal models. Initial results with mesenchymal precursor cell implantation in patients with long bone fractures and with coronary artery disease support the safety of the GMP process and the cells. Clinical protocols using these allogeneic cells will optimise culture methodologies and biological scaffolds/matrices for enhancing survival of implanted cells.

Prof Silviu Itescu

Professor of Medicine, University of Melbourne, and Chief Scientific Adviser, Mesoblast Limited

Professor Itescu is the founder of Mesoblast Limited and Angioblast Systems, Inc. Most recently Director of Transplantation Immunology at New York's Columbia University Medical Center, Professor Itescu has established an outstanding international reputation in the fields of stem cell biology, autoimmune diseases, organ transplantation and heart failure. His experiences range from laboratory research to new drug development and clinical evaluation.

Professor Itescu recently pioneered novel approaches to the use of adult stem cells for the treatment of heart disease and is leading international collaborative trials in this area. Professor Itescu was an advisor on cell therapy for cardiovascular diseases to both the US President's Council on Bioethics and the US FDA Biological Response Modifiers Advisory Committee (BRMAC).

He has consulted for many international pharmaceutical companies and has been an advisor to biotechnology and health care investor groups.

THE ROLE OF MSC IN THE TREATMENT OF ACUTE GRAFT VERSES HOST DISEASE.

Mesenchymal Stem Cells (MSC) are derived from the non-haemopoietic compartment of the bone marrow. They possess the ability to differentiate into multiple mesodermal lineages including bone, cartilage, muscle and fat, which has led to the initiation of clinical trials evaluating their role in tissue repair. In addition to their potential role in tissue repair and regeneration, MSC have unique immunomodulatory properties. In vitro studies demonstrate they are both immunogenic and immunosuppressive. This property has been exploited in the treatment of severe acute graft versus host disease (aGVHD) with encouraging preliminary results.

We are participating in a European multicentre Study to address whether MSCs show efficacy in the treatment of steroid refractory aGVHD. MSCs are cultured using a standardised protocol and reagents in an attempt to produce cellular products with common characteristics. In Adelaide, we have treated two patients who developed steroid refractory aGVHD following unrelated donor bone marrow transplantation. Outcomes will be presented and future studies discussed.

Dr Ian Lewis,

Senior Consultant Haematologist, Royal Adelaide Hospital and Institute of Medical & Veterinary Science.

Dr Ian Lewis graduated in medicine from the University of Adelaide and undertook training in haematology at the Royal Adelaide Hospital (RAH) and the Institute of Medical and Veterinary Science (IMVS). After completing a PhD he undertook further training at the University of Minnesota where he developed an interest in stem cells, in particular in cord blood stem cells.

He is currently a Senior Consultant Haematologist at the RAH and IMVS with clinical interests in stem cell transplantation and the treatment of leukaemia. He continues to have a strong research focus and is especially interested in developing techniques for the expansion of cord blood stem cells as well as exploring alternative uses of stem cells.

MSC STUDIES IN NON-HUMAN PRIMATES

We have established two main model systems to study MSC biology: a NOD/SCID mouse and non-human primate muscle injury model; and a non-human primate stem cell mobilisation assay. Using these two models we have shown that virally-marked MSCs can repopulate injured muscle by means of transdifferentiation or fusion. This technology, along with a means of accessing MSCs from peripheral blood, may provide therapeutic opportunities to treat muscle and other degenerative disorders as well as offering 'cellular factories' for gene expression *in vivo*.

Prof John Rasko

Program Head, Centenary Institute of Cancer Medicine & Cell Biology

Professor Rasko is a Haematologist who directs Cell and Molecular Therapies at Royal Prince Alfred Hospital and heads the Gene and Stem Cell Therapy Program at the Centenary Institute of Cancer Medicine & Cell Biology, University of Sydney. His was the first formal appointment in clinical gene therapy in Australia.

Professor Rasko has a productive track record in gene therapy, experimental haematology and cell biology. His research has been successful in uncovering new mechanisms of leukemia, understanding blood hormones and their mechanisms of action, and clinical trials of new biological therapies for cancer and bleeding disorders. Most recently he led a team which identified the gene for Hartnup Disease reported in Nature Genetics. He has received many awards for medical research, teaching and public service. He serves on Hospital, Sydney University Medical Faculty, state and national bodies including the Gene Technology Technical Advisory Committee; and ARC, NH&MRC and Cancer Council Grant Review Panels.

NSW Stem Cell Network

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