

**DEPARTMENT OF PATHOLOGY
THE UNIVERSITY OF MELBOURNE
Bachelor of Science (Honours) & Post-graduate Diploma of
Science
(531-496 and 531- 497)
Final Defense of Thesis Seminars
Tuesday, 13th May 2008**

Time	Title	Speaker
1.30 – 2 pm	THE EFFECT OF ACTIVIN A IN THE FORMATION OF ENDODERM FROM CORD BLOOD-DERIVED STEM CELLS	Peter Van Kooy
2 – 2.30 pm	GENERATING FZD-SPECIFIC SCFV ANTIBODIES BY PHAGE DISPLAY	Michelle Palmieri
2.30 – 3 pm	THE EFFECT OF HISTONE DEACETYLASE INHIBITORS ON HYPERTROPHIC STIMULI IN CARDIOMYOCYTES	Ann Jui-En Lin
3.0 – 3.30 pm	Afternoon Tea	

Venue
Peter MacCallum Seminar Room
Level 4, Medical Building
The University of Melbourne

THE EFFECT OF ACTIVIN A IN THE FORMATION OF ENDODERM FROM CORD BLOOD-DERIVED STEM CELLS

Peter Van Kooy, Dr. Faten Zaibak, Prof. Robert Williamson; Department of Pathology, University of Melbourne

Cystic fibrosis (CF) is a common severe genetic disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Chloride transport in the lungs is reduced, leading to infection and respiratory failure. Emerging research on directed stem cell differentiation into endodermal cells that precede mature lung epithelial cells may provide new therapeutic options.

The transforming growth factor-beta (TGF- β) family member activin A (AA) is a robust *in vitro* inducer of endodermal cells from embryonic stem cells (ESCs), but has never been investigated in adult stem cells. We studied unrestricted somatic stem cells (USSCs), highly proliferative, immunologically safe, multipotent adult stem cells from human umbilical cord blood. We isolated and characterised USSCs, and investigated the effects of AA, to test the hypothesis that AA-induced USSCs would form endodermal cells for potential use in CF cell therapy. We also assessed whether AA-treated USSCs, when further induced, form mature lung epithelial cells that express the CFTR gene.

Seven untreated USSC lines were profiled for various markers using reverse transcription polymerase chain reaction (RT-PCR) analysis. We show that all USSC lines express the mRNA coding for the main subunit of the AA receptor, as well as the broad endodermal-associated marker Gata6. Variable expression of primitive streak marker MixL1, and the early neuronal-associated marker Nestin was also found. None express the definitive endodermal-associated markers Sox17, Foxa2, or Ttf1, nor the mature endodermal lung markers SPC or CFTR.

USSCs were then cultured in basal medium supplemented with 2% fetal calf serum (FCS) and four different AA concentrations. Controls were cultured in basal medium supplemented with 2% or 30% FCS alone. Cells were harvested for gene expression profiling using RT-PCR and/or quantitative real time PCR analysis, and were studied using functional differentiation assays to assess the potential of induced and non-induced USSCs to form cells of the three germ line layers.

AA-treated USSCs were found not to express the endodermal markers Sox17, Foxa2 or Ttf1. Sox17 was expressed in initial experiments, however, this observation could not be reproduced in multiple replicate tests. AA-treated USSCs that were subjected to endodermal, mesodermal (bone), and ectodermal (neuronal) differentiation assays still induced expression of the SPC and Brachyury genes, and upregulated Nestin, the respective markers of successful differentiation. This indicates that AA-treated USSCs retain their multipotency.

This study shows that untreated USSCs express early mesendodermal, endodermal, and ectodermal-associated markers by RT-PCR, however, studies at the protein level will be needed to analyse the proportion of cells expressing these markers before ascribing functional properties. Contrary to our hypothesis, we also demonstrate that USSCs induced with AA in low serum conditions do not express the definitive endodermal markers Sox17, Foxa2 and Ttf1, and retain the multipotency inherent in untreated USSCs. Further studies could analyse the variability between AA, FCS, and USSC batches, which may have affected this study's outcome. The inability of USSCs to commit to endodermal differentiation following AA treatment, despite genetically expressing a main component of the AA-TGF- β signalling pathway, suggests a possible difference between ESCs and adult stem cells that has yet to be elucidated. Further studies in both USSCs and ESCs, analysing differentiation triggers and their relevant signalling pathways, should provide further insights into the factors that control and regulate adult stem cell fate.

GENERATING FZD-SPECIFIC SCFV ANTIBODIES BY PHAGE DISPLAY

Michelle Palmieri. Supervisor: Dr Francesca Walker

Cancer is the uncontrolled growth of tissue, due to the inability to maintain normal cellular homeostasis. In colorectal cancer, the Wnt signalling pathway and its components are central to the pathogenesis of the disease. Activation of the canonical Wnt pathway by Wnt ligands and Frizzled receptors, result in the accumulation of β -catenin and in the transcription of Wnt target genes, enabling tumourigenesis. Thus expression of, and ligand binding to Frizzled (Fzd) receptors, specifically Fzd 7, is central to tumour development in colorectal cancer.

To better understand the role of Fzd receptors and specifically Fzd 7 in the development of colorectal carcinomas, there is a need to monitor Fzd protein expression, localisation and interactions by means such as immunofluorescence and immunoblotting. This research has been hampered by lack of suitable high affinity antibodies to the receptor. Conventional means of raising antibodies to Fzd receptors are hindered by the low levels of Fzd expression on cells and by the high degree of sequence homology between species. Phage display is a relatively novel means of generating high affinity, specific antibodies to cell surface receptors and is particularly suited to poorly immunogenic antigens. The successful isolation of antibodies by this technique requires expression of the antigen at high level on a solid support and the availability of paired samples with and without expression of the target antigen. This project explored generation and expression of Fzd 7 in mammalian cells and the feasibility of phage display as a technique in antibody production.

A flag-tagged Fzd 7 construct was cloned into the pcDNA3 mammalian expression vector and expressed in transiently transfected HEK 293T cells and in stably transfected BaF/3 cells. Using the HEK 293T cells, Fzd 7-flag protein was shown to be expressed at high levels by FACS analysis, and to be present on the cells as a monomer and a dimer by immunoprecipitation and western blotting. The feasibility of phage display as a means of generating specific antibodies was investigated utilizing a well defined cell-surface receptor system, flag-tagged EGFR expressed on BaF cells. Using the BaF/EGFR system, it was shown that multiple rounds of selection using a phage library are capable of identifying and enriching a pool of specific phage to a cell surface protein. Selection for Fzd 7 specific phage was less successful and is still ongoing. This work has set the foundations for the selection and identification of a specific, high affinity Fzd 7 antibody with the potential to expand the current knowledge of Wnt signalling in colorectal cancer or as a means of targeted therapy.

THE EFFECT OF HISTONE DEACETYLASE INHIBITORS ON HYPERTROPHIC STIMULI IN CARDIOMYOCYTES

Ann Jui-En Lin Supervisors: Dr Tom Karagiannis (Peter MacCallum Cancer Centre) and A/Prof Assam El-Osta (Baker Heart Research Institute)

BACKGROUND-Cardiac hypertrophy is an adaptive response to a number of intrinsic and extrinsic stimuli. Physiological hypertrophy (such as that induced by excessive exercise), is not thought to be harmful, however, pathological hypertrophy (such as that induced by various anti-cancer chemotherapeutics) is associated with arrhythmia, heart failure and sudden death.

Recent studies have demonstrated that epigenetic modifications play a role in regulating cardiac growth [1, 2]. This suggests a potential role for epigenetic modifying compounds such as histone deacetylase (HDAC) inhibitors in regulating cardiac hypertrophy.

HDAC inhibitors are emerging therapeutics for cancer and much research is aimed at investigating the use of these drugs with conventional chemotherapeutics, including doxorubicin which is known to induce cardiac hypertrophy [3]. This prompted our decision to investigate the effects of combinations of a HDAC inhibitor and doxorubicin on cardiac toxicity and hypertrophy.

HYPOTHESIS-The hypothesis of the research project is that histone deacetylase inhibitors may potentially counteract the effects of anthracycline-induced cardiotoxicity in cancer therapy.

We specifically postulate that histone deacetylase inhibitors can reduce doxorubicin-mediated hypertrophy in cardiomyocytes.

AIMS-The aim of this study was to investigate the hypertrophic effects of doxorubicin in rat H9c2 cardiomyocytes. Furthermore, the effects of the well-known histone deacetylase inhibitor Trichostatin A (TSA, a natural antifungal antibiotic), on doxorubicin-induced hypertrophy in the rat H9c2 cardiomyocytes was also investigated.

METHODS AND RESULTS-H9c2 cells were differentiated to cardiac specific phenotype by chronic 10nM all-trans-retinoic acid (RA) treatment, as demonstrated by microscopy, nuclear staining and real-time polymerase chain reaction (RT-PCR). Exposure of differentiated H9c2 cells to doxorubicin, results in cell death and hypertrophy as indicated by the increased cell size, protein content and fetal regulatory gene activation.

The effects of TSA on doxorubicin-induced cardiac hypertrophy and gene expression was also analysed using RT-PCR. Experimental evidence indicates that TSA alters the expression of cardiac specific genes in H9c2 cells. Unexpectedly, the combination of doxorubicin and TSA treatment has synergistic effects on the change in gene expression on various representative genes.

CONCLUSION-The findings of this research project indicate that TSA augments doxorubicin-mediated cardiac hypertrophy, increasing the expression of fetal regulatory genes (ANP and MLC-2v), and concurrently decreasing dominant adult genes (α -MHC). Further research is required to understand the complex relationship between epigenetic modifications, cardiac hypertrophy and chemotherapy-mediated cardiotoxicity.

1. Chang, S., et al., *Histone Deacetylases 5 and 9 Govern Responsiveness of the Heart to a Subset of Stress Signals and Play Redundant Roles in Heart Development*. Mol. Cell. Biol., 2004. **24**(19): p. 8467-8476.
2. Backs, J. and E.N. Olson, *Control of Cardiac Growth by Histone Acetylation/Deacetylation*. Circ Res., 2006. **98**(1): p. 15-24.
3. Mallinson, J., *Cardiotoxicity Associated With Doxorubicin*. Cancer Nursing Practise, 2003. **2**(9): p. 30-34.