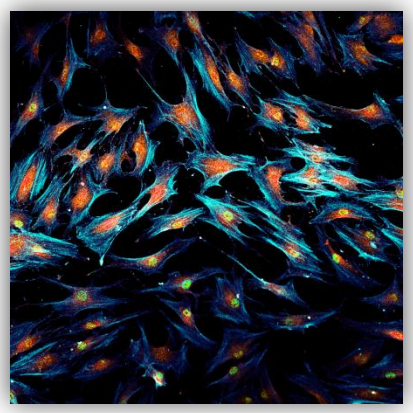
Characterisation of Small Leucine Rich Proteins Gene and Protein Expression in Mesenchymal Stem Cell Differentiation into Osteoblasts, Adipocytes and Chondrocytes



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DECLARATION

I declare this thesis is my own account of my research and contains as its main content, work which has not been previously submitted for a degree at any tertiary education institution.

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MANUSCRIPTS

Currently in submission

Jenny Z. Wang, Joshua R. Lewis, Lawrence J. Liew, Anthony C. Buzzai, Jeremy Tan, Gerard Hardisty, Jeffrey M. Hamdorf, Minghao Zheng, Richard L. Prince. **Estradiol effects on cellular proliferation and extracellular calcification in adipose tissue-derived stem cells during osteogenesis**.

Currently in preparation

Anthony C. Buzzai, Jenny Z. Wang, Joshua R. Lewis, Sarah J. Etherington Richard L. Prince. **Characterisation of Small Leucine Rich Proteins Gene and Protein Expression in Mesenchymal Stem Cell Differentiation into Osteoblasts**

Oral presentations

Combined Biological Sciences Meeting 2013. Perth, Australia. **The gene expression of Small Leucine Rich Proteins during the osteogenesis of human mesenchymal stem cells.**

ABSTRACT

This thesis is directed to understanding the role of Small Leucine Rich Proteins (SLRPs) in the cell biology of mesenchymal tissue in particular bone and cartilage. SLRPs are a family of 17 biologically active macromolecules which form the extracellular matrix in a variety of tissues and may play a role in bone and cartilage biology and diseases, in particular osteoporosis. It was hypothesised that:

1) The gene and protein expression of specific SLRPs will be up-regulated during the development of bone and cartilage.

2) During osteogenesis, the location of these SLRPs shows a pattern of distribution within the extracellular matrix.

3) Osteogenesis related SLRPs are specific to the cell development of that tissue.

To investigate the first hypothesis, a bioinformatics study of a human osteosarcoma cell was initially used to determine the gene expression on all 17 SLRP members. The six highest expressed members Lumican, Epiphycan, Tskushi, Biglycan Decorin, and Osteomodulin (OMD) were selected for further analysis. To investigate the second hypothesis, the gene expression of these six selected members were analysed using real time quantitative reverse transcriptase polymerase chain reaction in both long term (up to 28 days) and short term (up to 7 days) osteogenesis of donor matched human adipose and bone marrow mesenchymal stem cells. These results showed the increase in expression of OMD in osteogenic stimulated media. As a result of these studies OMD was selected for further study, as a potential biomarker of osteoblasts.

The gene expression of OMD was only increased significantly in osteoblast-like cells compared to other mesenchymal stem cell lineages including cartilage and adipose tissue. Protein expression of OMD was further investigated by western blotting. This was followed by confocal microscopy to further understand the expression of this protein. It was found through both methods that the protein expression of OMD was increased during osteogenesis, reflecting the gene expression previously observed.

In conclusion, it was shown that the gene and protein expression of OMD was increased specifically during osteogenesis, and therefore could be used as a marker of osteogenesis of mesenchymal stem cells. Furthermore, its role in osteogenic development should be further studied to understand its role in osteogenesis.

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ACKNOWLEDGEMENTS

The image on the front cover of this thesis shows stem cells I had cultured undergoing cell death. This image is not only a reminder of the tough moments throughout my honours year, but a reminder of those who helped me get through them.

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ABBREVIATIONS

Bone Mineral Density	BMD
Adipose Derived Stromal Cells	ADSCs
Asporin	ASPN
Biglycan	BGN
Bone Marrow Stromal Cells	BMSCs
Bone Morphogenic Protein	BMP
Chondroadherin	CHAD
Decorin	DCN
Epiphycan	EPYC
Extracellular Matrix	ECM
Extracellular Matrix Protein 2	ECM2
Glyceraldehyde-3-Phosphate Dehydrogenase	GAPDH
Glycosaminoglycan	GAG
Interleukin	IL
Leucine Rich Repeats	LRRs
Lumican	LUM
Mesenchymal Stem Cells	MSCs
Nyctalopin	NYX
Opticin	OPTC
Osteogenic media	OSM
Osteoglycin	OGN
Osteomodulin	OMD
Phosphate Buffered Saline	PBS
Podocan	PODN
Podocan-Like Protein	PODNL1

Polymerase chain reaction	PCR
Proline-Arginine-Rich End Leucine Rich Repeat Protein	PRELP
Quantitative real time reverse transcriptase PCR	qRT-PCR
Small Leucine Rich Proteins	SLRPs
Sodium Dodecyl Sulphate	SDS
Transforming Growth Factor Beta	TGF-β
Tsukushi	TSKU
Tumour Necrosis Factor Alpha	TNFα