The Interaction between Vitamin D and Extracellular Calcium on Osteogenic Differentiation

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B.Sc, M.Biotechnology

Thesis submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

October, 2014

The Discipline of Medicine
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Thesis abstract

While the role of vitamin D in the prevention of rickets in children and osteomalacia in adults has been well demonstrated, its benefit in the treatment of osteoporosis is subject to controversy. Clinical trials of vitamin D supplementation to prevent fractures have been conducted with mixed results and some meta-analyses have indicated limited benefit in reducing fracture risk. The most consistent beneficial effects of vitamin D have been obtained when combined with calcium supplements. 1,25-dihydroxyvitamin D acts on the three major types of bone cells (osteoblasts, osteoclasts and osteocytes) to initiate either catabolic or anabolic actions on bone. To elucidating the potential benefits of vitamin D to bone health, this study examined direct actions of vitamin D metabolites on bone cells focussing on stimulation of in vitro osteogenic differentiation. Two cell culture models, representing immature and mature stage of osteoblasts, were employed to investigate the role of vitamin D on osteogenic differentiation. The regulation of a variety of gene expressions and modulation of mineral deposition by these cells, were used as key readouts.

In chapter 3, vitamin D was observed to play an inhibitory role on mineral deposition by the immature calvarial bone-derived osteoblast-like cells (Calvarial cells) but did not exert any suppressive effect on the mature osteoblast/early osteocyte cell line, MLO-A5. Thus the actions of vitamin D appear to be dependent on either the stage of cell maturation or their skeletal origin.

The studies using Calvarial cells were expanded in chapter 4 by utilising cells derived from genetically modified mouse lines, including the global vitamin D receptor (VDR) knockout (VDRKO) and the over-expression of VDR in osteocalcin-expressing cells (OSVDR), in comparison to cells derived from wild-type animals. The active hormone form, 1α,25-dihydroxyvitamin D3 (1,25D), promoted a mature cell phenotype at
physiological levels (around 30 pM) dependent on the level of $Vdr$ mRNA. However, in OSVDR cells with high levels of VDR, a pharmacological concentration of 1,25D (1 nM) appeared to stimulate de-differentiation of the osteoblast phenotype by down-regulating the expression of mature osteoblast/osteocyte genes. $Enpp1$ and $Tnap$ were identified as key genes to modulate mineral deposition in these models.

In chapter 5, the cell line MLO-A5 was again utilised, here for studying the interaction between vitamin D and extracellular calcium on osteoblasts. Both endogenous and exogenous sources of 1,25D, either alone or interacting with extracellular calcium, increased mineral deposition and the expressions of maturation-related genes. Extracellular calcium altered vitamin D metabolism by MLO-A5 cells. Again, key genes associated with mineral deposition were $Enpp1$ and $Tnap$.

Data from this study confirm the stimulatory actions of vitamin D on osteogenic differentiation and identified an interaction with extracellular calcium levels. Mineral deposition was found to be dependent on 1,25D modulation of $Enpp1$ and $Tnap$ expressions. A highly novel finding was that the extracellular calcium concentration modulates the metabolism of vitamin D and the maturation of these cells. These data help to address the controversy on the actions of vitamin D on osteoblast differentiation and mineralisation and improve our understanding of their biology.
Declaration

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Dongqing Yang
Acknowledgements

Firstly, I would like to thank my supervisors Professor Howard Morris and Associate Professor Gerald Atkins. Back to the time I was looking for a PhD position, it was very lucky that Howard kindly offered me the chance to study in his laboratory and also Gerald agreed to be my co-supervisor to guide me in the cell biology works. During the study period, both Howard and Gerald made available an incredible amount of intelligent, effort and time to discuss my project, inspire my scientific thinking and improve my academic writing skill. Also, only with their continuous encouragement, could I deal with the difficulty and frustration I encountered during scientific research, enabling me to reach the completion of my PhD study.

Secondly, I would like to forward my acknowledgement to Associate Professor Paul Anderson and Dr Andrew Turner in the Musculoskeletal Biology Research Laboratory, the University of South Australia. Without their patience on teaching me and answering my numerous questions, I would never have been able to accomplish my project. I also would like to thank Ms Rebecca Sawyer and Dr Nga Lam in the Musculoskeletal Biology Research Laboratory, the University of South Australia, as well as Dr Asiri Wijenayaka, Miss Renee Ormsby, Dr Masakazu Kogawa, Dr Nobuaki Ito and Dr Matt Prideaux, from Bone Cell Biology Group, the University of Adelaide, for their kindly guidance on the daily bench work from time to time.

I gratefully acknowledge Professor Lynda Bonewald, University of Missouri, Kansas City, MO, USA, for the provision of the MLO-A5 cell line, made available to Associate Professor Gerald Atkins through a pre-existing collaboration. Especially, I would like to give my acknowledgement to Professor Hong Zhou from the ANZAC Research Institute, Concord, NSW, Australia, for accepting me into her laboratory and generously teaching
me the skill of establishing osteoblast-like culture from neonatal mouse skull bone, which was a very important *in vitro* model in my project.

I am giving my most special thankfulness to my wife Jiangqin Wei for her unconditional understanding, forgiveness and encouragement every single day during this period. Without her love and support both physically and mentally, it would have been absolutely impossible for me to finish my study and make this achievement. Last but not least, I also would like to forward my gratefulness to all of our family members in China for their constant encouragement and support for us both.
**Publications and Presentations**


**Yang D**, Turner A, Anderson PH, Morris HA, Atkins GJ. Vitamin D metabolites and extracellular calcium promote mineral deposition by the mature osteoblast cell line MLO-A5. (Submitted for publication).

Published Abstracts:


Oral Presentations:

**Yang D**. The role Vitamin D in the proliferation and differentiation of osteoblasts. The 6th (2010) Clare Valley Bone Meeting, Clare, SA, Australia.
**Yang D.** The role of calcitrol in the differentiation of osteoblasts *in vitro*. The 7th (2012) Clare Valley Bone Meeting, Clare, SA, Australia.

**Yang D.** Differential effects of 1,25-dihydroxyvitamin D (1,25D) on *in vitro* mineral deposition: Interaction between osteoblast stage of maturation and culture medium calcium concentration, Australian Society for Medical Research (ASMR) Scientific Meeting, 2012, Adelaide, SA, Australia.

Poster Presentations:


**Yang D**, Atkins GJ, Turner AG, Anderson PH, Morris HA. The role of calcitriol in the differentiation of osteoblast *in vitro*. The 15th Workshop on Vitamin D, June 20 – 22, 2012, Houston, TX, USA.