

The Role of Vitamin D Receptor in Osteoblasts and Bone Mineralisation

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A thesis submitted for the degree of

Doctor of Philosophy to

The University of Adelaide

December 2012

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ABSTRACT

Age-related bone loss is associated with a change in bone remodelling characterised by decreased bone formation relative to bone resorption. It is well described that age-related bone loss is accelerated as a consequence of vitamin D deficiency, a process which can be replicated in rodent studies. While vitamin D has been shown to play important roles for adequate bone mineralisation and the prevention of osteoporosis, the exact mechanisms remain controversial. It is clear that vitamin D is necessary for the stimulation of intestinal calcium and phosphate absorption, maintenance of calcium homeostasis and supply of calcium and phosphate for bone mineralisation. However, vitamin D has also been shown to directly act on bone cells to promote mineralisation as well as regulate bone resorption. The question of the essential nature of the *in vivo* role for the direct actions of vitamin D on bone has proven to be difficult to resolve. The only published mouse model which addresses the direct actions of vitamin D in osteoblasts is the osteoblast-specific vitamin D receptor transgenic mouse, or OSVDR mouse. Using this transgenic mouse model, it has been reported that the enhanced vitamin D activity in osteoblasts promotes bone formation and mediates reduction in bone resorption most likely through reduced RANKL signalling of osteoclastogenesis. The reported overall bone phenotype of the OSVDR was increased vertebral trabecular bone as well as increased cortical bone volume leading to increased bone strength. In contrast to the findings in OSVDR mice, global VDR knockout mice can mineralise osteoid in the presence of high levels of dietary calcium and phosphate, therefore many have concluded that the role for direct vitamin D activity in bone cells is redundant. This view however, does not take into account the fact that vitamin D activity in bone cells may play a permissive role to optimise bone health by modulating mineralisation and bone resorption.

Thus, the studies conducted in this thesis are aimed to further address the role of osteoblastic VDR in bone remodelling and bone architecture. Specifically, these studies aimed to further

establish the phenotype of the OSVDR mouse model utilising 3D micro-CT analyses as well as establish the role of vitamin D activity in osteoblasts during vitamin D deficiency and dietary calcium depletion. The effects of these physiological interventions on OSVDR mice are described in terms of bone structure, cellular activities, biochemical parameters, and gene expression profiles of bone and other organs involved in calcium and phosphate homeostasis. The overall hypothesis is that VDR activity in mature osteoblast lineage is important to regulate processes of bone remodelling and maintenance of an optimal skeletal structure.

The data presented within these chapters showed that the phenotype of increased bone mineral volume is present in more regions of bone, which was not previously recognised. Furthermore, during vitamin D deficiency, while bone loss occurs in wild-type mice, OSVDR mice maintain both cortical and trabecular bone volume, indicating that bone loss due to vitamin D deficiency is due, at least in part, to reduced vitamin D activity in osteoblasts. In contrast to vitamin D deficiency, the effects of low calcium stress in OSVDR mice results in bone loss comparable to wild-type mice, which is likely to be due to a disruption of bone remodelling, since we observed lowered osteoblast, osteoclast and osteocytes activities. Intriguingly, low calcium fed OSVDR mice demonstrate a marked increase in serum fibroblast growth factor 23 (FGF23) levels, resulting in suppressed renal 1,25-dihydroxyvitamin D (1,25D) synthesis, and reduced expression of intestinal calcium absorption genes. Thus, the inappropriately low 1,25D-mediated intestinal calcium absorption in OSVDR mice, fed low calcium, may further contribute to the reduction in bone mineralisation and bone volume. These data suggest that in addition to the reported direct action of vitamin D activity in osteoblasts to regulate bone turnover, VDR-mediated activity in osteoblast also plays a role in the endocrine feed-back mechanism of renal 1,25D synthesis, which may contribute to the maintenance of bone mineral and the resulting bone phenotype.

In summary, the findings from this thesis implicate the essential role of vitamin D and VDR in osteoblasts either directly or indirectly impacts on bone homeostasis, including osteoclast

activity, osteoblast differentiation, osteocyte activity, bone FGF23 production and renal feed-back signalling.

DECLARATION

“This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to **Nga Ngoc Lam** and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text”

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Nga Ngoc Lam

ACKNOWLEDGMENTS

Dr Paul Anderson

Thanks to my supervisor, Dr Paul Anderson who has provided me with support, enthusiasm and encouragement throughout my PhD study. It was a privilege to work in your laboratory and I have very much enjoyed the research project. Thank you for your advice, knowledge, guidance and reassurance, all of which have been invaluable.

*

Dr Peter O'Loughlin

Thanks to Dr Peter O'Loughlin for being my supervisor. I truly appreciate all your advice and help with my PhD project and the thesis.

*

Prof Howard Morris

Thank you for your enthusiasm, encouragement and invaluable intellectual advice and guidance throughout my studies and to the preparation of this thesis.

*

SA Pathology (IMVS)

To all the members of the Musculoskeletal Biology Research group, I offer sincere thanks for your advice and assistance, support and friendship throughout my PhD journey.

*

Garvan Institute

Thankyou to Dr Paul Baldock and Prof Edith Gardiner for providing the OSVDR transgenic mouse model

*

Family and friends

I would like to express everlasting gratitude to my family and friends. My parents Hoan and Oanh who have raised me into the person I am today, who have always offered encouragement, advice and unconditional love. Thanks to my sisters and brother, Tien, Hao

and Tra who have always been there for support and love. Thank you to my wonderful partner, Thien for all your patience, understanding, support and love throughout the years and for helping me fulfil my dreams and pursue happiness. I love you all.

INTERNATIONAL AND NATIONAL SCIENTIFIC MEETINGS AND AWARDS ARISING FROM WORK PRESENTED IN THIS THESIS

PRESENTATIONS

International

Oral: **Lam NN**, Sawyer RK, Anderson SR, Morris HA, O'Loughlin PD, Anderson PH.
Skeletal effects of increased osteoblastic VDR during calcium-deprivation in a mouse model.
American Society for Bone and Mineral Research, Toronto, Canada, 2010

Poster: **Lam NN**, Sawyer RK, Anderson SR, Morris HA, O'Loughlin PD, Anderson PH.
Osteoblast-specific VDR over-expression protects against bone loss due to vitamin D-deficiency. **International Bone & Mineral Society combined with Australian & New Zealand Bone and Mineral Society conference, Sydney, Australia, 2009**

National

Oral: **Lam NN**, Sawyer RK, Anderson SR, Morris HA, O'Loughlin PD, Anderson PH.
Increased bone VDR during low dietary calcium mediates renal negative feedback and impairs osteoclast and osteoblast activities in a mouse model. **Australian Health and Medical Research Congress, Melbourne, 2010**

Poster: **Lam NN**, Sawyer RK, Anderson SR, Morris HA, O'Loughlin PD, Anderson PH.
Skeletal effect of increased osteoblastic VDR during calcium-deprivation in a mouse model.
Australian & New Zealand Bone and Mineral Society Conference, Adelaide, 2010

Poster: **Lam NN, Sawyer RK, Anderson SR, Morris HA, O'Loughlin PD, Anderson PH.**
Skeletal effect of increased osteoblastic VDR during calcium-deprivation in a mouse model.
6th Clare Valley Bone Meeting, McLaren Vale, 2010

Poster: **Lam NN, Sawyer RK, Anderson SR, Morris HA, O'Loughlin PD, Anderson PH.**
Osteoblast-specific VDR over-expression protects against bone loss due to vitamin D-deficiency. Australian Society for Medical Research Conference, Adelaide, 2009

Poster: **Lam NN, Sawyer RK, Anderson SR, Morris HA, O'Loughlin PD, Anderson PH.** The study of osteoblastic VDR in a mouse model. **Australian & New Zealand Bone and Medical Society Conference, Melbourne, 2008**

AWARDS

- **American Society for Bone and Mineral Research: *Young Investigator Travel Award*, 2010**
- **Molecular and Experimental Pathology Society of Australasia: *Travel Award*, 2010**
- **Australian Society for Medical Research: *Best Poster Award in Healthy Aging*, 2009**
- **Australian & New Zealand Bone and Medical Society: *Travel Awards*, 2008 and 2009**
- **Department of Physiology, University of Adelaide. *Travel awards*, 2008-2010.**