The Regulation of the Sclerostin Gene and the Catabolic Effects of Sclerostin Protein on Bone

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THESIS ABSTRACT

Age and disease-related bone loss is a major health issue. Bone tissue is constantly remodelled throughout life in order to maintain a healthy skeleton and bone loss is caused by an imbalance in the remodelling process. Bone remodelling is a highly coordinated process between osteoclasts, osteoblasts and osteocytes, with bone targeted for renewal being resorbed by osteoclasts and the resorbed bone replaced by the activities of osteoblasts and osteocytes. During the synthesis of new bone organic matrix, osteoblasts become embedded and differentiate into osteocytes. Osteocytes were previously thought to be terminally differentiated, quiescent cells. However, a wealth of recent evidence suggests that osteocytes play important and dynamic roles.

Recently, the osteocyte expressed protein, sclerostin, was identified to be a major regulator of bone formation. Various pharmaceutical companies are currently in the process of developing therapies to neutralise sclerostin, in order to reverse its anti-anabolic effects on bone. In pre-clinical and clinical studies to date, neutralising sclerostin had bone anabolic effects, and although anti-catabolic effects were also observed, these were usually reported as incidental events. Stemming from observations made by our group of pro-catabolic stimuli up-regulating sclerostin expression, it was hypothesised that sclerostin may have a catabolic action in addition to its anti-anabolic actions. Subsequent work identified the pre-osteocyte/osteocyte as cellular targets of sclerostin, and gene microarray analyses of osteocyte-like cells treated with recombinant sclerostin, led to the discovery of two novel mechanisms, by which sclerostin may act in a catabolic manner.

As presented in Chapter 2, the work undertaken for this thesis demonstrated that sclerostin promotes osteocyte support of osteoclast formation and activity, consistent with recent reports by other groups that suggest osteocytes play a central role in regulating the formation and activity of osteoclasts.

As presented in Chapter 3, sclerostin can also increase the expression by osteocytes of resorption-related molecules, in particular carbonic anhydrase 2. The importance of this observation is that acidification of the extracellular space by osteocytes could promote osteocytic release of mineral and increase of the osteocyte lacunar size, a process termed 'osteocytic osteolysis'. The results presented in Chapter 3 provide the first mechanistic evidence for this process.

As presented in Chapter 4, 1α,25-dihydroxyvitamin D (1,25D) was also identified as a regulator of *SOST*/sclerostin expression and a putative vitamin D response element (VDRE) was shown to be present in the proximal 6.3 kb *SOST* promoter.

In summary, the novel work presented in this thesis expands our knowledge of the activity and regulation of sclerostin. Together, these findings suggest that a subset of pro-catabolic stimuli may induce sclerostin expression, which in turn may act to promote both osteoclastic and osteocytic removal of bone. This research has implications for the pharmacological inhibition of sclerostin, which is currently being pursued commercially. In embarking on such therapy, it is essential to understand the biology of sclerostin as completely as possible.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or tertiary institution to Asiri Wijenayaka 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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PUBLICATIONS

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Atkins GJ, Rowe PS, Lim HP, Welldon KJ, Ormsby R, **Wijenayaka AR**, Zelenchuk L, Evdokiou A, Findlay DM. (2011) *Sclerostin is a locally acting regulator of late-osteoblast/preosteocyte differentiation and regulates mineralization through a MEPE-ASARM-dependent mechanism*. J Bone Miner Res. PMID: 21312267.

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PRIZES AND AWARDS

- 2010 Adelaide University International Postgraduate Research Scholarship (IPRS)
- 2011 Amgen ANZBMS Outstanding Abstract Award, Basic, presented at the Joint Meeting of the International Osteoporosis Foundation and the Australian New Zealand Bone and Mineral Society Gold Coast, QLD, September 2011.
- 2013 ANZBMS Roger Melick Young Investigator Award 2013, presented at the Annual Scientific Meeting of the Australian New Zealand Bone and Mineral Society, Melbourne, VIC, September 2013.
- 2013 ASBMR President's Poster Competition finalist at the Annual Meeting of the American Society for Bone and Mineral Research, Baltimore Convention Center, Baltimore, MD, USA, October 2013.

Abbreviations

1,25D	1α,25-dihydroxyvitamin D_3 (1,25D) / 1,25$(OH)_2$D
ACP5/Acp5	tartrate-resistant acid phosphatise
ANZBMS	Australian and New Zealand Bone and Mineral Society
APC	Adenomatous polyposis coli
ASBMR	American Society for Bone and Mineral Research
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BMD	bone mineral density
BMPs	bone morphogenetic proteins
BMSCs	bone marrow stromal cells
BMU	basic multicellular unit
bp	base pairs
BSP-1	bone sialoprotein-1
CA2/Car2	carbonic anhydrase 2
CBP	cAMP response element-binding protein
ChIP	Chromatin Immunoprecipitation
CSF-1	colony stimulating factor 1
CTSK/Ctsk	cathepsin K
Cx43	connexin-43 ()
DAPI	4',6-diamidino-2-phenylindole
DKK1	Dickkopf-related protein 1
DMEM	Dulbecco's Modified Eagle Medium
DMP-1	dentin matrix acidic phosphoprotein 1

Dsh	disheveled
ECD	extracellular domain
ECRs	evolutionary conserved regions
EMSA	electrophoretic mobility shift assays
FCS	foetal calf serum
FGF23	fibroblast growth factor 23
FITC	fluorescein isothiocyanate
Fn14	factor-inducible gene 14
Fzd	frizzled
GFP	green fluorescence protein
GSK3b	glycogen synthase kinase 3 beta
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIF1α	hypoxia-inducible factor 1-alpha
hMSC	human mesenchymal stem cell
hOCy	osteocyte-like cells
IGF	insulin-like growth factors
kb	kilo base
kDa	kilo dalton
LDLa	laminin-G-like domain
LRP	low density lipoprotein
MAPK	mitogen activated protein kinase
M-CSF	macrophage colony stimulating factor
MEPE	matrix extracellular phosphoglycoprotein
mRNA	messenger ribonucleic acid
NCBI	National Center for Biotechnology Information

NHBC	normal human bone derived cells
NHMRC	National Health and Medical Research Council of Australia
OCN	osteocalcin
ODF	osteoclast differentiation factor
OPGL	osteoprotegerin ligand
OPN	osteopontin
OPPG	osteoporosis pseudoglioma
OS	osteosarcoma
OSM	Oncostatin M
OSX/Osx	osterix
OVX	ovariectomized
PBMC	peripheral blood mononuclear cells
PBS	Phosphate-Buffered Saline
PGE_2	prostaglandin E2
PHEX	phosphate-regulating gene with homologies to endopeptidases on the X-chromosome
pHi	intracellular pH
pHo	extracellular pH
PPi	pyrophosphate
PTH	parathyroid hormone
RANK	receptor activator of NF-κB
RANKL	receptor activator of NF-κB ligand
rh	recombinant human
rhSCL	recombinant human sclerostin
RT-PCR	Reverse transcription polymerase chain reaction

RUNX2	runt related transcription factor 2 ()
RXR	retinoid X receptor
SCL	sclerostin
sCTx	serum C-telopeptide of collagen
SDS-PAGE	Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis
SIBLING	small integrin binding N-linked glycoprotein
siRNA	small interfering RNA
TBE buffer	Tris/Borate/EDTA Buffer
TCF/LEF	T cell factor/lymphoid enhancer binding factor
TGFβ	Transforming growth factor-beta
TK	thymidine kinase
TNF	tumor necrosis factor alpha
TNSAP	tissue non-specific alkaline phosphatase
TRANCE	TNF-related activation-induced cytokine
TRAP	tartrate resistant acid phosphatase 5
TSS	transcription start site
TWEAK	TNF-like weak inducer of apoptosis
TZDs	thiazolidinediones
UHMWPE	ultra-high molecular weight polyethylene
VDR	vitamin D receptor
VDRE	vitamin D response element
Wnt	wingless integration
αMEM	α-Minimum Essential Media