Role of androgens in vascular smooth muscle cell calcification

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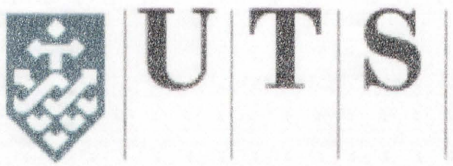
A thesis submitted in fulfilment of the requirements for the degree of Master of Science



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"I have not failed. I've just found 10,000 ways that won't work"

Thomas Edison

1847 - 1931

Certificate of authorship/originality

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Abbreviations

ALP	Alkaline phosphatase
AP-1	Activating protein-1
AR	Androgen receptor
ART	Androgen replacement therapy
β-GP	β-Glycerophosphate
BCASMC	Bovine coronary artery cells
BMP	Bone matrix protein
BSP	Bone sialoprotein
Ca	Calcium
Cbfa1	Core-binding factor alpha-1
CPA	Cyproterone
CVC	Calcifying vascular cells
CVD	Cardiovascular disease
Dex	Dexamethasone
DHT	5α-dihydrotestosterone
E_2	17-β-estradiol
EC	Endothelial cells
ECM	Extra-cellular matrix
ERK1/2	Extracellular signal-related kinase 1/2
ERSD	End-stage renal disease
ERα	Estrogen receptor-α
ERβ	Estrogen receptor-β
FBS	Foetal bovine serum
GPR30	G-protein coupled receptor 30
HASMC	Human aortic smooth muscle cells
HDL	High-density lipoprotein
HDL-C	High-density lipoprotein cholesterol
HERS	Heart and Estrogen/Progestin Replacement Study
HF	Hydroxyflutamide
HRT	Hormone replacement therapy
Hsp	Heat shock proteins
IL-6	Interleukin-6
JNK	c-Jun N-terminal kinase
LDL	Low-density lipoprotein
LDL-C	Low-density lipoprotein cholesterol
MAPK	Mitogen-activated protein kinase
MASMC	Mouse artery smooth muscle cells
MGP	Matrix-Gla protein
MMP	Matrix metalloproteinases
MPP	MPP dihydrochloride hydrate
Msx2	Msh homebox-2
NFκB	Nuclear factor κB
NO	Nitric oxide
OPG	Osteoprotegerin
OPN	Osteopontin
Osx	Osterix

Pi	Inorganic phosphate
PI3K/Akt	Phosphoinositide-3 kinase
PHTPP	Phenyl-bis triflouromethyl pyrazole pyrimidin
POCS	Polycystic ovary syndrome
PPi	Inorganic pyrophosphate
RANKL	Receptor activator of nuclear κB ligand
Runx2	Runt-related transcription factor
SRE	Steroid response element
SMC	Smooth muscle cells
T	Testosterone
TBP	TATA box-binding protein
(TNF)α	Tumour necrosis factor-α
VSMC	Vascular smooth muscle cells
WHI	Women's Health Initiative
WHI-CACS	Women's Health Initiative Coronary-Artery Calcium Study

Abstract

Calcification is a common feature of advanced atherosclerotic lesions and is a clinically significant predictor of cardiovascular events. Coronary calcification is more prevalent in men than age-matched women. However, atherosclerotic calcification increases in postmenopausal women, who present with lower levels of estrogen, suggesting that sex hormones play a critical role in its pathogenesis and progression. This has implications for hormone therapy treatment that is used to treat age-related conditions such as osteoporosis and menopause Extensive observational studies into estrogen replacement therapy have revealed that postmenopausal women treated with estrogen exhibit less extensive atherosclerotic calcification. The effects of androgens on atherosclerotic calcification have, however, received little attention and consequently its mechanisms remain poorly understood. This study therefore explored the effects of androgens on atherosclerotic calcification.

In vitro studies postulate vascular smooth muscle cell (VSMC) differentiation into mineralising osteoblast-like cells as a key mediator of atherosclerotic calcification. Given the gender disparity in atherosclerotic calcification we hypothesised that androgens promote differentiation of VSMC into mineralising osteoblast-like cells. Therefore, the aims of this study were to 1) examine the effects of androgens in vascular smooth muscle cell differentiation and calcification and 2) elucidate the molecular mechanisms of androgen action in this process, using phosphate-induced bovine and murine *in vitro* models of calcification.

This study demonstrated that co-treatment of bovine coronary artery smooth muscle cells (BCASMC) with phosphate and testosterone (T) and dihydrotestosterone (DHT) promoted calcification. Investigation of the molecular mechanisms underlying calcification in the bovine model revealed T-stimulated calcification was estrogen receptor (ER) driven. DHT, however, mediated its effects via the androgen receptor (AR). Further investigation of molecular mechanisms showed DHT regulated ALP activity whereas T did not. T,

therefore, promoted calcification in an ER-driven, ALP independent pathway in contrast to DHT, which mediated its effects via an AR-driven, ALP dependent pathway.

A primary mouse cell-based calcification model was also established. In contrast to the bovine model, it was found that T and DHT treatment did not promote calcification in the murine model. The lack of androgen promotion of calcification in this model was associated with the absence of ALP activity. The conclusion drawn from the bovine model, of a mechanistic role for ALP in the DHT/AR driven mineralisation but not for T-driven mineralisation, suggested that in the murine cells an ER pathway is not functioning.

In conclusion, the studies presented in this thesis demonstrate that T and DHT promote differentiation of vascular smooth muscle cells into osteoblast-like cells capable of mineralisation. T and DHT mediate calcification via alternative pathways that can involve AR and ERs. A potential mechanistic role for ALP in DHT/AR-driven mineralisation has been established.