



UNIVERSITÀ DEGLI STUDI DI PADOVA

SEDE AMMINISTRATIVA: UNIVERSITÀ DEGLI STUDI DI PADOVA

DIPARTIMENTO DI SCIENZE MEDICO-DIAGNOSTICHE E
TERAPIE SPECIALI

SCUOLA DI DOTTORATO DI RICERCA IN
SCIENZE MEDICHE, CLINICHE E SPERIMENTALI
INDIRIZZO: SCIENZE CARDIOVASCOLARI
CICLO XXI

Contribution of Interstitial Valve Cells to Aortic Valve Calcification

Coordinatore: Ch.mo Prof. Gaetano Thiene

Supervisore: Ch.mo Prof. Paolo Pauletto

Dottorando: Dott. Marcello Rattazzi

Ai miei genitori,

INDEX

Abbreviations

1. Riassunto	p. 1
2. Summary	p. 4
3. Introduction	p. 6
4. Epidemiology of aortic valve calcification	p. 8
5. Aortic valve developmental biology and functional structure	p. 12
5.1 The developing aortic valve	p. 12
5.2 The adult aortic valve	p. 15
6. Mechanisms of vascular calcification	p. 20
6.1 Induction of bone formation	p. 21
6.2 Loss of inhibition	p. 24
6.3 Cell death	p. 28
6.4 Circulating calciprotein particles	p. 30
7. Pathogenesis of aortic valve calcification	p. 33
8. Aim of the study	p. 41
9. Methods	p. 42
9.1 Bovine tissue collection and VIC isolation	p. 42
9.2 Treatments of uncloned and cloned VIC	p. 43
9.3 VIC seeding on collagen scaffolds	p. 43
9.4 Immunocytochemical and histochemical studies	p. 43
9.5 Calcium deposition assay	p. 45
9.6 Alkaline phosphatase activity assay	p. 45
9.7 Flow cytometry analysis	p. 45
9.8 Proteomic analysis	p. 46
9.9 Western blotting analysis	p. 47
9.10 Measurement of reactive oxygen species production	p. 48
9.11 Statistical analysis	p. 48

10. Results	p. 49
10.1 Phenotypic cell profile of intact aortic valve leaflet	p. 49
10.2 Isolation and characterization of BVIC clones	p. 49
10.3 Endotoxin and phosphate effects on ALP activity expression and calcium deposition in uncloned BVIC	p. 51
10.4 Selective response of BVIC clones to endotoxin and phosphate treatment	p. 54
10.5 Calcification of collagen scaffolds by BVIC clones	p. 60
10.6 Modification in cytosolic protein profile in clonal cells acquiring a pro-calcific phenotype	p. 61
10.7 Reactive oxygen species production in pro-calcific clonal cells	p. 64
11. Discussion	p. 67
12. References	p. 75
Appendix	

Abbreviations

AB: apoptotic bodies
ACE: angiotensin-converting enzyme
ADMA: asymmetric dimethylarginine
ALP: alkaline phosphatase
AngII: angiotensin II
ApoE^{-/-}: apolipoproteinE deficient mice
AT1: angiotensin receptor-1
aVIC: activated VIC
BMI: body mass index
BMP: bone morphogenic proteins
BVIC: bovine interstitial valve cells
CAD: coronary artery disease
Cbfa-1: core binding factor alfa-1
Chm-I: chondromodulin-I
CVC: calcifying vascular cells
DDAH: dimethylarginine dimethylaminohydrolase
EC: endothelial cells
ECM: extracellular matrix
EMT: endothelial mesenchymal transition
Ennp1: ectonucleotide pyrophosphatase/phosphodiesterase1/PC-1
Ennp1^{-/-}: Ennp1 deficient mice
ESRD: end-stage renale disease
eVIC: embryonic VIC
FGF-23: fibroblast growth factor-23
FGF-23^{-/-}: FGF-23 deficient mice
HA: hydroxyapatite
Hsp: heat shock protein
ICAM-1: intracellular adhesion molecule-1
IL: interleukin
LDL: low density lipoprotein
LDLr^{-/-}: LDL receptor deficient mice
LPS: lypopolisaccaride/endotoxin
Lrp5: LDL receptor-related protein5
MGP: matrix-gla protein
MGP^{-/-}: MGP deficient mice
MMP: matrix metalloproteinase
Msx2: Msh homeobox 2
MV: matrix vescicles
NAFTc1: Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
NMM: non muscle myosin
NO: nitric oxide
NOS: nitric oxide synthase
Notch1: Notch homolog 1, translocation-associated
obVIC: osteoblastic VIC
OC: osteocalcin
ON: osteonectin
OPG: osteoprotegerin
OPG^{-/-}: OPG deficient mice
OPN: osteopontin
OPN^{-/-}: OPN deficient mice
oxLDL: oxidized LDL
Pi: inorganic phosphate
PPi: inorganic pyrophosphate
pVIC: precursor VIC
qVIC: quiescent VIC
RANK: receptor activator of NF-kB
RANKL: receptor activator of NF-kB ligand
RANKL^{-/-}: RANKL deficient mice
RAS: renin-angiotensin system
ROS: reactive oxygen species
Runx2: runt-related transcription factor 2
SMA: smooth muscle α -actin
SMC: smooth muscle cells
SMemb: embryonic smooth muscle heavy chain
SMM: smooth muscle myosin
SOD[Cu-Zn]: superoxide dismutase [Cu-Zn]
Sox-9: SRY-box 9
TGF- β : transforming growth factor- β
TIMP: tissue inhibitor of metalloproteinases
TLR: toll-like receptor
TNF α : tumor necrosis factor- α
VCAM-1: vascular cell adhesion molecole-1
VEC: valve endothelial cells
VEGF: vascular endothelial growth factor
VIC: valve interstitial cell
vWF: von Willebrand Factor

