Identification and characterization of the mediators of the calcification paradox

6. SUMMARY

This thesis focuses on the identification and characterization of novel inhibitors of the pathophysiological process of vascular calcification (VC).

By characterization of CBF, the conditions and concentrations under which this peptide inhibits VC was identified. In addition, we show that this peptide inhibits calcification by hindering transdifferentiation of SMCs by interacting with PIT-1 to inhibit the activation of NF-κβ and its downstream BMP2/p-SMAD pathway, effectively reducing the expression of osteogenic genes and inhibiting apoptosis while promoting metabolism.

Next, CBF (1-8), a small fragment of CBF, was identified which is a more potent inhibitor of medial vascular calcification compared to CBF. The impact of CBF (1-8) on VC was demonstrated and the mechanisms by which CBF (1-8) inhibits VC were illustrated in this thesis. CBF (1-8) reduces apoptosis and promotes cellular metabolism and wound healing thereby reducing VC. In addition, it prevents VC by reducing the transdifferentiation of SMCs to osteoblast-like cells by reducing the expression of osteogenic genes via the BMP2 and β-catenin pathways.

Moreover, CBF (1-8) reverses the loss of bone density caused by VC inducing conditions in VDN rats. The effect of CBF (1-8) on bone density is validated by the absence of any significant difference in enamel and dentine of these animals as they are not actively remodelled, demonstrating that CBF (1-8) promotes mineralization in the actively remodelled bones such as the tibia and the cementum in the dentures.

CBF (1-8) is derived from the protein chromogranin A and might be on of the missing link in our current knowledge about the calcification paradox. Moreover, CBF (1-8) is the first peptidic mediator which links VC to bone mineral density *in vivo*. Understanding the mechanisms by which CBF (1-8) functions, will help utilize the regulatory balance between VC and bone mineralization enabling us to tilt the balance towards bone mineralization by maintaining plasma CBF (1-8) concentration. In addition, the identification and characterization of these previously unknown peptides open novel options for VC therapy without or -at least- limited a negative impact on bone density.

In future, these findings have to be validated in human cohorts for future therapeutic applications. As calcification paradox is a complex process, subsequent investigation of the key factors affecting VC will help to identify specific pathology associated substances affecting bone density. Further, these substances have to be characterized in the context of their impact on bone density using functional bioassays and/or humanized models to find the mechanisms involved.