Interplay between inflammation and calcification in cardiovascular diseases

ABSTRACT

Cardiovascular calcification has been linked to all-cause mortality and is a broadly adopted predictor of cardiovascular (CV) events. Rather than a mere by-product of the changing disease environment, calcification impacts actively the disease progression and pathogenesis as it predominates both in early- and late-stages, through mediating tissue biomechanical destabilisation and directly impacting tissue inflammation. However, its clinical contribution to the fate of the disease remains to be elucidated. Emerging body of evidence from both basic and clinical research has demonstrated the significance of the innate immune system in cardiovascular diseases (CVDs). Here, inflammation and calcification are engaged in a vicious cycle particularly at early-stages, whereas in advanced-lesions, large calcifications linked with suppressed inflammation and plaque stability. However, this interaction during disease progression remains largely elusive. The aim of this thesis is to investigate the interplay between inflammation and calcification in advanced atherosclerosis and calcific aortic valve disease (CAVD).

Study I explores gene and protein expression signatures and biological pathways of advanced CAVD lesions in order to characterise the underlining mechanisms associated with the disease pathology. Multi-omics integration of overlapping transcriptome/proteome molecules with miRNAs, identified a unique CAVD-related protein-protein 3D layered interaction network. After addition of a metabolite layer, Alzheimer's disease (AD) was identified in the core of the gene-disease network. This study suggests a novel molecular CAVD network potentially linked to amyloid-like structures formation.

Study II characterises osteomodulin (OMD) in the context of atherosclerosis, chronic kidney disease (CKD) and CAVD. Plasma OMD levels were correlated with markers of inflammation and bone turnover, with the protein being present in the calcified arterial media of patients with CKD stage 5. Circulating OMD levels were also associated with cardiac valve calcification in the same patients and its positive signal was detected in calcified valve leaflets by immunohistochemistry. In patients with carotid atherosclerosis, plasma OMD levels were increased in association with plaque calcification as assessed by computed tomography. Transcriptomic and proteomic data analysis showed that OMD expression was upregulated in atherosclerotic compared to non-atherosclerotic control arteries, and particularly in highly calcified plaques, where its expression correlated positively with markers of vascular smooth muscle cells (VSMCs) and osteoblasts. *In vivo*, OMD was

enriched in VSMCs around calcified nodules in aortic media of nephrectomised rats and in plaques from *ApoE*$^{-/-}$ mice on warfarin. *In vitro* experiments revealed that exogenous administration of recombinant human OMD protein repressed the calcification process of VSMCs treated with phosphate by maintaining the VSMC contractile phenotype along with enriched extracellular matrix (ECM) organisation, thereby attenuating VSMC osteoblastic transformation.

Study III analyses OMD expression in human carotid plaques and particularly its link with future CV events. Transcriptomic analysis revealed that OMD levels were increased in plaques from asymptomatic patients compared to symptomatic ones, with high levels being associated with fewer CV events in a follow-up analysis.

Study IV investigates the link between mast cell (MC) activation and key features of human plaque vulnerability, and the role of MC in VSMC-mediated calcification. Integrative analyses from a large biobank of human plaques showed that MC activation is inversely associated with macrocalcification and positively with morphological parameters of plaque vulnerability. Bioinformatic analyses revealed associations of MCs with NK cells and other immune cells in plaques. Mechanistic *in vitro* experiments showed that calcification attenuated MC activation, while both active and resting MCs induced VSMC calcification and triggered their dedifferentiation towards a pro-inflammatory- and osteochondrocyte-like phenotype.

Overall, this thesis demonstrates that the underlying mechanisms of CVD related to inflammation and calcification can be comprehensively characterised by integration of large-scale multi-omics datasets along with cellular and molecular assays on one side, and disease specific biomarkers and advanced diagnostic imaging tools on the other. In summary, these studies not only indicate that advanced-calcification is a stabilising factor for plaque and disease progression but also, unveil novel insights into the cardiovascular calcification pathobiology, and offer promising biomarkers and new therapeutic avenues for further exploration.