178 **ER STRESS IN VASCULAR CALCIFICATION**

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Vascular calcification is a pathological process of deposition of calcium and phosphate crystals in the form of hydroxyapatite (HAp) in the blood vessel wall. Its presence in the vessel wall causes stiffness and leads to left ventricular hypertrophy and increases cardiovascular morbidity and mortality. It is a serious health problem common in ageing populations and widely prevalent in pathological conditions such as atherosclerosis, diabetes and chronic kidney disease.

Vascular calcification is a regulated, cell-mediated process, similar to bone formation that involves osteogenic transdifferention of vascular smooth muscle cells (VSMCs) characterized by expression of bone-specific genes in the calcified vessel. However, the exact mechanisms responsible for triggering this process are unknown.

The endoplasmic reticulum (ER) is involved in the production, correct folding and secretion of newly synthesized proteins in cells. ER stress occurs as a result of unfolded protein accumulation or an increased demand for protein secretion. It leads to activation of a signalling pathway called the unfolded protein response (UPR), mediated by three main ER stress transducers IRE1, PERK and ATF6. Importantly, ER stress has recently been implicated in bone development. Therefore, we hypothesized that phenotypic conversion and calcification of VSMCs can be regulated by ER stress.

Human primary VSMCs were treated with tunicamycin and thapsigargin and expression of 15 bone markers in response to ER stress was examined by Western blotting and quantitative real-time PCR.

ER stress was shown to downregulate expression of BMP2, a key bone morphogen. It was shown to upregulate expression of Osterix, an obligate bone specific transcription factor, as well as its downstream targets bone sialoprotein, alkaline phosphatase and osteoprotegerin in VSMCs. However little effect was observed on

expression of Runx2, thought to be crucial for VSMC osteogenic transdifferentiation. In addition, the two different ER stress inducers were found to have differential effects on activation of these bone markers. Using siRNA knock-down it was established which branches of the UPR potentially play a role in mediating these effects on bone gene expression. Alkaline phosphatase was found to be regulated by ATF4, a transcription factor downstream of PERK. The downregulation of BMP-2 by ER stress was mediated by both ATF6 and ATF4.

Taken together this data suggests that ER stress may play a role in VSMC osteogenic transdifferentiation via non-canonical transcriptional pathways.