Medial Vascular Calcification Inhibition through the Wnt/β-Catenin Pathway

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Statement of Purpose: According to the World Health Organization (WHO) cardiovascular disease is the number 1 leading cause of death globally, with an estimation of over 15 million deaths in 2015. During cardiovascular disease, vascular calcification starts with a buildup of plaque within the medial layer of the arteries resulting in reduced blood flow and wall stiffening. Intimal and medial are two prominent types of calcifications. Intimal calcification, or atherosclerosis, usually involves intimal thickening resulting from endothelial damage due to high blood pressure, smoking, etc. Alternatively, medial calcification or arteriosclerosis is characterized by the stiffening of the walls within the medial layer of the arteries due to calcification in the area. It has been hypothesized that stress generated from intimal calcification causes vascular smooth muscle cells, lining the medial layer of the arteries, to undergo a phenotypic switch causing them to lose their contractility and induce medial calcification. Recent research has shown that the process in which vascular smooth muscle cells calcify and turn into osteoblast-like cells identical to the formation of bone. Although there is still no clear understanding of the mechanism, researchers have hypothesized that the Wnt/ β-catenin pathway plays a role in the phenotype change. The canonical (Wnt/β-catenin) pathway is the most studied pathway and is implicated in many developmental processes, including bone metabolism. Within this pathway, Wnt binds to Fzd and the LRP 5/6 coreceptor complex to activate Dsh, which in response causing the inactivation GSK3, a "cytoplasmic destruction complex" which is responsible for regulating the phosphorylation of the β-catenin protein and targeting it for degradation. My research group has developed an *in vitro* cell culture model to induce calcification in human vascular smooth muscle cells. The objective of this study is to characterize the in-vitro model of vascular smooth cell calcification and confirm Wnt-signaling as it affects vascular smooth muscle cell calcification.

Methods: 3mM Sodium Phosphate Dibasic Anhydrous was added to Human Aortic Smooth Muscle cells to induce calcification. To determine if Wnt-signaling was expressed or inhibited was determined by Immunohistochemistry (IHC) staining at the 7-day and 14-day time points for Alpha Smooth Muscle Actin (∝SMA), Axin1, and RUNX2. β-Catenin proteins were measured by using an ELISA Assay and read on a standard microplate reader at 450nm.

Results: When the calcification was measured at the 3-day and 7-day time point; the 7-day calcification had much higher calcium content. ∝SMA in the 14-day control stain showed more expression, while the 14-day calcification showed no ∝SMA expression. For AXIN1, negative regulator of the Wnt-signaling pathway, calcification increased as AXIN1 decreased. RUNX2 increased in the cell's nucleus while calcification increased, In the ELISA assay β-Catenin had a higher concentration in the calcification sample than the control.

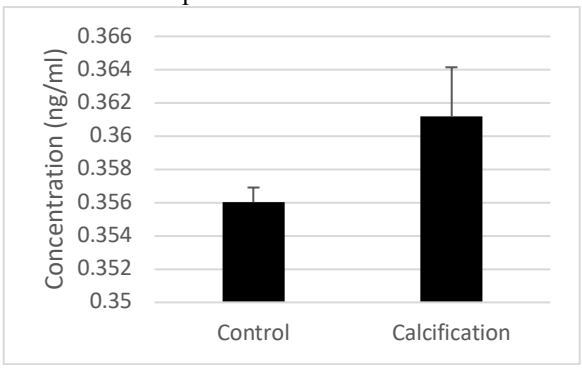

Figure 1: β-Catenin protein concentration is higher in calcified smooth muscle cells compared to control.

Conclusion: Inorganic phosphate (3mM) successfully induced calcification in human vascular smooth muscle cells. Immunohistochemistry showed decreased expression of ∝SMA and AXIN and an increased expression of RUNX2 in calcified cells. The decrease in ∝SMA and the increase of RUNX2 expression is consistent with previous studies regarding the switch to osteoblast-like cells and the involvement of Wnt-signaling. AXIN showed decreasing expression as calcification increased, although it is usually always present in some capacity. As previously mentioned, the role of AXIN is to promote degradation of β-catenin to prevent Wnt-signaling. Therefore, the decrease of AXIN could be due to suppression because of the accumulation of β-catenin. We can, however, conclude that Wnt-signaling is involved based on the increasing expression of RUNX2 and the early expression of AXIN. This conclusion leads us to hypothesize that WNT could be a possible therapeutic target in treating vascular calcification.