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Arteriosclerotic Calcification: *A Serpi(n)ginous Path To Cardiovascular Health?*

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Cardiovascular sclerosis increasingly afflicts our aging, dysmetabolic population – and this hardening of our hearts and arteries has significant physiological consequences[1]. Myocardial stiffening reduces diastolic ventricular filling and function necessary for robust cardiac output during systole[2]. Arterial stiffening impairs Windkessel physiology – the rubbery elasticity of conduit vessels that ensures smooth distal tissue perfusion throughout the cardiac cycle. Thus, in addition to the diastolic heart failure associated with cardiac sclerosis, a type of diastolic perfusion failure occurs with arteriosclerotic conduit vessel stiffening. The inability to store kinetic energy as potential energy in elastic conduit vessels during systole reduces the sustained pressure differential necessary to drive smooth distal perfusion throughout diastole -- and is manifested by increased arterial pulse wave velocity (PWV) during systole[1].

One clinical consequence of diastolic vascular perfusion failure can be well-appreciated in the central nervous system. In the Dallas Heart Study, increased aortic stiffness as quantified by PWV strongly portends increased brain MRI white matter hyperintensity volume[3], a signature of ischemic (not hemorrhagic) histology[4], independent of other cardiovascular risk factors including systolic blood pressure[3]. Cognitive decline is a clinical feature of conduit vessel stiffness[5, 6] and white matter hyperintensity volume[4]. Large conduit artery biomechanics reflect the composite contributions of mural material properties, geometric properties, and dynamic endothelial and neuroendocrine inputs that control global and regional tissue perfusion[7]. Arterial calcification -- an active form of tissue biomineralization – has emerged as one important pathogenic feature of conduit vessel stiffening[8, 9]. Over the past 2 decades, elegant work forthcoming from research teams at UCLA has identified that powerful signals provided by bone morphogenetic proteins (BMP) of the TGF-beta superfamily play critically important roles in arterial calcification[10]. Matrix Gla protein

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(MGP), a secreted calcium binding protein that inhibits BMP function in a Gla-(gamma-carboxyglutamate) dependent fashion, functions as a vascular co-morphogen and rate-limiting negative regulator of arterial mineral deposition in murine disease models[11, 12]. *MGP*-null mice die precociously with pan-arterial calcification and aortic rupture[13]. MGP clearly impacts the BMP-directed osteochondrogenic programming; however, results from other groups have highlighted that the spectrum of MGP-regulated vascular actions relevant to arterial calcification may in fact extend beyond BMP modulation to encompass elastin matricrine signaling[14] and osteogenic degradation products[15]. Since under-carboxylated MGP tracks arterial stiffness in humans[16], a better understanding of MGP actions may yield novel therapeutic approaches to arteriosclerotic disease and its consequences.

In this issue of *Circulation Research* [17], Yao, Boström, and colleagues once again advance our understanding of MGP actions in arteriosclerotic disease[18]. They identify that MGP serves to restrict expression and activities of vascular serine protease that promote the endothelial-mesenchymal transition (EndMT) – a key contributor to the cells and signals that drive arterial calcium deposition. In response to metabolic insult such as hyperglycemia, previous work from this group identified that MGP deficiency enabled endothelial cell (EC) phenotypic plasticity and subsequent osteogenic trans-differentiation, with concomitant induction of multiple Yamanaka factors including *Sox2*[18]. However, the regulatory circuits conveying this response were uncharacterized. Detailed vascular assessment by electron microscopy revealed early post-natal degradation of the remodeling arterial internal elastic lamina (IEL) in *MGP*−/− mice with upregulation of Twist and *Slug/snai2* – key markers and mediators of the EndMT [17]. Implementing gene array analyses to interrogate for potential mediators, the team identified that mRNAs encoding a select cohort of five serine proteases -- *elastases* 1 and 2 and *kallikreins* 1, 5, and 6 – were markedly upregulated with MGP deficiency in aortic tissues. *In vitro*, treatment of human aortic ECs with this 5-protease protein cocktail upregulated the expression of key markers of multipotency – including *Sox2*[17] -- along with the EndMT, thereby phenocopying the actions of MGP deficiency[18]. RNAi targeting either the protease pentad or *Sox2* abrogated osteogenic trans-differentiation of human aortic ECs induced by MGP knockdown. Importantly, BMP4- and glucose-induced EndMT was abrogated by administration of serpinA1 (alpha-1 antitrypsin) or diisopropylfluorophosphate – broad-specificity serine protease inhibitors – and this inhibitory action was fully reversed by transduction with a Sox2-expressing lentivirus. *In vivo*, EC-specific deletion of *Sox2* reduced arterial calcification in the global MGP-null background – and administration of serine protease inhibitors including serpinA1 reduced arterial calcification and delayed precocious cardiovascular death in *MGP*-null mice. Thus, the authors newly discover that a cadre of serine proteases participate in the EndMT and the *Sox2*-dependent phenotypic plasticity that drives arterial calcification in the absence of MGP – and demonstrate that serine protease inhibition limits arteriosclerotic disease and demise in this enlightening vasculopathy model[17].

The precise proteases driving arterial calcification as responsive to serine protease inhibition *in vivo* have yet to be unambiguously identified, and future studies will undoubtedly focus upon this important aspect. However, it is intriguing to reflect upon the responses to recombinant serpinA1 in *MGP*−/− mice and their significant implications. Firstly, serpin-

based biologics – e.g., serpinA1 (a.k.a alpha-1 antitrypsin, AAT), C1 esterase inhibitor, etc. – have found important FDA-approved therapeutic niches in molecular medicine[19]. Given the results of Yao et al[17], one can envision potential serpin-based strategies to reduce arteriosclerosis in high-risk states such as chronic kidney disease and/or diabetes. Secondly, while AAT deficiency engenders neutrophil elastase-mediated emphysema in mid-life that is responsive to AAT replacement (augmentation), the pharmacology of serpinA1/AAT is more complex[19]. SerpinA1/AAT targets multiple proteases beyond neutrophil elastase– including certain kallikreins and cathepsins[19, 20]– that are involved with inflammation as well as vascular elastin matrix turnover. Considering the emerging role for kallikreins in the epithelial-mesenchymal transition[21] and those upregulated in MGP-null mice[17], it is probable that some aspect of the beneficial response to protease inhibition may accrue via modulation of protease- activated receptor signaling in addition to support of IEL integrity and reduction in osteogenic elastin degradation products[15]. Thirdly, SerpinA1 expression is increased in human atherosclerotic plaques, where human genetics points to relevant contributions to cardiometabolic disease risk[22]. Thus, while it remains to be determined whether serpinA1/AAT administration is effective in preclinical models of cardiometabolic disease, given the results in MGP-deficient mice[17] and the broad substrate specificity and clinical safety profile of serpinA1/AAT[19], potential repurposing for arteriosclerosis deserves additional preclinical and clinical investigation. However, since calcium deposition is not the only determinant of vascular stiffness[7], it will be important to directly assess cardiovascular compliance and function. Finally, regardless of underlying mechanisms, the feed-forward reciprocal relationship between endothelial *Sox2* and vascular protease expression discovered in *MGP*-null mice[17] highlights the potential efficacy achieved by targeting this regulatory linchpin as strategy to preserve aortic endothelial phenotype -- and thus conduit vessel integrity, compliance, and function. As such, a new pharmacological pathway is blazed[17] wherein serpin therapy might help preserve vascular health and end organ function in our patients afflicted with arteriosclerotic disease[3].

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