Vascular Calcification: An Age-Old Problem of Old Age

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Vascular calcification, once considered a passive consequence of aging, is now recognized to be a highly regulated process akin to bone formation. Vascular calcification is prevalent across ethnicities and age groups and observational studies show an interaction with aging in asymptomatic adults and in individuals with established coronary artery disease.\(^1,2\) Recent findings from the HORUS study have shown that the link between aging and vascular calcification is an age-old association. In this study, 137 mummies up to 4,000 years old were examined with CT scans. Vascular calcification was present in 47/137 or 34% of the mummies and the age at the time of death correlated positively with the presence of vascular calcification as well as the number of vascular beds with calcified vessels.\(^3\) In the modern era, the incidence of vascular calcification has been shown to increase with advancing age and has been reported to be <5% annually for individuals <50 years of age to >12% for individuals >80 years of age.\(^2\)

When present, vascular calcification portends a worse clinical outcome; a meta-analysis of 218,000 patients found a 3.94-fold higher risk for cardiovascular mortality and a 3.41-fold higher risk for any cardiovascular event.\(^4\) Thus, understanding how aging influences the pathobiology of vascular calcification may have far-reaching implications for associated cardiovascular morbidity and mortality.

To understand the cellular and molecular mechanisms that underlie aging-related vascular calcification, investigators have begun to focus on the vascular pathophenotype associated with Hutchinson-Gilford Progeria Syndrome (HGPS). This rare genetic disorder is the result of a point mutation in the *LMNA* gene that generates an abnormal variant of prelamin A that retains its farnesyl group and is known as progerin.\(^5\) The disease is characterized, in part, by accelerated aging with early atherosclerosis and vascular calcification. To date, the causative mechanism(s) for precocious vascular calcification in this disease have not been elucidated fully. In this issue
of *Circulation*, Villa-Bellosta *et al* report that vascular calcification in HGPS results from decreased levels of extracellular inorganic pyrophosphate, an inhibitor of hydroxyapatite crystal formation and ectopic calcification. Using the *Lmna*<sup>G609G</sup> knock-in mouse model of HGPS, which expresses progerin and has pronounced aortic medial calcification, the investigators found that low levels of extracellular inorganic pyrophosphate occurred as a result of impaired synthesis as well as increased hydrolysis to inorganic phosphate. Pyrophosphate synthesis was impaired by substrate availability; HGPS vascular smooth muscle cells (VSMC) generated lower levels of mitochondrial ATP than controls. They also observed a concomitant increase in the expression of tissue non-specific alkaline phosphatase, which hydrolyzes pyrophosphate to inorganic phosphate. The obligate role of progerin in dysregulation of pyrophosphate metabolism was confirmed by retroviral-mediated gene transfer to force expression of progerin in wild-type VSMC. Progerin-expressing VSMC recapitulated the HGPS-VSMC phenotype with decreased pyrophosphate levels, aberrant pyrophosphate metabolism, and mineralization. While these studies do not tell us specifically how progerin induces mitochondrial dysfunction or upregulates alkaline phosphatase expression, the findings do identify pyrophosphate as a previously unrecognized mediator of vascular calcification in HGPS and may have broader applicability to our understanding of aging-related vascular calcification.

The results from this study performed using a disease model of accelerated aging to investigate vascular calcification may be extrapolated to explain this process under normal aging conditions. This is underscored by the fact that prelamin A is expressed in VSMC as they age in the absence of the HGPS genotype. Prelamin A has been detected in blood vessels isolated from older individuals or from young patients with chronic kidney disease on dialysis who often have phenotypically aged vasculature. In these vessels, prelamin A colocalizes with senescent
and calcifying VSMC. In vitro studies have demonstrated that prelamin A is increasingly expressed in pre-senescent cells through a mechanism involving increased oxidative stress, which itself has been implicated in the pathogenesis of vascular calcification. The role of prelamin A in modulating senescence has been established using HPGS-induced pluripotent stem cells. Under basal conditions, progerin and epigenetic changes associated with premature aging are absent; however, when these cells are differentiated towards VSMC, progerin levels increase and cells return to a senescent state. While it is not known if senescence per se dysregulates pyrophosphate metabolism in VSMC, it is clear that senescent VSMCs achieve a calcification phenotype. Senescent VSMC have increased expression of the osteoblast transcription factor Runx2 and alkaline phosphatase, and have an enhanced capacity to mineralize compared to non-senescent cells.

Evidence to indicate that dysregulation of vascular pyrophosphate metabolism is an aging-related process or that pyrophosphate levels decline with age is limited. Small observational studies of normal healthy subjects or individuals with chronic kidney disease on dialysis found only a weak inverse correlation between age and plasma pyrophosphate levels. Despite this, there is abundant experimental evidence that firmly links perturbations in pyrophosphate metabolism with vascular calcification. Genetic deletion of genes that regulate phosphate metabolism in mice has established that extracellular inorganic pyrophosphate does play a key role in regulating vascular calcification (reviewed in ). The finding that HPGS-VSMCs synthesize less pyrophosphate owing to decreased ATP levels also implicates mitochondrial dysfunction in aging-related calcification. Mitochondrial dysfunction exists in HPGS and proteomic analysis of murine HPGS adipocytes demonstrate modifications in the expression profile of mitochondrial proteins related to lipid metabolism, the tricarboxylic acid
cycle and oxidative phosphorylation, and oxidant stress.\textsuperscript{16} ATP levels are also known to be decreased in HPGS fibroblasts with levels being only 50\% of that measured in control subjects.\textsuperscript{17} As mitochondrial dysfunction and decreased ATP production has been demonstrated in calcifying VSMC exposed to high levels of inorganic phosphate,\textsuperscript{18} it is interesting to speculate that dysregulated pyrophosphate metabolism may induce mitochondrial dysfunction. Other plausible explanations to explain the decrease in mitochondrial ATP generation include a decline in mitochondrial biogenesis, which occurs with advancing age,\textsuperscript{19} a reduction in mitochondrial mass, or an increase in mitophagy.

Is dysregulation of pyrophosphate metabolism the only mechanism to explain aging-related vascular calcification? This is unlikely as other studies examining calcifying senescent VSMC have implicated activation of the DNA damage response and acquisition of a senescence-associated secretory phenotype as the mechanism for calcification. These prelamin A-expressing VSMC appear to regulate calcification in a paracrine manner by secreting pro-calcifying factors and cytokines, including interleukin-6, bone morphogenetic protein-2, and osteoprotegerin.\textsuperscript{9} While pyrophosphate metabolism was not investigated in the aforementioned studies, elevated levels of inorganic phosphate are known to modulate VSMC transition to a calcifying phenotype and hyperphosphatemia-induced nanocrystals have also been shown to increase expression of bone morphogenetic protein-2 in VSMC.\textsuperscript{20,21} Thus, dysregulation of pyrophosphate metabolism may act in concert with the DNA damage response and other known pro-calcifying mechanisms to promote aging-related vascular calcification.

What can we conclude from these studies? First, it is likely that vascular prelamin A expression may emerge as a biomarker of vascular aging. This may be of importance given the divergence between chronological and biological age in many disease states associated with
vascular calcification. Secondly, extracellular inorganic pyrophosphate levels and indices of phosphate metabolism should be evaluated when examining aging-related calcification. Whether or not pyrophosphate modulates other known mechanisms of vascular calcification or has predictive value for the development of calcification requires additional study. Finally, the therapeutic efficacy of exogenous pyrophosphate administration to prevent vascular calcification may be limited by the propensity for pyrophosphate to induce calcification in non-vascular tissues when in excess. This suggests that targeted therapies, such as vascular gene transfer, to maintain pyrophosphate levels may offer a greater therapeutic benefit to ameliorate vascular calcification with an improved side-effect profile. While aging is inevitable, findings from this and other similar studies will ensure that vascular calcification is not.

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