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EDITORIAL COMMENT

PLucKing at Vascular Fibrosis*

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The adventitia is the most complex layer of the vessel wall, comprising a loose arrangement of extracellular matrix (ECM) fibers and a diverse phenotype of cells including fibroblasts, stromal progenitors, pericytes, and immune cells (1). The adventitial fibroblast is the most well-studied of the adventitial cell population and contributes to vascular remodeling in response to hemodynamic stress. Following transverse aortic constriction or pressure overload stress to the murine heart, adventitial fibroblasts are activated, deposit ECM, and contribute to perivascular fibrosis around the coronary arteries. Adventitial fibroblasts are also motile and have been shown to contribute to atherosclerotic lesions by migrating into the intima or fibrous cap of atherosclerotic lesions. In murine models of Duchenne's muscular dystrophy, adventitial fibroblasts also contribute to perivascular fibrosis. Thus, a diverse range of vascular or hemodynamic stressors can activate adventitial fibroblasts and lead to perivascular fibrosis. Studies in humans with nonischemic heart failure have demonstrated that perivascular fibrosis strongly correlates with impaired blood flow in the left anterior descending coronary artery, demonstrating the adverse effects of vascular fibrosis on luminal flow (2). Activation of adventitial fibroblasts is thought to be similar to that of interstitial cardiac fibroblasts. Following activation, adventitial fibroblasts secrete ECM, inflammatory cytokines, and chemokines, and express smooth muscle actin to adopt a myofibroblast phenotype. Thus, targeting the adventitial fibroblast to attenuate perivascular fibrosis remains an attractive target to mitigate adverse vascular remodeling development, but large gaps in our knowledge remain about the molecular mechanisms of adventitial fibroblast activation.

In this issue of *JACC: Basic to Translational Science*, Li et al. (3) identify polo-like kinase 4 (PLK4) as a novel regulator of vascular fibroblast activation. PLK are a family of serine/threonine protein kinases that play an important role during cell division and are named from the phenotype observed in *Drosophila* mutants where PLK mutants resulted in abnormal spindle poles (4). Within the PLK family, PLK4 has little homology to the other members and regulates centriole duplication during cell division. PLK1, the most well-studied gene in this family, in addition to its role in cell division, is thought to contribute to lung fibrosis. The investigators thus decided to examine the role of PLKs in fibroblast activation and in particular the effects of PLK1 and PLK4 on myofibroblast differentiation of adventitial fibroblasts.

The investigators isolated adventitial fibroblasts from the rat aorta, stimulated them with PDGF AA to induce myofibroblast formation, and then observed that genetic or pharmacological inhibition of PLK4 with a highly selective inhibitor abolished the expression of αSMA, a canonical marker of myofibroblast formation. Serum response factor (SRF) acts as a master transcription factor that induces αSMA transcription, and SRF is itself activated by myocardin-related transcription factor A (MRTF-A). The investigators observed that inhibition of PLK4 abolished PDGF AA–induced increase in MRTF protein, as well as SRF transcriptional activity, suggesting that PLK4 likely uses the MRTF/SRF axis in inducing myofibroblast formation of adventitial fibroblasts. Conversely, increased PLK4 expression

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increased MRTF-A levels, whereas mutant PLK4 did not induce any increase in MRTF-A protein.

Because PDGF AA stimulates PDGFRα, the investigators determined whether activation of PDGFRα signaling induced activation of PLK4 by examining PLK4 phosphorylation. Inhibition of PDGFRα signaling with Crenol led to decreased phosphorylation of PLK4. Considering these observations, the investigators then investigated mechanisms of PDGFRα-mediated regulation of PLK4 activation. Activation of PDGFRα by PDGF AA led to phosphorylation and activation of a host of MAPK pathway kinases. Using siRNA, the investigators demonstrated that silencing of p38 led to significant reduction in PLK4 phosphorylation. These in vitro observations thus establish a signaling cascade of adventitial fibroblast activation, where PDGF AA-mediated activation of PDGFRα induces p38 activation, which in turn leads to PLK4 phosphorylation and subsequent activation of the MRTF-A/SRF axis for increased αSMA transcription and adoption of a myofibroblast phenotype.

Having determined upstream mechanisms inducing PLK4 activation and mechanisms downstream of PLK4 leading to increased αSMA expression, the investigators next investigated mechanisms of regulation of PLK4 expression. These investigations were based on the rationale that in pulmonary fibrosis, FoxM1, a transcription factor, regulates PLK1 transcription. However, the investigators observed that FoxM1 did not regulate PLK4 expression. They then examined the role of bromodomain and extra-terminal domain (BET) genes in regulating PLK4 expression. BET proteins play an important role in reading the chromatin code and have been implicated in cardiac fibrosis; inhibition of BET proteins with JQ1 has been demonstrated to attenuate cardiac fibrosis (5). The investigators demonstrate that treatment of adventitial fibroblasts with JQ1 attenuated PDGF AA-mediated myofibroblast formation and abrogated increase in PLK4 and MRTF-A protein levels. Increased levels of αSMA and MRTF-A expression by forced expression of PLK4 was also blocked by JQ1. Considering these observations, the investigators concluded that BET proteins were likely regulating PLK4 expression, though other combinatorial scenarios of BET proteins exerting transcriptional control on multiple other genes cannot be ruled out from the data presented.

In a final set of experiments to determine the physiological significance of their observations in vivo, they induce balloon angioplasty injury of carotid arteries in rats and apply a thermosensitive gel containing the PLK4 inhibitor CenB around the injured segments of the arteries. The investigators measured collagen deposition at 7 days and observed significantly reduced adventitial collagen deposition in the CenB-treated animals compared with vehicle-treated control animals, with concomitant reduction in adventitial thickness. The investigators also observed decreased vimentin and adventitial αSMA expression. However, there was no difference in the degree of neointimal hyperplasia, and no functional data on vascular mechanics are provided to determine whether inhibition of PLK4 leads to functional benefits. Notwithstanding, the paper provides new insight into the noncanonical function of PLK4 as a regulator of vascular fibrosis. Because the mechanisms of vascular and interstitial cardiac fibrosis overlap, and BET domains have been shown to contribute to interstitial cardiac fibrosis, the observations made by Li et al. (3) raise the question of whether PLK4 regulates cardiac fibrosis and myofibroblast activation following injury or hypertrophy. The health and mechanics of the vessel wall need to be ascertained with long-term application of PLK4 inhibitors. It is possible that adverse effects may arise secondary to off-target effects on other vascular cells. However, if the functional benefits of PLK4 are confirmed in vivo with more extensive studies using multiple models of injury, the observations made here could lead to rethinking of the role of PLKs and PLK4 from mediators in cellular spindle formation to regulators of myofibroblast differentiation in pathological states. Cardiovascular therapeutics may soon *PLucK* away at vascular fibrosis.

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