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Don't Judge Books by Their Covers: Vascular Smooth Muscle Cells in Arterial Pathologies

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Vascular smooth muscle cells (VSMC) play important physiological and pathophysiological roles. Two articles in the current issue focus on the latter in atherosclerosis[1]and in pulmonary arterial hypertension (PAH)[2]. Notably, Allahverdian et al.[1] report that in human coronary artery atherosclerotic plaques, many cells typically classified as macrophages are likely to be of VSMC origin, and Ricard et al.[2] report that in PAH, VSMC contributing to obstructive remodeling of the distal branches of the pulmonary artery likely arose from pericytes.

Though studies of macrophages in atherosclerosis far outnumber those of VSMC, there is nonetheless a trove of data, including from humans, going back more than 50 years that clearly support an important role for VSMC in both acute and chronic pathologies of the vessel wall (e.g., [3-5]). Of particular relevance to the two articles under discussion is the concept of VSMC phenotypic plasticity, which has support from classic ultrastructural studies [5] and more recent lineage tracing experiments [6]. While the latter studies support the phenomenon of VSMC phenotypic plasticity, they have been limited to experimental animal model systems that may not faithfully recapitulate all aspects of human pathology. Indeed, atherosclerosis in coronary or cerebral arteries is rarely examined in mice, whereas these sites are of great interest in studies of the human disease. Thus, whether *human* VSMC undergo phenotypic conversions in the context of atherosclerosis remains an unanswered question.

Now, as alluded to above, in this issue of *Circulation*, Allahverdian et al provide evidence for the phenotypic conversion of VSMC of the coronary artery to a macrophage-like state during human atherogenesis [1]. Through special fixation of the coronary vessels from

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explanted hearts, the authors were able to preserve intracellular lipid deposition and, by using smooth muscle alpha actin (ACTA2) as a marker of VSMC, show up to 50% of intimal cells being both lipid engorged and ACTA2+. By categorizing lesions as early (AHA Type I or II) or advanced (AHA Type III or IV), Allahverdian et al showed that the level of ABCA1 in ACTA2+ intimal cells was reduced in late lesions, suggesting lower levels of this reverse cholesterol transporter might explain the accumulation of lipid in ACTA2+ intimal cells. Their findings are consistent with our previous demonstration of the acquisition by mouse aortic VSMC of macrophage-like features after they were cholesterol loaded *in vitro* [7].

They went on to use the macrophage marker, CD68, to show that ~18% and 40% of intimal CD68 positive cells were also positive for ACTA2 in early and advanced atheromas, respectively. Finally, the authors discovered that of the CD68+ cells, only ~35% were positive for the leukocyte marker CD45, suggesting that a population of “macrophages” exist in human atheromas that are not of the myeloid lineage. Rather, as the authors conclude, a significant proportion of intimal cells represent once contractile VSMC that underwent phenotypic conversion to a macrophage-like state.

The findings of Allahverdian et al, then, provide yet another piece of evidence to support the phenotypic conversion of VSMC into other cell types. Additional studies with other definitive markers, such as the SMC isoforms of myosin heavy chain and calponin, will be valuable to further strengthen their findings. These are important because of a recent report of ACTA2 staining in activated macrophages of bone marrow [8], which could conceivably seed an evolving atheroma. In addition, it will be critical to perform lineage tracing studies to show more definitively the conversion of a once contractile VSMC into a macrophage-like cell. In this regard, Gomez and colleague have developed an elegant lineage tracing tool by combining in situ hybridization with the proximity ligation assay to track an indelible epigenetic mark (H3K4me2) over the *MYH11* locus in evolving neointimal lesions of human coronary arteries [9]. Another important endeavor will be to further characterize the VSMC-macrophage chimeric cell type using cell sorting and RNA-seq technology. Finally, the mechanism of ABCA1 down-regulation (and that of VSMC differentiation markers) should be explored in human atheromas and other arteriopathies. For example, it would be important to know whether levels of Myocardin, a molecular switch for the VSMC differentiated state [10], changes in human vascular diseases where VSMC undergo phenotypic conversions. Perhaps reduced levels of Myocardin (or its targets, including microRNAs and long noncoding RNAs) sensitize the VSMC for phenotypic conversion. It should also be considered that a macrophage-like cell may not be necessarily be a plaque villain, if it exerts, for example, a high level of efferocytosis, an activity considered to be disease-limiting in mouse models [11]. Ultimately, then, the question will be whether to intervene to thwart or encourage VSMC phenotypic conversions as a means of preventing or reversing advanced atheromatous disease. The work of Allahverdian et al. contributes important information, especially just how prevalent the phenomenon is in human plaques, to take into consideration is answering this question.

Turning to PAH, in Ricard et al. the theme shifts from VSMC assuming characteristics of foam cell macrophages to pericytes in the distal pulmonary artery as a source of VSMC-like cells[2]. The authors studied lung tissues from patients with PAH, and extended their

investigations to a murine retinal angiogenesis model, as well as to pericytes in culture. One striking feature of PAH is the obstructive remodeling of the distal pulmonary arteries, which includes the appearance of cells expressing smooth muscle-restricted markers in normally non-muscular small diameter vessels. This is thought to result from proliferation and migration of pulmonary arterial smooth muscle cells, but also potentially from cellular transdifferentiation[12], further evidence for which is provided in the present article.

Pericytes are the cells mainly on the external surface of small blood vessels, but given their elongated and multibranched morphology, they contact and communicate with endothelial cells (EC). As the authors note, they are well-established regulators of vascular development, stabilization, maturation, and remodeling, through important roles in EC growth and proliferation, as well as in VSMC contraction and blood flow control[2]. The authors now propose that the aforementioned cellular transdifferentiation component that contributes to the expansion of the smooth muscle cell population in the distal pulmonary artery in PAH is the conversion of pericytes to these cells.

There is a strong basis for this proposal. As reviewed in the article, even without invoking a transdifferentiation process, there are close biochemical, morphological, and functional relationships (notably contraction, particularly with the hypoxia present in the distal arterial vasculature in PAH) between pericytes and VSMC (e.g., [13, 14]). Pericytes, however, are typically abundant in the microvasculature, such as in capillaries, with some in arterioles. A key finding making plausible the scenario the authors envision, then, is that there is excessive pericyte coverage in distal arteries in human PAH.

This was accomplished by studying lung specimens from patients with idiopathic (iPAH) and heritable (hPAH) forms of the disease, with control samples of non-diseased regions from lung cancer patients. Using common markers of pericytes (NG2 and 3G5), they found ~2X more pericytes/vessel in PAH patient samples, independent of disease form. Using a mouse model of PAH, they found qualitatively similar results, but with even a more striking increase (up to 6X) in pericyte coverage compared to control vessels. The basis for this was next investigated first by establishing cultures of human pulmonary ECs isolated from patients with iPAH and controls. The conditioned medium from the cells sourced from the iPAH patients significantly stimulated the migration and proliferation of human pulmonary pericytes in vitro. Much of these effects could be attributable to the FGF-2 and IL-6 in the conditioned medium. The effects of FGF-2 and IL-6 on vessel pericyte coverage were extended in vivo with a mouse model of retinal angiogenesis.

As interesting as these results are, there is still the issue of the pericytes acquiring features of VSMC. The authors turned to the role of TGFβ in this process because of the well-known effects of this factor on promoting the contractile state and other characteristics of human VSMC (e.g., [15]) and that its signaling pathway has been shown to be activated in experimental models of PAH (e.g., [16]). Consistent with this, the authors found in their PAH tissue samples increased immunostaining for p-SMAD2, with ~45% of pulmonary pericytes being positive (vs ~6% in control tissues). Furthermore, in cultured pulmonary pericytes, TGFβ increased their expression of classical VSMC markers, *Acta2* and *Cnn1*. To extend these findings in vivo, the pericyte differentiation state in the lung tissues was examined. In

a similar strategy to Allahverdian et al., the authors used an immunostaining approach. In control tissues, pericytes were strongly positive for marker 3G5 and negative for VSMC markers. Many PAH pericytes around remodeled pulmonary arteries, in contrast, exhibited low levels of the marker NG2, but were strongly positive for TAGLN (aka SM22α) and ACTA2. To further support the suggestion that pericytes were the source of the VSMC-like cells in the remodeled arteries, the authors turned to a mouse model expressing an NG2-driven marker protein. After a 21 day exposure to hypoxia (to simulate PAH), by flow cytometry there were ~3X more pulmonary pericytes positive for ACTA2 than in the normoxic samples.

The first major finding of the studies- the increased pericyte coverage of the distal pulmonary artery in PAH- is strongly supported by relatively straightforward histological studies. The mechanisms for this in humans are, by necessity, more indirect. While FGF2 and IL-6 secreted by EC promoted pericyte proliferation and migration in vitro, in vivo there may be additional factors in play, especially PDGF signaling, which, as noted by the authors, has been implicated in pericyte recruitment and PAH pathogenesis. Another possibility raised is differentiation into pericytes of bone marrow-derived or tissue resident progenitor cells. Turning to the second major finding- that VSMC-like cells in PAH are derived from pericytes- there are also a number of important questions. TGFβ is a well known promoter of the VSMC phenotype, but its source and the molecular mechanism of the increased signaling pathway in PAH remains to be established. Further, there may be other VSMC-promoting factors to consider (e.g., NOTCH). The contribution to the expanded VSMC population of EC transitioning into mesenchymal cells expressing ACTA2 in PAH[17, 18] also needs to be determined.

Nevertheless, even with their limitations, these two reports are fascinating to consider in terms of cell plasticity. VSMC in atheroma are traveling, if not already there, to a macrophage-like state, and in PAH, the VSMC-like cells have pericytes in their rear view mirror. It is likely that similar phenomena occur as part of both normal development as well as in other pathologies. Further studies to establish this and the molecular mechanisms involved will not only illuminate fundamental biological processes, but ultimately may open up new therapeutic approaches.

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