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Title: Progenitor cells, bone-marrow-derived fibrocytes and endothelial-mesenchymal transition - new players in vascular fibrosis

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Progenitor cells, bone-marrow-derived fibrocytes and endothelial-mesenchymal transition - new players in vascular fibrosis

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Tissue fibrosis, defined as an excessive accumulation of extracellular matrix components leading to the destruction of organ architecture and impaired function, affects virtually every tissue and organ in the body, including the arteries. Vascular fibrosis of small and large arteries contributes to arterial remodeling, important in the development and complications of hypertension (1). Fibrogenesis is an active process that involves accumulation of structural proteins (collagen and fibronectin) and adhesion proteins (laminin and fibronectin), expression of adhesion molecules and integrins, and remodeling of the extracellular matrix (2,3). Healthy arteries are surrounded by perivascular adventitial tissue comprising collagens I and III in the intima, media and adventitia, with collagen types I, III, IV and V in the endothelial and vascular smooth muscle cell basement membranes (4). These fibrillar proteins maintain vascular integrity and normal vascular tone and function. In hypertension, accumulation of collagen and fibronectin and extracellular matrix reorganization lead to increased stiffness of the vessel wall (5,6). Initially these processes are adaptive and reversible and may compensate for higher blood pressures, but with time and progressive increases in blood pressure, this becomes maladaptive and decompensated, leading to arterial stiffness that contributes to hypertension-associated target organ damage. These events have been demonstrated in many experimental models of hypertension and in hypertensive patients and have been attributed to activation of ERK1/2, p38MAPK, TGF β , SMAD pathways, oxidative stress and dysregulation of matrix metalloproteinases (MMPs) (7). Decreased activation of MMPs and increased activity of TIMPs leads to reduced collagen turnover and consequent accumulation, with thickening and remodeling of the vascular wall.

Vascular fibrosis is a dynamic and active phenomenon, where a pro-

inflammatory, oxidative milieu, triggered by pro-hypertensive stimuli, lays the foundation for fibrosis and activation of extracellular matrix-producing cells. Until recently the process seemed fairly simple where adventitial fibroblasts and myofibroblasts were considered the major collagen-producing cells in the vascular wall (8,9). What is becoming increasingly evident is that a whole array of cells have potential to produce ECM proteins. In fact, myofibroblasts are differentiated from various precursors including adventitial fibroblasts, pericytes, phenotypic transition of endothelial cells, phenotypic modulation of vascular smooth muscle cells and recruitment of circulating multipotent monocytes and fibrocytes (10). However, the scenario continues to become more complex, as demonstrated by Wu et al (11) who demonstrate that three previously unidentified cell types, including Sca-1+ progenitor cells, bone marrow-derived infiltrating fibrocytes and cells of endothelial origin (endothelial-to-mesenchymal transition) are major vascular-fibrosing cells in hypertension. These findings underscore the complexity of fibrogenesis and highlight the heterogeneous cell pool that contributes to ECM production, vascular fibrosis and arterial stiffening.

Moreover, and rather intriguingly, it seems that pre-differentiated resident fibroblasts represent only a minor fraction of ECM-producing cells in hypertension, with Sca-1+ cells and bone marrow-derived cells accounting for over 50% of aortic collagen-producing cells and cells of endothelial origin contributing around 25% (11). These findings further indicate that not only are there multiple types of ECM proteins (elastin, fibrin, fibronectin) and collagens (I, III, IV) that contribute to fibrosis (12) but that the process is highly regulated and involves transformation, recruitment and activation of different types of collagen-producing cells.

Stem cell antigen-1 (Sca-1) is an 18 kDa mouse glycosyl phosphatidylinositol-anchored cell surface protein of the Ly6 gene family (13). It was originally identified as an antigen that was upregulated on activated lymphocytes and is commonly used as a marker of hematopoietic stem cells. Beyond the hematopoietic system, Sca-1 is expressed in a mixture of stem, progenitor and differentiated cell types, in various tissues and organs including the heart and vessels (14). Sca-1⁺ adventitial cells are embryonic hematopoietic cells and have the capacity to differentiate into various vascular cell types, including vascular smooth muscle cells (13,14). In the heart, resident Sca-1⁺ cells may play a regenerative role post myocardial infarction and in the vascular wall (15), resident Sca-1⁺ cells have been implicated in remodeling associated with arteriosclerosis (16). Resident vascular adventitial macrophage Sca-1⁺ progenitor cells are abundant in atherosclerotic lesions in hyperlipidemic ApoE(-/-) and LDL-R(-/-) mice (17). Taken together these data suggest that Sca-1⁺ cells play a role in atherogenesis. Extending these findings, Wu et al (11) show that resident vascular Sca-1⁺ cells also contribute to vascular fibrosis in Ang II-induced hypertension. Factors that trigger such events have not been clearly elucidated, but the fact that Sca-1⁺ cells were found to be a major source of collagen in aortic fibrosis in hypertension indicates that highly regulated systems must be involved. While these intriguing findings highlight a key role for embryonic Sca-1⁺ hematopoietic-derived cells in vascular fibrosis in a mouse model of hypertension, the clinical relevance remains unclear, because Sca-1⁺ antigen is absent in humans (14).

Not only were resident Sca-1⁺ progenitor cells identified to be a significant source of collagen in vascular fibrosis (11), but bone marrow-derived circulating fibrocytes were found to be especially important and to constitute the majority of cell types responsible for ECM production in the aorta in Ang II-induced hypertension.

This is not a new finding, since others have demonstrated that bone marrow-derived circulating progenitor cells are recruited to sites of vascular injury and to assume endothelial, smooth muscle-like and fibroblast-like phenotypes (18). Fibrocytes, which express leukocyte antigen CD45, produce ECM components and ECM-modifying enzymes, such as MMPs, and can differentiate into myofibroblasts and play a role in vascular remodeling and fibrosis in pulmonary hypertension. In Ang II-induced hypertension, fibrocytes seem to be especially important in vascular fibrosis, since Wu et al found that the majority of collagen I-producing cells of the aorta are CD45+Col I+ bone marrow-derived fibrocytes (11). What still needs to be identified are the specific factors involved in the recruitment and retention of these cells, although an underlying pro-inflammatory environment could be important because chemokines and cytokines seem to attract circulating fibrocytes to the vascular wall (11).

Wu et al (11) described a third novel mechanism contributing to aortic fibrosis in Ang II-induced hypertension, involving endothelial-to-mesenchymal transition (EMT), a process whereby differentiated endothelial cells undergo a phenotypic conversion to matrix-producing fibroblasts and myofibroblasts. EMT is usually preceded by and closely associated with inflammation and may be an adaptive response to endothelial injury (19). Underlying vascular inflammation in hypertension may stimulate signaling pathways such as TGFb/Smad, integrin-linked kinase (ILK) and Wnt/b-catenin, which are critically involved in the process of EMT (20). Interestingly, many of these signaling molecules are themselves implicated in the production of ECM proteins. Hence molecular mechanisms promoting EMT are similar to those that drive fibrosis, and as such may be putative targets of antifibrotic therapy.

While a new paradigm in aortic fibrosis in hypertension has been defined (11),

there are a number of questions that still need to be answered. Firstly, do similar cellular populations participate in vascular fibrosis of small arteries, the vessels that contribute to increased resistance and blood pressure elevation? Secondly, do Sca-1+ cells, bone marrow-derived fibrocytes and ETM cells in the vascular wall produce different types of ECM proteins and collagens? Thirdly, do the cell types have a distinct localization in the aorta and are they activated at different time points during fibrogenesis? Fourthly, what triggers the activation of these apparently unrelated cells to become pro-fibrogenic and finally what are the signaling pathways and mechanisms that regulate these different cell types to produce collagen and other ECM in a regulated and organized manner in the vascular wall?

Our previous notion that adventitial resident fibroblasts are the cellular origin and backbone of vascular fibrosis clearly needs to change and based on the findings of Wu et al (11), we need to now think of fibrosis as a complex multi-cellular phenomenon where recruitment, differentiation and transformation of various cell types define the ECM in hypertension. Moreover, from a therapeutic viewpoint, this new paradigm highlights the potential importance to target multiple cell types and different systems in the prevention of vascular fibrosis and aortic stiffening in hypertension.

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Figure legend

Diagram demonstrating a role for multiple cell types in vascular fibrosis in hypertension. Pro-hypertensive factors, such as angiotensin II (Ang II) and proinflammatory stimuli, stimulate activation of bone marrow-derived fibrocytes, Sca-1+ vascular cells, resident fibroblasts and myofibroblasts as well as endothelial-to-mesenchymal transition (EMT). Inflammation in particular stimulates EMT. Activation of these cells is associated with stimulation of pro-fibrotic signaling pathways, leading to production of extracellular matrix (ECM) proteins, such as collagens and fibronectin. These processes lead to vascular fibrosis and arterial stiffening in hypertension. ILK, integrin-linked kinase; MAPK, mitogen-activated protein kinases; ROS, reactive oxygen species. The study of Wu et al (11) suggests that the major cell types contributing to vascular fibrosis in Ang II-induced hypertension involve bone marrow-derived fibrocytes and Sca-1+ cells.

