The pathophysiological basis of vascular disease

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The dynamic nature of the vascular wall

Cardiovascular disease (CVD) remains the leading global cause of death and in 2017 an estimated 17.8 million deaths globally were attributed to CVD, and the number continues to grow [1]. Vascular diseases are the dominant component of CVD and adaptive and maladaptive remodeling of the vascular wall represent key processes in vascular diseases and are the major focus of therapeutic interventions. Vascular remodeling is an active process that leads to structural changes and requires cell growth, cell death, and cell migration, as well as degradation and reorganization of the extracellular matrix scaffold (ECM) in the vessel wall [2]. This adaptive process occurs in physiological conditions to maintain blood pressure hemostasis; however, in pathological conditions, such as hypertension, restenosis, and atherosclerosis, these adaptive changes instead initiate pathological vascular alterations [3, 4]. The cellular processes that contribute to the vascular remodeling that occurs in atherosclerosis and other CVD pathologies can be induced by inflammatory cytokines, hemodynamic stimuli, growth factors, and vasoactive hormones.

There are multiple cell types within the vessel wall that play a role in the vascular remodeling process: (1) endothelial cells (ECs), which comprise the innermost cell monolayer of the vessel and are in direct contact with blood flow, (2) vascular smooth muscle cells (VSMCs), which form the tunica media, provide structure, and regulate arterial vascular tone, and (3) fibroblasts and inflammatory cells, such as macrophages, are found in the adventitia layer, which functions to provide structural support to the blood vessel and is paramount to the outside-in inflammatory response to vascular injury that contributes to vessel remodeling [5]. It is now well understood that all of these cell types and layers of the vascular wall play a role in the vascular growth and repair adaptive processes. Within this editorial, we discuss the major contributions of each of these cell types on the vessel remodeling process and will emphasize recent progress in the field, highlighting the new findings published within this special issue on vascular biology.

Endothelial cells

The endothelial cell monolayer makes up the innermost cell layer of the artery and these arterial ECs are exposed to cyclical, perpetual wall shear stress as the direct result of blood flow across the ECs during the normal cardiac cycle. ECs are able to sense changes in shear stress through specialized mechanosensors that translate flow patterns into intracellular signals and this has been reviewed previously [6, 7]. Atherosclerosis is a primary underlying cause of CVD and is a lipid and chronic inflammation driven disease [8], and it is now well accepted that local hemodynamic forces regulate site-specific atherosclerotic plaque formation. Atherosclerotic plaques primarily form at arterial sites exposed to disturbed or oscillatory shear stress, such as the outer walls of blood vessel bifurcations, the inner curvatures of vessels, and downstream of stenoses [9].

ECs in the regions of oscillatory flow have increased expression and activation of transcription factors that regulate proliferation, inflammatory protein expression, and reactive oxygen species (ROS) production, including nuclear factor-κB (NF-κB), and activator protein (AP-1) [10]. The recent paper by Wang Y, et al. identifies a shear-sensitive transcription factor, ZBTB46, expressed in ECs exposed to laminar shear stress that is downregulated in

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ECs in response to the disturbed flow in vitro and in vivo in the partial carotid ligation model of accelerated atherosclerosis [11]. Interestingly, they also show that ZBTB46 functions to block EC proliferation by suppressing cell cycle promoters like the cyclins (CCN A2, B1, D1, and E2), cyclin-dependent kinases (CDKs 1, 2, 4, and 6), and phospho-retinoblastoma (pRb), while also promoting increased expression of the cell cycle inhibitor p21 [11]. Altogether, these data suggest that ZBTB46 may be a key regulator of shear-mediated EC proliferation and atherosclerotic plaque formation [11].

High blood pressure, or hypertension, fundamentally occurs when the heart must pump against higher peripheral resistance, which occurs due to structural and functional changes in large conductive and small resistance arteries. Clinical cases of hypertension often present with enhanced sympathetic nerve activation [12], resulting in increased vasoconstriction and reduced vasodilation. There are a number of vasoactive molecules that also play a role in the maintenance of a balance between vasoconstriction and vasorelaxation. Nitric oxide (NO$^{\bullet}$) is one of these molecules and is released by ECs to promote vasodilation via signaling to VSMCs. Laminar shear stress promotes NO$^{\bullet}$ production through effects on both endothelial NO$^{\bullet}$ synthase (eNOS) expression and activity through Krüppel-like factor 2 (Klf-2) and NF-κB [13, 14]. This increased NO$^{\bullet}$ production, in turn, inhibits further flow-induced NF-κB activation [14]. It is well known that chronic consumption of high-fat meals contributes to obesity, atherosclerosis, and endothelial dysfunction. Interestingly, Benson et al. demonstrate in their manuscript that a single high-fat meal in humans is enough to increase circulating triglyceride, cholesterol, free fatty acid levels, and circulating myeloperoxidase (MPO) activity and that these factors altogether contribute to impaired endothelial-dependent flow-mediated dilation [15]. It is known that MPO is able to reduce the bioavailability of NO$^{\bullet}$, thus inhibiting the vasodilatory functions of NO$^{\bullet}$. Interestingly, these new findings show that RBCs are one source of MPO and that free fatty acids induce MPO release from cells [15], which may ultimately contribute to endothelial-dysfunction and atherosclerosis.

Vascular smooth muscle cells in vessel remodeling

A primary vasoactive molecule that functions to regulate blood pressure through promotion of vasoconstriction is angiotensin II (Ang II). Numerous drugs developed to treat hypertension specifically target the renin-angiotensin-aldosterone system (RAAS), including angiotensin-converting enzyme (ACE) inhibitors designed to block Ang II production. Clinical studies have shown that ACE

inhibitors are beneficial in reversing conductive vascular remodeling caused by hypertension and decreasing arterial stiffness [16, 17]. In a seminal paper published in 1994 by Griendling et al. it was shown that Ang II stimulation of VSMCs increases NADPH oxidase (Nox) activity and reactive oxygen species (ROS) production [18], more specifically production of superoxide, which can be spontaneously or catalytically converted to the more stable and freely diffusible hydrogen peroxide [19]. This finding opened up an entire field of exploration into the role of reactive oxygen species and vascular health and disease [20, 21]. It has since been demonstrated that virtually all cells in the vessel wall produce ROS in varying amounts and in response to various stimuli [22, 23]. ROS are essential to the normal function of the vessel wall (NO$^{\bullet}$ for example), but a number of diseases are the result of an imbalance between the generation and scavenging of ROS, resulting in ROS levels in excess of endogenous antioxidant systems that contribute to a number of vascular pathologies [24]. Indeed, we now understand that superoxide generated by ECs, VSMCs, and macrophages can react with NO$^{\bullet}$ to generate the free radical peroxynitrite (ONOO$^{\bullet}$-), which functionally decreases the bioavailability of NO$^{\bullet}$ and thus impairs vascular relaxation and vasodilation to promote vascular pathophysiology [25]. In pathologies such as hypertension, ROS also function as mediators of VSMC growth, which contributes to vascular remodeling.

The progression of hypertension involves two different types of vascular remodeling: inward eutrophic remodeling and hypertrophic remodeling [26]. Both forms of remodeling frequently coexist in different vascular beds. Eutrophic remodeling is characterized by a reduction in the vessel and thickening of the media, thus leading to increased media:lumen ratio, but usually shows little change in medial cross-sectional area. Hypertrophic remodeling is characterized by an increase in wall cross-sectional area and involves both an increase in VSMC size and enhanced accumulation of ECM proteins, such as collagen and fibronectin [27, 28]. The reorganization of the media that occurs in both types of remodeling (eutrophic and hypertrophic) is mediated by phenotype modulation of VSMCs, migration, cellular growth, apoptosis, and ECM production and rearrangement [25]. One of the consequences of hypertension is the induction of the expression of inflammation, fibrosis, and hypertrophy related genes in VSMCs.

VSMC remodeling in conductive and resistance arteries has recently been reviewed in detail [2], but we briefly discuss some of the contributing factors below. IκBα (inhibitor of kappa B) functions to inhibit NF-κB activation. Inhibitor kappa B kinase 2, or IKK2, functions by phosphorylating IκBα, which subsequently leads to IκBα ubiquitination and proteosomal degradation, allowing for NF-κB activation [29]. An interesting paradigm shift was recently proposed,

which is that IKK2 can also serve as a myosin light chain kinase (MLCK) to promote phosphorylation of myosin light chain and initiation of VSMC contraction [30, 31]. However, it was unclear if IKK2 actually served as a MLCK or functioned to regulate myosin light chain phosphatase (MLCP) activity. Kwon et al. utilized rat mesenteric arteries and pressure myograph studies to demonstrate that IKK2 promotes vessel contractility by phosphorylating the myosin phosphatase target subunit MYPT1 and thus activating MLCP, suggesting that IKK2 plays a role in VSMC contractility that may be independent of NF-κB regulation [32].

Ang II-induced hypertension increases activity of the NF-κB, AP-1, and CRE-binding protein (CREB) transcription factors in VSMCs leading to the upregulation of monocyte chemoattractant protein 1 (MCP-1), transforming growth factor β (TGFβ), osteopontin (OPN), fibronectin (FN1), and collagens (COL). The actin cytoskeleton participates in cellular responses mediated by Ang II and mechanotransduction [33, 34]. More specifically, the actin-binding protein cofilin and its upstream activator slingshot phosphatase (SSH1) serve as key regulators of actin dynamics [35, 36]. SSH1 expression is upregulated during neo-intimal formation and has been linked to inflammatory signaling. The manuscript by Williams et al. utilized a novel SSH1$^{-/-}$ mouse to determine its importance in Ang II mediated vascular remodeling [37]. SSH1$^{-/-}$ animal characterization showed no baseline differences in aortic contractility, mean blood pressure, or aortic structure [37]. Interestingly, the loss of SSH1 exacerbated baseline and AngII-induced increases in fibronectin deposition and osteopontin expression, as well as TGFβ1-induced expression of OPN. However, no differences in Ang II-mediated TGFβ or collagen production were detected between SSH1$^{-/-}$ and control animals [37], suggesting that the actin regulator SSH1 functions to block increases in expression of specific fibrotic genes typically induced by Ang II in hypertension.

During remodeling, VSMCs undergo a process known as de-differentiation prior to migration and proliferation. One setting in which this commonly occurs is in neo-intimal hyperplasia. The de-differentiation of VSMCs from a contractile phenotype to a more proliferative and/or migratory phenotype typically precedes neo-intimal formation. Of note, ROS play a clear role in the regulation of VSMC phenotypic switching, which was comprehensively reviewed by Durgin et al. in "Redox control of vascular smooth muscle cell function and plasticity", in which the authors discuss the current understanding of the role of vascular Noxes in VSMC differentiation state [38]. In a study by Datla et al., they investigated the role of Poldip2, a multi-function protein with numerous roles in the vasculature, in the neo-intimal formation process [39]. They first establish in stage II human atherosclerotic plaques that

Poldip2 is highly expressed in the endothelium and in the underlying VSMCs, where Poldip2 co-localized with proliferating cell nuclear antigen (PCNA) [39]. Furthermore, they clearly show in a femoral-artery wire injury model that the lack of Poldip2 (heterozygous mice) decreases neo-intimal formation and ROS production in vivo and VSMC proliferation in vitro and in vivo, potentially via increased expression of the cell cycle inhibitor p21 [39]. One proposed mechanism by Datla et al. is the regulation of hydrogen peroxide production from Nox4 by Poldip2, since it was previously shown that Poldip2 increases Nox4 activity and ROS generation [40] and VSMC migration [41]. However, it is likely that other mechanisms are a play, since Poldip2 has also been linked to other functions [42]. Increased VSMC proliferation and migration are also required for arterial remodeling in the pulmonary setting. Dipeptidyl peptidase IV (DPP-4), also known as CD26, is a 110 kDa type II transmembrane glycoprotein expressed by multiple cell types including VSMCs and ECs and DPP-4 inhibitors have been shown to have a plurality of beneficial effects, including suppression of VSMC proliferation and neo-intimal formation [43, 44]. Xu et al. investigated in their recent manuscript if DPP-4 inhibition would also have beneficial effects with respect to pulmonary artery remodeling [45]. Using an experimental model of pulmonary hypertension, they show that inhibition of DPP-4 with Sitagliptin attenuates right ventricular systolic pressure, right ventricle remodeling, hypertrophy of the medial layer in pulmonary arteries, and decreased inflammatory cell infiltration in vivo [45]. Additionally, they show that DPP4 inhibition blocks PDGF-mediated proliferation and migration in pulmonary VSMCs [45].

Excessive ROS production is also a primary contributor to diabetic vascular complications, including nephropathy and atherosclerosis, in part through chronic activation of the Janus kinase/Signal transducer and activator of transcription (JAK/STAT) signaling pathway. Unresolved activation of the JAK/STAT pathway increases expression of genes involved in cell proliferation, fibrosis, inflammation, and oxidative stress. Suppressors of cytokine signaling, known as the SOCS family of proteins, are endogenous regulators of JAK/STAT and are potential points for therapeutic intervention. Lopez-Sanz et al. investigated if SOCS-1 targeted therapies have beneficial effects in an STZ-induced murine model of diabetes [46]. They found that the use of adenovirus to overexpress SOCS-1 or a peptidomimetic was able to significantly lower superoxide production levels, DNA oxidation, and Nox subunit expression in vivo and in vitro [46]. Additional studies showed that SOCS-1 blocked high-glucose-mediated Nox complex assembly and Nox subunit expression [46]. SOCS-1 treatment also increased antioxidant enzyme expression, which correlated with a reduction in renal damage, atherosclerosis, and

inflammation in vivo, suggesting that SOCS-1-targeted therapies may be an alternative strategy to limit oxidative stress and the progression of diabetic micro- and macro-vascular complications [46].

Increased intrahepatic vascular resistance is thought to contribute to the progression of non-alcoholic fatty liver disease or steatosis and portal hypertension seems to pre-cede increases in inflammation and fibrosis in this pathol-ogy. However, the exact mechanisms underlying vascular dysfunction in non-alcoholic fatty liver disease remain unknown. In the study from Van der Graaff et al. we learn that mean arterial blood pressure is lower while portal blood pressure is higher in a rat model of steatosis, and that maximal contraction force in aortic rings from these animals is also markedly reduced [47]. The authors ultimately demonstrate that, in contrast to liver cirrhosis, arterial hypo-reactivity in steatosis is NO•-independent and, instead, appears to be mediated by cyclooxygenase 2 (COX-2)-related mechanisms, whereas the increased intrahepatic vascular resistance appears to be related to hyper-reactivity to vasoconstrictive mediators [47]. However, it what vaso-constrictive mediators are at play in this setting remain to be established.

In addition to ROS, Notch signaling has been implicated in neo-intimal formation after vascular injury and Notch signaling is implicated in pro-inflammatory gene expression and is linked to atherosclerotic disease progression. How-ever, Notch signaling in VSMCs during atherosclerosis development is not well established. Therefore, Davis-Knowlton et al. set out to characterize VSMCs from human atherosclerotic lesions, to examine Notch expression levels, and to quantify cellular responses to Notch signaling [48]. Specifically, they found that medial and intimal VSMCs and plaque-derived VSMCs all express Notch2 and Notch3, whereas ECs primarily express Notch1 [48]. The Liaw group previously established that Notch signaling stimu-lated by Jagged1 in healthy VSMCs induces differentiation/contractile marker expression via both Notch2 and Notch3 and that Notch2 signaling suppressed VSMC proliferation [49]. In this recent study, they demonstrate that while Jagged1 stimulation of healthy VSMCs increased levels of smooth muscle α-actin, this effect is lost in endartectomy-derived VSMCs [48], the reasons for which remain to be investigated.

While many of the remodeling effects described above have been in the pathophysiologic setting, many of these same remodeling effects are also necessary for physiologic processes. The formation of collateral blood vessels is one example in which vascular remodeling is a good thing. Vessel occlusions caused by atherosclerosis, such as those that occur in peripheral artery disease, result in a loss of oxygen to the downstream tissues, also known as ischemia. The body's endogenous response to ischemia is to develop collateral blood vessels to bypass these sites of obstruction, restore blood flow and preserve tissue function [50]. Importantly, a patient's ability to form functional collateral vessels improves outcomes and survival [51]. Collateral vessel formation requires many of the same remodeling mechanisms described above working in concert [52–54], including cytokine signaling, ECM remodeling, and cell proliferation, and migration [55, 56]. Often patients with other co-morbidities, such as diabetes, do not effectively form collateral blood vessels [57]. Therefore, understanding the biologic mechanisms that promote functional collateral vessel formation to restore blood flow may help identify novel therapeutic targets for non-invasive interventions. OPN, a secreted glyco-phospho-protein, was previously shown to be highly upregulated in response to ischemia in a ROS-dependent manner and was established as a critical mediator of post-ischemic collateral formation [58]. How-ever, it was recently established that humans express three OPN isoforms as a result of alternative mRNA splicing to generate: OPNa, OPNb, and OPNc [59–61] and how each of these isoforms affect collateral formation is investigated in the manuscript by Lee et al. [62]. Utilizing a loss-of-func-tion, gain-of-function in vivo approach, they demonstrate that OPN isoforms differentially affect limb perfusion and functional limb use, where OPNa and OPNc significantly improve both of these endpoints [62]. Furthermore, they go on to show that the OPNa- and OPNc-mediated increases in perfusion are primarily driven by increases in arteriogenesis via pro-migration and pro-survival effects on macrophages [62].

Vascular calcification contributes to decreases in vessel wall elasticity and is a highly regulated biological process involving the downregulation of VSMC contractile pro-teins, including SMA and SM22, and the upregulation of bone-related proteins like Runx2 and BMP2 [63]. Hyalur-onan (HA) is an ECM structural protein that has been shown inhibit osteoblast differentiation. Kong et al. inves-tigated if HA affects osteogenic differentiation and calcifi-cation of VSMCs and found that low and high molecular weight HA treatment significantly reduced VSMC calcifi-cation in a dose-dependent manner [64]. HA treatment decreased alkaline phosphatase activity, decreased expres-sion of Runx2 and BMP2, and blocked BMP2 signaling, while overexpression of the HA synthase, HAS2, also inhibited calcification [64]. In contrast, inhibition of HA synthesis or knockdown of HAS2 increased calcification [64].

Conclusion

This special issue of *Laboratory Investigation* includes a spectrum of papers that address some of the fundamental

issues in cardiovascular biology. One common theme for many of the manuscripts is the contribution of inflammation and repair to cardiovascular pathology. Indeed, many aspects of cardiovascular pathology reflect a maladaptive inflammatory process that is critical in the development and repair of the cardiovascular system. Evolution has selected for highly efficient developmental and repair mechanisms involving production of reactive oxygen species, calcification, recruitment of inflammatory cells, etc. that lead to adverse outcomes in the disease state. As a result, a better understanding of these fundamental mechanisms provides us with a broader understanding of the cardiovascular system throughout the life cycle.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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