Blood vessels are continuously subjected to dynamic mechanical forces, including stretch and shear stress that increase with elevated blood flow, pressure, or viscosity. Under physiological conditions, eg, during vascular development, mechanical stress in the arterial wall regulates critical parameters of vascular function and thus plays a crucial role in keeping the balance between blood supply and tissue oxygen demand. Several protective mechanisms have evolved to maintain the physiological integrity and structure of vessels, such as adaptive changes of the vasomotor tone. In contrast to these physiological processes, sustained or chronic alterations in blood pressure and/or flow frequently lead to irreversible phenotypic changes of the vascular wall and finally vascular remodeling.\(^1,^2\)

The process of vascular remodeling is characterized by increased vascular smooth muscle cell (VSMC) growth, migration, and extracellular matrix deposition, as well as altered secretion of fibrotic growth factors by endothelial cells (EC).\(^2,^3\) Ultimately, these mechanisms are responsible for impaired vessel relaxation, sustained vasoconstriction, and vascular inflammation in diseases such as atherosclerosis and coronary artery disease. Taken together, these phenomena present as key contributors of decreased lumen size, increased vessel resistance, and account for the process of vascular remodeling.

Although the structural changes that are induced by mechanical forces have been elucidated nicely in the past, particular attention has now focused on the plethora of signaling cascades that initiate and perpetuate the vascular remodeling process in response to strain. Cascades that have been investigated recently include reactive oxygen species (ROS),\(^4\) nitric oxide (NO),\(^6\) nuclear factor \(\kappa\)B (NF-\(\kappa\)B),\(^7,^8\) epidermal growth factor receptor (EGFR),\(^9\) the mitogen-activated protein (MAP) kinase,\(^10\) and protein kinase C (PKC)\(^11\) pathways, as well as the formation of focal adhesions that are comprised of integrins bound to intracellular scaffold and signaling proteins.\(^12\) GTPases, Cdc42, and RhoA are also mediators of shear stress–induced cellular alterations.\(^13\)

In this issue of Circulation Research, Lemarie et al offer novel insight into the signaling pathways that determine the vascular response to mechanical strain.\(^14\) The authors report that ROS-dependent transforming growth factor (TGF)-\(\alpha\) activation, which signals via EGFR, directly activates the transcription factor NF-\(\kappa\)B under conditions of high pressure. Although NF-\(\kappa\)B has been shown to be a pivotal regulator of VSMC survival and proliferation under normal or high stretch/pressure conditions, the main focus of this contribution was the molecular mechanism triggering NF-\(\kappa\)B activation in response to mechanical strain.

The authors demonstrate increased superoxide production in mouse carotid arteries that were maintained at high intraluminal pressures (150 mm Hg), compared with arteries maintained at normal intraluminal pressure (80 mm Hg). This effect was abolished after treatment with the NADPH oxidase inhibitor apocynin, indicating that strain-induced ROS production was dependent on NADPH oxidases. Similar effects were observed using the antioxidant flavin inhibitor DPI. Decreased levels of the endogenous NF-\(\kappa\)B inhibitor IK-B\(\alpha\), as well as increased phosphorylation of the NF-\(\kappa\)B subunit p65, were documented after treatment with both inhibitors, suggesting ROS-dependent NF-\(\kappa\)B activation.

It was reported previously that increased ROS generation can induce intracellular signaling cascades via EGFR activation.\(^15\) Here, Lemarie et al present additional evidence in that ROS act upstream of high pressure–dependent EGFR phosphorylation, because the NADPH oxidase inhibitor apocynin inhibited EGFR phosphorylation. Similarly, an EGFR kinase inhibitor abolished EGFR phosphorylation but did not affect high-pressure–induced ROS production. High pressure failed to activate NF-\(\kappa\)B in vessels obtained from Wa2 mice, which carry an inactivating mutation in the EGFR kinase domain, or from mice treated with an EGFR kinase inhibitor. Thus, it is suggested for the first time that TGF-\(\alpha\)-induced EGFR activation is implicated in NF-\(\kappa\)B activation under high-pressure conditions.

The next question that the authors addressed in this study was whether angiotensin II and its AT\(_1\) receptor isotype, which are activated at high blood pressure, transactivated EGFR via ROS and thus induced NF-\(\kappa\)B activation. It was shown, however, that high intraluminal pressure activated NF-\(\kappa\)B even in the presence of an AT\(_1\) antagonist, suggesting angiotensin II-independent pathway. To further elucidate the ligands of strain-activated EGFR, the authors therefore turned their attention to the EGFR ligands TGF-\(\alpha\), heparin-binding epidermal growth factor (HB-EGF), or epiregulin.\(^16,^17\) After confirming the localization of these ligands in the vessel wall under normal and high pressures, Lemarie et al used 3 animal models to investigate the functional relevance of these ligands: TGF-\(\alpha\) mutant mice (Wa1), as well as HB-EGF knockout and epiregulin-deficient mice. It turned
out that in vessels of Wal mice, high pressures failed to activate NF-κB, whereas this response was retained in wild-type, HB-EGF, or epiregulin-deficient mice. These results clearly demonstrated that high pressure induced a TGF-α–dependent EGFR and NF-κB activation.

TGF-α is usually found as a precursor molecule that is anchored to the plasma membrane. The action of proteases, such as ADAM17 (TACE), is required for its activation and thus biological activity. TGF-α shows sequence homology with EGF and both ligands can compete for binding to EGFR, thereby stimulating similar intracellular signaling cascades that include the Akt and MAP kinase pathways. Interestingly, treatment of strained arteries with a matrix metalloproteinase (MMP) inhibitor prevented the strain-dependent release of TGF-α, phosphorylation of EGFR, and subsequent activation of NF-κB in the study by Lemarie et al. Taken together, the model proposed as a result of these studies suggests that increased vascular strain/pressure leads to an initial ROS production/release, which induces MMP-dependent TGF-α release that finally interacts with EGFR and activates NF-κB–dependent signaling, resulting in vascular remodeling.

This is an interesting and promising concept that is supported by several findings in the literature, albeit in different biological systems and signaling cascades. In particular, it is known that ROS can induce the release of active HB-EGF via an MMP-mediated mechanism, resulting in EGFR-dependent Akt activation and enhanced cell proliferation. Furthermore, Shao and Nadel have recently reported that ROS-dependent TGF-α release and EGFR activation can play a critical role in inflammatory airway diseases. More specifically, human neutrophil elastase can induce ROS production via a PKC-dependent pathway in human airway epithelial cells. Likewise, ROS can activate TACE and mediate TGF-α release, resulting in EGFR phosphorylation and the enhanced production of mucins.

A logical follow-up question arising as a result of the studies performed by Lemarie et al addresses the source and potential pharmacological modification of ROS generation in the vasculature, particularly in the VSMC. It has been shown that ROS are abundantly produced in the vasculature as a result of NADPH oxidase activity. Several growth factors and cytokines, such as angiotensin II, stimulate NADPH-dependent ROS production. More information, however, is now required with respect to the distinct mechanisms involved in mechanical stress-dependent NADPH oxidase activation, as well as the NADPH oxidase isoforms involved in this response.

In conclusion, Lemarie et al offer a new mechanism for vascular NF-κB activation in response to mechanical forces by demonstrating that normally inactive TGF-α is activated by ROS-dependent MMP activity and responsible for EGFR-dependent NF-κB activation. This process involves IKK degradation, p65 phosphorylation, and finally VSMC proliferation and vascular remodeling. The current study thus provides novel mechanistic insight into high pressure–dependent NF-κB activation by TGF-α and offers the exciting opportunity to specifically interfere with strain-induced vascular remodeling by modifying TGF-α activation.

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References


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