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Direct Role for Smooth Muscle Cell Mineralocorticoid Receptors in Vascular Remodeling: Novel Mechanisms and Clinical Implications

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Abstract

The mineralocorticoid receptor (MR) is a key regulator of blood pressure. MR-antagonist drugs are used to treat hypertension and heart failure, resulting in decreased mortality by mechanisms that are not completely understood. In addition to the kidney, MR is also expressed in the smooth muscle cells (SMCs) of the vasculature, where it is activated by the hormone aldosterone and affects the expression of genes involved in vascular function at the cellular and systemic levels. Following vascular injury due to mechanical or physiological stresses, vessels undergo remodeling resulting in SMC hypertrophy, migration, and proliferation, as well as vessel fibrosis. Exuberant vascular remodeling is associated with poor outcomes in cardiovascular patients. This review compiles recent findings on the specific role of SMC-MR in the vascular remodeling process. The development and characterization of a SMC-specific MR-knockout mouse has demonstrated a direct role for SMC-MR in vascular remodeling. Additionally, several novel mechanisms contributing to SMC-MR-mediated vascular remodeling have been identified and are reviewed here, including Rho-kinase signaling, placental growth factor signaling through vascular endothelial growth factor type 1 receptor, and galectin signaling.

Keywords

Mineralocorticoid receptor; Aldosterone; Vascular injury; Vascular remodeling; Smooth muscle cells

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Conflict of Interest
Jenny B. Koenig and Iris Z. Jaffe declare that they have no conflict of interest.

Compliance with Ethics Guidelines
Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction

In addition to the critical role of the vasculature as a conduit to deliver oxygen and nutrients, blood vessels also regulate regional blood flow, modulate vascular tone and systemic blood pressure, and function as a barrier to prevent thrombosis and infection. The vessel includes two communicating layers: the inner lining of endothelial cells that are in contact with circulating blood, and the outer smooth muscle cells (SMCs) that control vascular contraction. In larger vessels, this is further surrounded by adventitia that is composed of fibroblasts and extracellular matrix (ECM) and contributes to vascular structural characteristics.

When the endothelium is damaged, the vessel undergoes a healing process, termed vascular remodeling. When remodeling is exuberant, it can result in vascular stiffening (as seen in hypertension and aging) and can contribute to atherosclerosis, vein graft failure, restenosis after percutaneous vascular procedures, and cardiac transplant vasculopathy (reviewed in [1]). Endothelial damage can be caused by mechanical injury or by cardiovascular risk factors, including dyslipidemia, hypertension, diabetes, or toxins from smoking. In the area of endothelial damage, the normally quiescent SMCs proliferate and produce ECM that contributes to vascular fibrosis, stenosis, and stiffening. These adversely remodeled vessels have decreased compliance and decreased ability to vasodilate to enhance local blood flow, and may have decreased luminal area, thereby contributing to hypertension, regional ischemia, and decreased functional capacity. Although we have some knowledge about the mechanisms of vascular remodeling, our current cardiovascular therapies are still limited by the effects of adverse remodeling. Thus there is still a need to identify novel contributors to vascular remodeling that may serve as effective therapeutic targets to prevent cardiovascular disease.

The mineralocorticoid receptor (MR) is an intracellular transcription factor that binds the steroid hormone aldosterone, which acts in the kidney to enhance sodium retention and increase blood pressure [2]. Therefore, MR antagonists (such as spironolactone and eplerenone) are often used as antihypertensive drugs and to treat heart failure, a state of volume excess. However, in clinical trials, patients treated with MR antagonists have greater improvements in their cardiovascular health than would be attributable to modest declines in volume and blood pressure [3–6], thus prompting further research into the roles of aldosterone and MR in vascular pathologies. Clinical data reveal that elevated aldosterone levels are associated with an increased incidence of atherosclerotic ischemic events (myocardial infarction and stroke) and cardiovascular mortality [7, 8]. Additionally, animal models of vascular injury show enhanced vascular remodeling with aldosterone infusion and decreased remodeling with MR antagonist treatment [9–12].

Due to the critical role of aldosterone and MR in regulating blood pressure, their detrimental effects on vascular remodeling have been previously attributed to hypertension with secondary vascular consequences. However, in addition to its presence in the kidneys, MR is also expressed in endothelial cells and SMCs of the vasculature in humans and rodents, supporting the possibility that MR in the vessels could contribute directly to the remodeling process. Activation of MR in SMCs *in vitro* has been shown to regulate the expression of

genes involved in vascular inflammation, fibrosis, and calcification [13–15] and to promote SMC proliferation [16,17]. Recent advances in our understanding of the specific and direct role of SMC-MR in the process of vascular remodeling *in vivo* and novel molecular mechanisms by which SMC-MR contribute to vascular remodeling will be summarized in this review.

Direct role for SMC-MR in vascular remodeling

The recent creation of mouse models in which the MR can be specifically deleted from the SMCs (SMC-MR-KO) has allowed directed study of the effects of SMC-MR in vascular remodeling in the setting of normal MR function in the kidney, other cells of the vasculature, and elsewhere. Our lab developed a model that allows for inducible deletion of SMC-MR in adulthood, thereby eliminating the potential that developmental effects of MR could have led to the observed changes in vascular function later in life. SMC-MR-KO mice have normal blood pressure when they are young and exhibit decreased blood pressure with aging relative to their wild-type counterparts, despite unchanged renal MR function [18]. Additionally, aged SMC-MR-KO mice have decreased vascular tone, and the aged vessels exhibit decreased contractile responses to thromboxane, angiotensin II, and calcium channel agonists [18]. Finally, SMC-MR-KO mice have an attenuated increase in blood pressure and superoxide production in response to angiotensin II infusion [18]. These findings suggest a role for extra-renal—and specifically SMC—MR in the vascular contractile changes that are associated with aging and oxidative stress. The characterization of this animal model has served as a foundation for further research on the role of SMC-MR in vascular injury and the remodeling process.

Vascular remodeling in response to injury can be modeled in animals by manually denuding a blood vessel of its endothelial cell layer. In multiple models of mechanical endothelial injury, aldosterone has been found to enhance the remodeling process, while MR antagonists attenuate it [9–12]. The specific role of SMC-MR has recently been explored using a wire-induced carotid endothelial denudation model in mice. In this model, aldosterone was infused at a low dose to achieve levels seen in patients with cardiovascular disease. Although this dose of aldosterone did not increase blood pressure, it still caused a significant increase in SMC proliferation, ECM deposition, and medial vessel thickening after injury, supporting the concept that the enhanced remodeling might be due to direct effects of aldosterone on the vasculature [10]. Indeed, in SMC-MR-KO mice, aldosterone infusion did not have any significant effect on vascular remodeling compared to vehicle infusion, supporting that SMC-MR is necessary for aldosterone-enhanced remodeling [19]. Additionally, even in the absence of excess aldosterone infusion, SMC-MR-KO mice have an attenuated fibrotic response to carotid wire injury relative to MR-intact littermates [19]. These data indicate that SMC-MR directly contributes to the vascular fibrotic response to mechanical injury in the presence of physiological levels of aldosterone and mediates the enhanced vascular remodeling response to aldosterone excess.

Another animal model of vascular remodeling is the response to hypertension induced by uni-nephrectomy/aldosterone/salt challenge. In this model, wild-type mice exposed to hypertension for 4 weeks develop increased stiffness and decreased distensibility of the

carotid artery. Using a model with constitutive SMC-MR deletion, it was recently demonstrated that these changes in vascular properties induced by the hypertensive stimulus in MR intact littermates are not observed in SMC-MR-KO mice [20]. This difference was not due to changes in the collagen-to-elastin ratio of the vessels, but may be attributable to the observation that this hypertension model induced an increase in expression of the integrin alpha-5-subunit in the carotid arteries that is not induced in the SMC-MR-KO mice [20]. Thus the presence of the MR in the SMCs is necessary for the vascular response to hypertension that results in altered vascular distensibility and increased stiffness, perhaps through its regulation of integrin protein expression. Since vascular stiffness is an independent risk factor for cardiovascular ischemic events and mortality in humans [21, 22], further exploration of the mechanisms by which SMC-MR directly contributes to vascular remodeling has important clinical implications.

Novel mechanisms by which SMC-MR contributes to vascular remodeling

The data presented above support a specific and necessary role for SMC-MR in the vascular injury response, including vascular remodeling and stiffening. A variety of mechanisms by which MR contributes to vascular remodeling have been previously identified and reviewed [23]. Here we focus on very recent data that has specifically implicated SMC-MR in new cellular signaling pathways associated with vascular remodeling. In addition to the integrin mechanism alluded to by Galmiche et al. [20], we review three additional novel SMC-MR regulated signaling pathways that contribute to vascular remodeling. These include Rho-kinase signaling, placental growth factor (PGF) signaling through vascular endothelial growth factor type 1 receptor (VEGFR1), and galectin signaling.

Rho-kinase signaling

Rho proteins are small G-proteins that activate Rho-associated kinases (ROCKs), which have been observed as important regulators of a range of SMC functions, including contraction, migration, proliferation, and apoptosis [24]. Additionally, ROCK activity has been linked to a range of clinical conditions involving vascular remodeling, including atherosclerosis, restenosis, and pulmonary hypertension [24]. Since 2008, substantial new data has revealed a role for Rho kinase in the pathogenesis of aldosterone-mediated vascular remodeling [25]. In cultured rat SMCs, aldosterone increases Rho-kinase activity, stress fiber formation, and SMC migration in an SMC-MR-dependent manner (evaluated through the attenuation achieved with the MR-antagonist eplerenone). These data implicate SMC-MR activation of Rho-kinase in the process of SMC migration. A mechanism for the activation of the Rho-kinase pathway by aldosterone has been proposed that involves c-Src phosphorylation [26]. Src is kinase that is synergistically phosphorylated in response to aldosterone and angiotensin II in cultured rat SMCs [26]. This effect is attenuated by eplerenone, irbesartan (an angiotensin II receptor antagonist), or PP2 (a selective Src inhibitor). Angiotensin II is also observed to act synergistically with aldosterone on SMCs to increase superoxide anion generation via NADPH oxidase and to increase translocation of RhoA to the membrane. Finally, co-stimulation with aldosterone and angiotensin II increase SMC migration in a c-Src and Rho-kinase-dependent manner. These data support aldosterone-activated SMC-MR and angiotensin II acting synergistically in vascular SMCs

to promote proliferation and migration via activation of a c-Src-dependent, redox-sensitive, Rho-kinase pathway (see Fig. 1, Panel A).

In humans, MR antagonism with eplerenone has also been shown to be beneficial in hypertensive patients with high levels of ROCK activation. In comparing different therapies for patients with hypertension, eplerenone and nifedipine (a calcium channel blocker) resulted in decreased ROCK activity and blood pressure, while losartan (an angiotensin II receptor blocker) exhibited only hypotensive effects [27]. Thus MR-antagonist treatment exerts modest effects on blood pressure (similar to other anti-hypertensive therapies), but provide additional vascular protection, perhaps in part through a reduction of SMC Rho/ROCK activity.

Placental growth factor signaling via VEGFR1

Placental growth factor (PGF) is a member of the vascular endothelial growth factor (VEGF) family that is involved in post-embryonic angiogenesis and wound healing. PGF has been previously identified as a vascular aldosterone-regulated gene [15]. PGF expression and vascular protein secretion are up-regulated in response to aldosterone in *ex vivo* treated mouse vessels and diseased human vessels [10]. Attenuation of this effect by spironolactone treatment and identification of an aldosterone-responsive MR binding site sequence upstream of the *Pgf* gene suggest that MR is the direct mediator between aldosterone and PGF expression. Aldosterone induction of PGF expression (and expression of its receptor *VEGFR1*) was further enhanced in vessels that had undergone endothelial denudation injury [10]. Furthermore, aldosterone-induction of PGF expression was prevented in vessels from SMC-MR-KO mice [19]. PGF is thus identified as a novel target of SMC-MR with enhanced expression in the setting of endothelial injury.

Further studies have revealed additional details about the mechanism by which the SMC-MR/PGF/VEGFR1 pathway contributes to the mechanism of aldosterone-enhanced vascular remodeling *in vivo*. The enhanced SMC proliferation, ECM deposition, and medial vessel thickening induced by aldosterone after wire injury in wild-type mice is prevented in PGF-KO mice [10]. Additionally, aldosterone-induced remodeling following wire injury is inhibited by treatment with VEGFR1- (but not VEGFR2-) blocking antibody [19]. Immunohistochemistry revealed that VEGFR1 is expressed exclusively on endothelial cells in healthy vessels and is expressed on SMCs only in response to vascular injury. Induction of VEGFR1 after injury is substantially attenuated in the SMC-MR-KO mice [19]. Collectively, these findings provide an explanation for why aldosterone enhances vascular remodeling only after endothelial injury and not when the endothelium is healthy and intact. MR activation of PGF is enhanced in the setting of vascular injury and disease, and the effects of PGF on SMC proliferation are only realized when its receptor, VEGFR1, is expressed on SMC in the area of injury in a SMC-MR-dependent manner (see Fig. 1, Panel B).

Galectin-3-mediated fibrosis

Galectin-3 is a small lectin protein that is expressed in many tissues. Previous studies have implicated galectin-3 in the development of cardiac fibrosis in heart failure models and have

suggested that the detrimental effects of aldosterone on cardiac fibrosis may also be mediated by galectin-3 (reviewed in [28]). Recently, a role for galectin-3 was identified in mediating the vascular remodeling effects in the aldosterone/salt hypertension model [29].

In cultured SMCs, aldosterone increased the expression of galectin-3 in a concentration-dependent and MR-dependent manner, suggesting that galectin-3 may be another SMC-MR-regulated gene [29]. In a rat model of hypertension induced by 3 weeks of aldosterone-salt challenge, inhibition of either MR or galectin-3 showed similar effects in attenuating the hypertension, inflammation, and vascular fibrosis. Finally, galectin-3 knock-out mice do not exhibit the aldosterone-induced increases in collagen I synthesis, vascular inflammation, or vessel fibrosis that are observed in their wild-type counterparts [29]. Thus MR regulates galectin in vascular SMCs, and both MR and galectin-3 are directly involved in the mechanism of aldosterone-mediated vascular fibrosis in this rat hypertension model (see Fig. 1, Panel C).

In humans, elevated serum levels of galectin-3 have been associated with adverse outcomes in cardiovascular patients [30]. However, the role of galectin-3 in vascular remodeling in humans remains unclear. In a study within the HF-ACTION clinical trial (which examined the effects of exercise training versus “usual” treatments for patients with chronic heart failure), there was no observed correlation between galectin-3 plasma levels and MR-antagonist use after adjusting for other prognostic predictors [31]. Further research is needed to clarify how the plasma levels of galectin-3 (and other molecules or proteins implicated in the vascular fibrosis process) are related to vascular outcome and/or response to different therapies. It is possible that galectin-3, PGF, VEGFR1, and other SMC-MR-regulated genes may be novel biomarkers of aldosterone-mediated vascular disease that could be used to predict and/or follow the effects of different doses of MR antagonists and other therapies.

Summary and clinical implications

In summary, recent advances in the study of mineralocorticoid receptors in vascular SMCs have enhanced our understanding of their role in the vascular injury response. The development and characterization of the SMC-MR-KO mouse has led to the discovery that SMC-MR is necessary for the adverse vascular effects of aldosterone on the responses to mechanical injury and to hypertension. In the absence of SMC-MR, aldosterone does not mediate its detrimental effects on SMC proliferation, vessel wall thickening, vascular fibrosis, and vascular stiffening. New mechanisms by which SMC-MR contributes to the vascular injury response have also recently been described, including pathways involving integrin alpha 5, Rho-kinase, PGF-VEGFR1, and galectin-3 (see Fig. 1). While further research is needed to elucidate the complete mechanism of the aldosterone-mediated vascular remodeling, the data presented above defend an important and direct role for the vascular SMC-MR and implicate several new players that may contribute to this phenomenon at a molecular and cellular level.

MR antagonists have traditionally been used to treat hypertension and heart failure; however, the data presented above demonstrating a direct role for SMC-MR in vascular remodeling may support the use of MR antagonists to prevent or treat vascular fibrosis and

stiffening that result from vascular injury in other disorders (reviewed in [33]). Indeed, in animal models, MR antagonists prevent coronary stent restenosis [12], adverse vein graft remodeling [9], and pulmonary vessel remodeling in pulmonary hypertension [32]. However, the use of systemic MR antagonists can cause unwanted effects, including hyperkalemia and gynecomastia, through MR inhibition in extra-vascular tissues. Since we are currently unable to directly target and specifically inhibit SMC-MR in a clinical setting, these extravascular effects limit the use of MR antagonists for vascular protection, and thus further work in cell type-specific drug delivery mechanisms is required. The potential to use MR antagonists to coat stents could be considered for localized mechanical vascular injury induced by angioplasty and stenting, particularly since aldosterone appears to specifically contribute to SMC proliferation but not to endothelial regrowth after vascular injury [19]. As cell type-targeted therapy is far from a reality, additional research on the cellular mechanisms by which SMC-MR contributes to vascular remodeling has the potential to identify the downstream molecules that could be the targets of novel anti-fibrosis drugs, including VEGFR1, Rho-kinase, galectin, and likely others.

In addition to drug targets, the vascular remodeling mediators downstream of SMC-MR may be valuable as biomarkers. Current MR-antagonist treatment is titrated to its effects on blood pressure lowering. However, MR antagonists may be able to have effects on vascular protection at different doses than those needed to decrease blood pressure. Treatment with MR antagonists for vascular indications could be initiated or monitored based on plasma levels of these SMC-MR-regulated biomarkers. Further studies are needed to determine whether secreted SMC-MR targets such as PGF or galectin-3 might be biomarkers to identify patients who could benefit from MR antagonists or as markers for treatment effect. If such biomarkers become available, patients may be able to gain the substantial vascular benefits of MR antagonism while avoiding potentially unnecessary effects on the kidneys or other tissues in the body.

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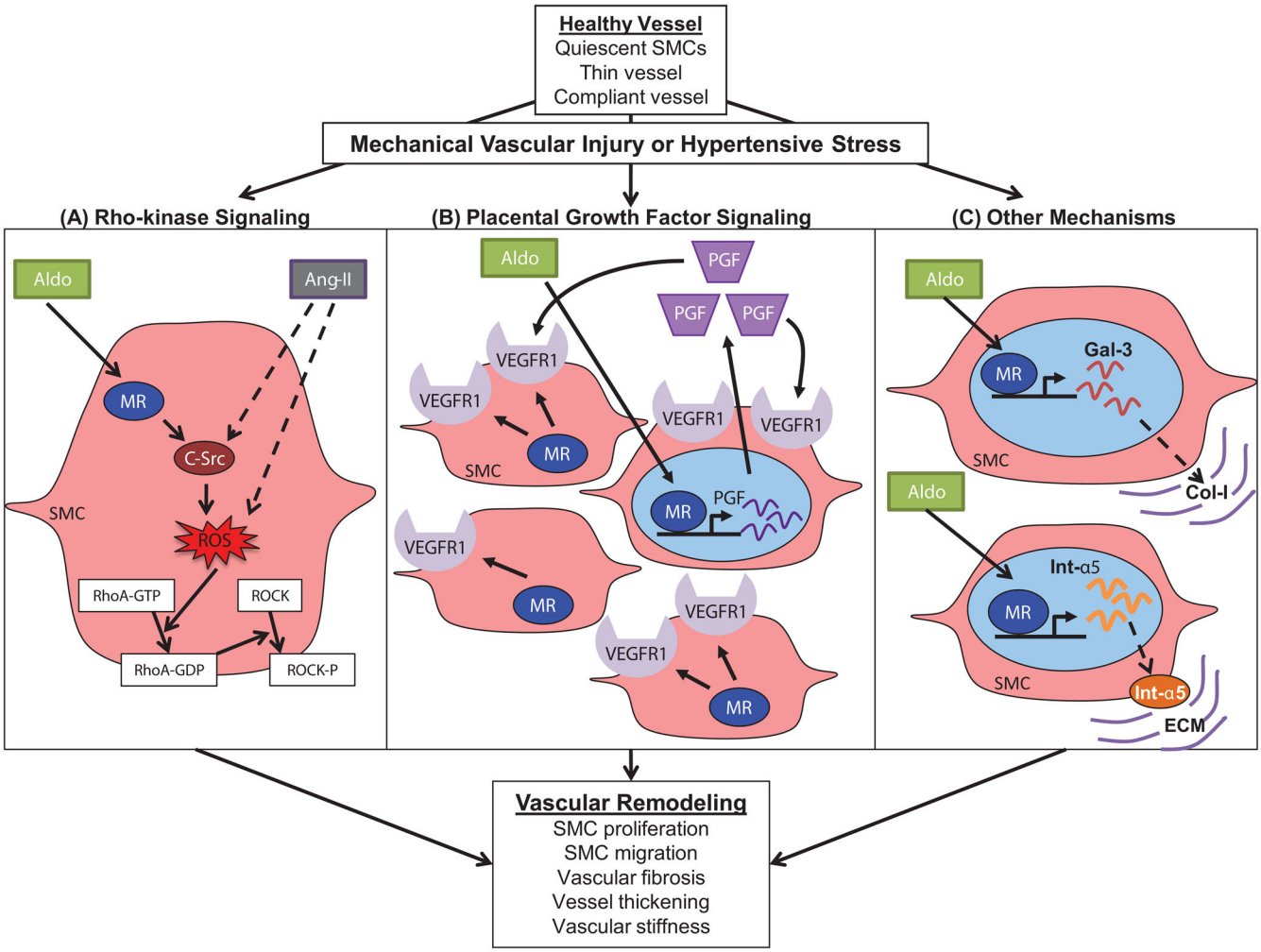

Figure 1. New mechanisms by which smooth muscle cell (SMC) mineralocorticoid receptor (MR) directly contributes to vascular remodeling

(A) Rho-kinase signaling is synergistically activated by aldosterone (Aldo) via SMC-MR and by angiotensin-II (Ang-II), leading to Rho-associated kinase (ROCK) activation and SMC migration. ROS = reactive oxygen species, ROCK-P = phosphorylated ROCK (activated form). **(B) Placental growth factor (PGF) signaling** contributes to vascular remodeling specifically in areas of vascular injury, where the vascular endothelial growth factor type 1 receptor (VEGFR1) is up-regulated in the SMC by MR. **(C) Other mechanisms** include galectin-3 (Gal-3) regulation by SMC-MR resulting in enhanced type I collagen (Col-I) expression in the extracellular matrix (ECM) and increased expression of the alpha-5 subunit of integrin (Int-α5) that interacts with the ECM to enhance vascular stiffness.