SRF/myocardin: a novel molecular axis regulating vascular smooth muscle cell stiffening in hypertension

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This editorial refers to ‘Inhibition of SRF/myocardin reduces aortic stiffness by targeting vascular smooth muscle cell stiffening in hypertension’ by Zhou et al., pp. 171–182.

Large artery stiffening is a main risk factor for chronic diseases including aging-related hypertension, heart failure, renal disease, stroke and dementia, which represent major causes of morbidity and mortality. Arterial stiffening is caused primarily by excessive fibrosis due to exuberant accumulation of collagen. More recently the mechanisms of arterial stiffening in hypertension were extended to proteins regulating vascular smooth muscle cell (VSMC) plasticity and cellular stiffness.¹

Despite earlier studies showing involvement of the cytoskeletal network, there is a gap in our knowledge regarding underlying mechanisms that initiate cellular stiffness and sustain this process. In this issue Zhou et al.² provide evidence that VSMC stiffness assessed by atomic force microscopy (AFM) is increased in spontaneously hypertensive rats (SHR) at the level of large arteries but not in small arteries. RNA and protein expression of serum response factor (SRF)/myocardin as well as their downstream targets caldesmon, calponin, and RhôA are increased in thoracic aorta from SHR compared with arteries from Wistar-Kyoto rats (WKY) at 4 months of age. The hypertension-induced increase in SRF represents one of the main mechanisms, as supported by normalization of cellular stiffness measurements in the presence of an inhibitor of SRF activity in both in vitro and in vivo conditions. Normalization of cellular stiffness is paralleled by an improvement of aortic elasticity preceding the reduction of blood pressure in SHR. This finding suggests that recovery of arterial mechanical properties in large arteries is causally involved in blood pressure reduction.

This article has revisited three major concepts in clinical essential hypertension confirmed in the SHR model recapitulating the arterial phenotype in hypertension: (i) there is no intrinsic stiffening of large arteries assessed by distensibility-pressure curves or elastic modulus; wall stress curves in patients with essential hypertension compared with age-matched normotensives (except in young hypertensives); (ii) arterial wall hypertrophy is accompanied by normal stiffness of the artery at a given blood pressure or circumferential wall stress; (iii) reduced stiffness of wall components appears to develop in small size resistance arteries from hypertensive patients or SHR, although there was some variability that depended on the origin of the arteries.³,⁴ In the present study, the increase in cellular stiffness at the level of the aorta has to be interpreted in light of the in vivo increase in arterial pressure. Based on similar increases in blood pressure in smaller muscular arteries, the lack of increase in cellular stiffness is more surprising. The prominent role played by VSMC tone in distal artery mechanical properties likely explains this result since AFM does not provide direct measurement of vascular tone. This close relation between vascular tone and arterial stiffness has been well demonstrated in knockout mice with conditional deletion of the VSMC SRF, a transcription factor regulating expression of smooth muscle genes involved in the maintenance of the contractile state.⁵

In this issue, Zhou et al.² use a new and less cytotoxic second-generation inhibitor of Rho/MRTF-A/SRF-mediated transcription CCG-100602⁶ to treat SHR. This drug inhibited markedly the overexpression (both at the RNA and protein levels) of SRF and its muscle-specific co-factor myocardin, which occurs in thoracic aorta but not in distal arteries from SHR compared with WKY rats. This inhibition of SRF and myocardin resulted in decreased expression of SRF-regulated stiffness-associated genes. To our knowledge, it is the first report on myocardin inhibition by this drug in pathological conditions. This study raises the question whether CCG-100602 inhibits the expression of myocardin by direct binding or via MRTF-A and MRTF-B, two members of the myocardin-related-transcription factor family. Myocardin is constitutively located in the nucleus whereas the nuclear localization of MRTF-A/B is controlled by actin polymerization (Figure 1). Additionally, myocardin suppresses MRTF-A expression via activation of miR-1.⁷ CCG-100602 has been reported to inhibit MRTF-A nuclear localization in myofibroblasts. It will be interesting in the future to examine the expression levels and nuclear/cytoplasm localization of MRTF-A/B related to actin remodeling to know if there is a reciprocal expression of MRTF-A/B and myocardin in hypertension. Differences between the targeting genes of SRF/myocardin and SRF/MRTF may account for the regional cellular stiffness in SHR. This study opens a new avenue of research regarding the opinions expressed in this article are not necessarily those of the Editors of Cardiovascular Research or of the European Society of Cardiology.
microRNAs involved in SRF/myocardin-mediated cellular stiffness regulating large and small artery cross-talk in hypertension.

Smooth muscle myosin light chain kinase (smMLCK) expression and phosphorylation of MLC are increased both in the aorta and mesenteric arteries of SHR rats. This is due to an insertion of 12-bp in the promoter adjacent to the CArG box which enhances SRF binding to the smMLCK promoter. Specific inhibition of MLCK activity and blocking Ras protein signalling which induces changes in actin dynamics and in vasoconstriction by regulating actomyosin contractility. In the nucleus, the myocardin/ SRF complex activates VSMC-specific contractile genes and miR-1. The hypertension-induced increase in SRF and myocardin in elastic arteries (EA) drives the increase in VSMC stiffness (red arrows). In the cytoplasm MRTF-A/B is sequestered by monomeric G-actin. Incorporation of monomeric G-actin into F-actin fibres stimulates nuclear translocation of MRTF-A/B, thus enhancing SRF transcription activity via their interaction. Additional studies are warranted to elucidate if there are differential avidities of the myocardin/ SRF and MRTF-A/B/ SRF in elastic and muscular arteries (MA). The imbalance towards the MRTF-A/B/ SRF complex is supposed to influence less VSMC stiffness in MA in hypertension (blue dashed arrows). This could be linked to the fact that MRTF/ SRF activity, but not myocardin, is regulated by actin treadmilling. In addition, there could be a reciprocal regulation between expression of myocardin and MRTF-A/B, inhibition of MRTF-A/B by miR-1 constitutes one way to reduce MRTF-A/B/ SRF signalling by myocardin.

The authors focused on the mechanisms underlying VSMC stiffness. However, responses of the endothelium to vascular stiffening promote Rho-driven SMC contraction and vasoconstriction. Thus, the contribution of the SRF signalling pathway in endothelial cells to arterial stiffness constitutes an interesting direction for new research on SRF. We could speculate that CCG-100602 differentially normalizes endothelial cell function according to the arterial site in SHR.

In conclusion, it is still very difficult to determine whether large artery stiffness is a cause or consequence of hypertension. Clearly, we are beginning to understand key VSMC signalling pathways in the molecular biology of arterial stiffness. The work of Zhou et al. raises the possibility that SRF is a relevant target to resolve in the absence of effective treatments to halt/reverse the arterial stiffening process.

**Figure 1** VSMC stiffness mechanisms occurring in hypertension depend on the activation of SRF/cofactors. Hypertension affects the extracellular matrix (ECM)-integrin-cytoskeletal axis by both increasing ECM accumulation as well as activity and expression of integrins. Intracellularly, this process involves focal adhesion-associated proteins (talin, FAK, paxillin, vinculin) and activation of RhoA/Rho kinase signalling which induces changes in actin dynamics and in vasoconstriction by regulating actomyosin contractility. In the nucleus, the myocardin/ SRF complex activates VSMC-specific contractile genes and miR-1. The hypertension-induced increase in SRF and myocardin in elastic arteries (EA) drives the increase in VSMC stiffness (red arrows). In the cytoplasm MRTF-A/B is sequestered by monomeric G-actin. Incorporation of monomeric G-actin into F-actin fibres stimulates nuclear translocation of MRTF-A/B, thus enhancing SRF transcription activity via their interaction. Additional studies are warranted to elucidate if there are differential avidities of the myocardin/ SRF and MRTF-A/B/ SRF in elastic and muscular arteries (MA). The imbalance towards the MRTF-A/B/ SRF complex is supposed to influence less VSMC stiffness in MA in hypertension (blue dashed arrows). This could be linked to the fact that MRTF/ SRF activity, but not myocardin, is regulated by actin treadmilling. In addition, there could be a reciprocal regulation between expression of myocardin and MRTF-A/B, inhibition of MRTF-A/B by miR-1 constitutes one way to reduce MRTF-A/B/ SRF signalling by myocardin.

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