Stretch-dependent smooth muscledifferentiation in theportal vein - role of actin polymerization, calcium signaling and microRNAs.

Albinsson, Sebastian; Bhattachariya, Anirban; Hellstrand, Per

Published in:
Microcirculation

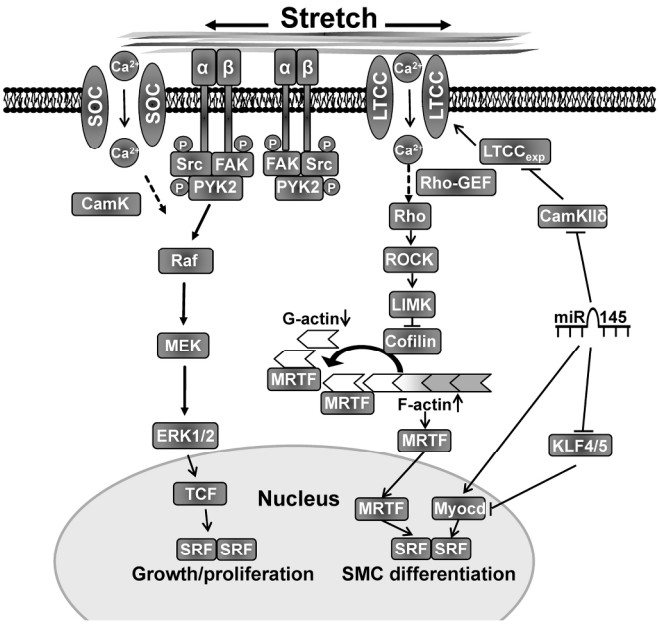
DOI:
10.1111/micc.12106

2014

Microcirculation
The Official Journal of the Microcirculatory Society, Inc., the British Microcirculation Society, and the Australia & New Zealand Microcirculation Society

Stretch-dependent smooth muscle differentiation in the portal vein - role of actin polymerization, calcium signaling and microRNAs

Journal:	*Microcirculation*
Manuscript ID:	UMIC-2013-0088.R1
Manuscript Type:	Invited Reviews
Date Submitted by the Author:	18-Oct-2013
Complete List of Authors:	Albinsson, Sebastian; Lund University, Experimental Medical Science Bhattachariya, Anirban; Lund University, Experimental Medical Science Hellstrand, Per; Lund University, Experimental Medical Science
Keywords:	smooth muscle, microRNA, actin polymerization, calcium, vascular
Note: The following files were submitted by the author for peer review, but cannot be converted to PDF. You must view these files (e.g. movies) online.	
DSCN3800.MOV	



209x148mm (300 x 300 DPI)

Stretch-dependent smooth muscle differentiation in the portal vein

- role of actin polymerization, calcium signaling and microRNAs

Sebastian Albinsson, Anirban Bhattachariya and Per Hellstrand

Abstract

The mechanical forces acting on smooth muscle cells in the vascular wall are known to regulate processes such as vascular remodeling and contractile differentiation. However, investigations to elucidate the underlying mechanisms of mechanotransduction in smooth muscle have been hampered by technical limitations associated with mechanical studies on pressurized small arteries, due primarily to the small amount of available tissue. The murine portal vein is a relatively large vessel showing myogenic tone that in many respects recapitulates the properties of small resistance vessels. Studies on stretched portal veins to elucidate mechanisms of mechanotransduction in the vascular wall have shown that stretch-sensitive regulation of contractile differentiation is mediated via Rho-activation and actin polymerization, while stretch-induced growth is regulated by the MAP-kinase pathway. In this review, we have summarized findings on mechanotransduction in the portal vein with focus on stretch-induced contractile differentiation and the role of calcium, actin polymerization and microRNAs in this response.

Introduction

The cells in the vascular wall are constantly subjected to mechanical forces exerted by the blood pressure and flow. While endothelial cells have the ability to sense changes in blood flow, smooth muscle cells are sensitive to changes in wall tension. The change in tension is detected by stretch-sensors in the plasma membrane such as stretch-sensitive ion channels, G-protein coupled receptors or integrins coupled to focal adhesion kinase, which then activate multiple signaling pathways in smooth muscle cells [38,39,46]. Mechanical stretch is one of several environmental cues that can regulate smooth muscle phenotype [51]. In contrast to striated muscle, smooth muscle cells have a remarkable ability to alter their phenotype, which is likely an important mechanism for the repair process following vascular injury and for the ability to develop new blood vessels to supply nutrients for growing tissues. However, phenotypic modulation of smooth muscle cells is also involved in the development and progression of vascular disease.

Smooth muscle mechanotransduction and vascular disease

An increase in blood pressure results in acute effects on smooth muscle function in small arteries as the cells contract in order to resist the increase in pressure and protect the capillary network from excessive and possibly damaging pressure levels. This intrinsic regulation of smooth muscle contraction in response to elevated pressure is termed the myogenic response, first described by the British physiologist William Bayliss in 1902 [11]. If the increase in blood pressure persists over a longer period of time, the smooth muscle cells and extracellular matrix will be rearranged as a thicker wall around a smaller lumen, a process that is referred to as inward vascular remodeling [1,37,44]. Similarly, prolonged stimulation with a contractile agonist results in inward vascular remodeling suggesting that smooth muscle tone is an important determinant for the remodeling response [43]. Like the acute myogenic response, the remodeling process may be an important mechanism to protect the capillaries from excess

pressure and flow. Narrowing the lumen of the vessel and increasing the wall thickness will, according to the law of Laplace, counteract the increased wall stress caused by the elevated intraluminal pressure. However, permanent inward remodeling of small arteries and arterioles will also result in elevated peripheral resistance and increased sensitivity to vasoconstrictors as analyzed in classical studies by Folkow (for reviews, see [27,49]). Recent studies have demonstrated the relevance of vascular remodeling as a predictor of future cardiovascular events [45,56].

Inward remodeling in essential hypertension is primarily eutrophic, i.e. showing a narrowed lumen and increased wall-to-lumen ratio with unaltered cross-sectional wall area. In secondary forms of hypertension, such as in renovascular disease and in diabetes mellitus, hypertrophic remodeling has also been reported [57,58]. Dilatation of the vessel wall causes increased strain and stimulates growth of the medial layer (outward remodeling). Both growth and remodeling depend on sensing of mechanical forces and knowledge regarding their signaling pathways is still far from complete. As shown in organ culture of pressurized arteries, inward remodeling can be elicited by active vasoconstriction [8], which is expected to activate multiple signal pathways related to trophic effects. In addition, recent studies suggest that the response may involve actin polymerization elicited by the Rho pathway as a key mechanism affecting cellular mechanical properties [48,55,64].

Models for studying mechanotransduction in smooth muscle

In order to study the isolated effects of mechanical stretch on smooth muscle phenotype and function, it is in some respects advantageous to use *ex vivo* or *in vitro* models. The primary reason for this is that an increase in blood pressure *in vivo* involves the activation of a number of homeostatic mechanisms that may influence smooth muscle phenotype. The most common method to evaluate long term effects of stretch in smooth muscle is to use cultured cells on flexible membranes. This technique has several advantages, including the possibility to precisely regulate the frequency and degree of stretch and the

possibility to transfect the cells in order to overexpress or silence proteins of interest. However, the effects of stretch are highly dependent on the 3D-environvent as well as on the differentiation state of the smooth muscle cells. The cell-cell and cell-matrix interactions as well as the phenotype of smooth muscle cells are fundamentally altered during culture of isolated cells. It is thus important to confirm findings in cell culture by using *in vivo* or *ex vivo* models with intact vascular tissue.

To study the effect of increased wall tension in intact vessels several laboratories have used models of pressurized rodent arteries in *ex vivo* organ culture. Seminal studies in this field were performed in large conduit vessels during the mid- to late-1990s showing that increased pressure causes hypertrophy as well as contractile differentiation of the vascular wall [12,13]. In small arteries and arterioles pressure myography has been extensively used to reveal mechanisms of contraction, myogenic reactivity and remodeling in combination with electrophysiological, imaging and molecular biological techniques as well as organ culture [8,22,35,72]. However, although, pressurization of small arteries is a relevant model for studying the effects of wall tension on the regulation of smooth muscle phenotype, the technical difficulty and the limited amount of material that can be obtained from small resistance arteries represent considerable challenges.

The relevance of the portal vein model for the study of vascular mechanotransduction

Both pre- and postcapillary small vessels develop myogenic tone, and although this is much less studied in the venous system, the main characteristics seem to be similar as recently shown in the rat mesenteric circulation [25]. Large conduit arteries and capacitance veins, on the other hand, are primarily regulated by neurogenic mechanisms and lack myogenic tone. A large vessel showing prominent myogenic tone is however the portal vein, which anatomically is interposed between two exchange vascular beds, the splanchnic capillaries and the liver sinusoids. The myogenic activity of the portal vein consists of phasic contractions triggered by bursts of action potentials, and this vessel has been extensively studied in

investigations fundamental for present-day understanding of structural, electrophysiological and mechanical properties of vascular smooth muscle [65].

The wall of the portal vein comprises a thin inner circular muscle layer and a thicker outer longitudinal layer. In the rat, these muscle layers are weakly developed at birth but the longitudinal muscle layer develops well organized contractile and cytoskeletal filaments as well as spontaneous activity in the first three postnatal weeks, whereas the circular layer is less filamentous, lacks spontaneous activity and is fully developed approximately one week later [67]. These differences may reflect stretch-regulated development primarily of the longitudinal muscle layer for propelling blood and maintaining the integrity of the vessel in the anatomical absence of major longitudinal supporting structures.

The contractile pattern of the portal vein resembles the vasomotion typical of small resistance vessels, and even though the portal vein may be at the extreme end in showing a phasic rather than a tonic (sustained) contractile pattern, it in several respects reproduces properties of myogenically active vessels in the microcirculation [26,65]. The portal venous pressure is around 7 mmHg in the rat, and following a partial obstruction by a loose ligature around the entrance to the liver hilus the pressure increases, accompanied within a few days by a rapid hypertrophy of the vessel wall [33,71]. The hypertrophy is associated with increased contractile force and expression of smooth muscle proteins after one week of obstruction [41,42,71]. Since the dominant muscle layer of the portal vein is longitudinal, the effects of intraluminal pressure may be approximated *in vitro* by distension of the vessel in the longitudinal direction. This allows investigation of long-term effects of stretch in organ culture of strips or whole vessels without a need for cannulation and pressurization. Due to the myogenic tone the vessels maintain contractile activity without addition of vasoconstrictors. In comparison with unloaded veins, larger cell size, ERK activation and increased DNA and protein synthesis, as well as longitudinal remodeling, were observed when the vein was loaded by a gold weight corresponding to the optimal

preload for active force production [80]. It should be noted that in this model the wall stress of the loaded veins is kept at the physiological level, whereas the absence of stress in the unloaded veins is unphysiological. The model is thus not one of hypertrophy but rather resembles a knock-down model to reveal the physiological role of a factor, wall stress in this case. In subsequent work we have extensively used this model to investigate signaling mechanisms associated with mechanical distension of the vascular wall. For up to five days of organ culture the spontaneous contraction of the portal vein is clearly visible as it moves the weight up and down in the culture tube (Suppl video 1). The myogenic activity increases with stretch [34] and it is likely that the active contraction in the portal vein (and in small resistance arteries) in response to an increased wall stress is an important factor for the effects observed following mechanical stimulation of smooth muscle cells.

Mechanical stretch of the portal vein recapitulates many of the effects that are observed in hypertensive animal models *in vivo*. The acute effects of stretch (<10 min) in the portal vein include activation of the ERK/MAPK and PI3K/Akt pathways [2,30,70]. Inhibition of these pathways prevents the stretch-induced increase in growth and protein synthesis in the portal vein. The Rho/ROCK pathway is also activated by stretch of the portal vein but with a slower time course (>6h) [3]. Thus, the signaling mechanisms that are activated in vascular smooth muscle during hypertension *in vivo* can at least in part be attributed to mechanical tension of the vascular wall. In the following we summarize our findings regarding the effects of mechanical stretch on smooth muscle differentiation, suggesting important roles of actin polymerization, calcium signaling and microRNAs in this process.

Role of actin polymerization for stretch-dependent contractile differentiation

Differentiation of smooth muscle cells is often characterized by contractile function and the expression of markers such as myosin heavy chain, α-actin, tropomyosin, calponin and SM22α. In mouse portal veins, the expression of all of these markers is induced by stretch, both at the mRNA and protein level. The gene expression of smooth muscle markers is regulated by the transcription factor serum response

factor (SRF) together with its co-factors myocardin and myocardin related transcription factors (MRTFs) [51]. The expression of myocardin is restricted to smooth muscle cells and cardiomyocytes whereas MRTF is ubiquitously expressed. Another interesting difference is that myocardin is predominantly found in the nucleus of the cell while MRTF shuttles between the nucleus and the cytoplasm depending on the level of actin polymerization [50,52]. Seminal work by Treisman and co-workers demonstrated that MRTF binds to globular actin (G-actin) in the cytosol and this binding prevents its nuclear translocation [47,63]. When actin is polymerized into filamentous actin (F-actin), MRTF is released from G-actin and translocated to the nucleus via the importin α/β1 heterodimer where it, like myocardin, potentiates the activity of SRF [50,52,75] (Figure 1).

Actin polymerization is regulated by multiple signaling mechanisms, including several pathways controlled by the small GTPase Rho. The most well-known downstream signaling pathway of Rho involves activation of Rho-kinase, which leads to phosphorylation of LIM-kinase and Cofilin as well as inhibition of myosin phosphatase resulting in calcium sensitization [66]. Cofilin is an actin depolymerizing factor, which is inactivated by phosphorylation at Ser3 by LIM-kinase. Rho is also involved in actin polymerization by promoting the activation of mDia and profilin, which facilitates actin filament assembly. By using a rhotekin pull-down assay, we found that mechanical stretch increases the level of GTP-bound, activated, Rho [3]. This activation subsequently results in an increased phosphorylation of cofilin, which is evident after 6h of stretch stimulation. Furthermore, stretch-induced inactivation of cofilin also stabilizes actin filaments and reduces the G-actin pool [3]. By measuring the stretch-induced protein synthesis of specific smooth muscle markers, identified by 2D-gel electrophoresis and MALDI-TOF, we could demonstrate that the increased Rho-activation and actin polymerization in response to stretch is associated with an increased expression of smooth muscle markers.

To investigate the importance of Rho signaling and actin polymerization for stretch-induced responses in the portal vein we used inhibitors of Rho (C2/C3 toxin) and Rho-kinase (Y-27632) as well as actin binding molecules such as Latrunculin B and Jasplakinolide. Latrunculin B binds to actin monomers and prevents them from polymerizing into actin filaments. Jasplakinolide, on the other hand, binds to actin filaments and prevents their dissociation into monomers. Jasplakinolide also enhances the rate of actin filament nucleation and both of these mechanisms result in a reduced pool of monomeric G-actin [15]. In portal veins, inhibition of Rho by C2/C3 and actin polymerization by Latrunculin B prevents the acute stretch-induced Erk1/2 phosphorylation, suggesting that an intact cytoskeleton is required for mechanotransduction in smooth muscle cells [79]. Furthermore, both C2/C3 and Latrunculin B inhibit stretch-induced synthesis of contractile smooth muscle proteins to the level of unstretched vessels demonstrating that actin polymerization plays a critical role for stretch-induced contractile differentiation [79]. In unstretched portal veins, stabilization of actin filaments by Jasplakinolide increases the synthesis of smooth muscle markers to the level of stretched portal veins [2].

Role of calcium signaling in contractile differentiation

Stretch of myogenically active smooth muscle depolarizes the cells and promotes calcium influx trough voltage-gated calcium channels [22,34,36]. The increase in intracellular calcium plays a fundamental role for contraction by activating the calcium-calmodulin dependent myosin light chain kinase (MLCK), which initiates smooth muscle contraction by phosphorylation of the myosin light chains. However, calcium influx via voltage-gated calcium channels can also promote activation of the Rho/ROCK pathway and subsequent activation of smooth muscle gene expression, although the molecular link is not known [53,61,73] In the specific case of Ang II-induced Rho activation, calcium-sensitive Rho-GEF activation involving janus kinase 2 (JAK2) or protein tyrosine kinase 2 (PYK2 have been suggested to play a role [29,78]. The source of calcium influx appears to be crucial for determining the effects on gene

expression. While calcium influx via L-type calcium channels can activate transcription of both smooth muscle markers and early response genes such as c-fos, influx via non-voltage-dependent, e.g. store operated calcium channels primarily drives the expression of genes associated with hypertrophy and proliferation [74]. The effect of calcium on growth response gene expression is dependent on calcium-sensitive signaling molecules and transcription factors such as calmodulin-dependent protein kinase II (CamKII) and cAMP response element-binding protein (CREB) [9].

Stretch of the portal vein results in simultaneous growth and contractile differentiation and we have shown that the stretch-induced growth response, measured as total protein synthesis, is reduced by 2-APB, an inhibitor of SOCE [54]. On the other hand, verapamil, an inhibitor of L-type calcium channels, reduces or prevents stretch-induced smooth muscle differentiation in the portal vein. This effect is likely mediated via inhibition of stretch-induced activation of the Rho pathway. The effect of stretch on smooth muscle differentiation and rho-activation is reproduced in the portal vein by depolarization using potassium chloride, which activates the L-type calcium channels [54]. This further strengthens the hypothesis that stretch-induced contractile differentiation involves activation of the Rho signaling pathway by an increased L-type calcium channel influx. The regulation of calcium signaling via L-type channels could thus be an essential component in mediating the effects of increased pressure on phenotype modulation and remodeling of vascular smooth muscle.

Role of miRNAs in stretch-induced contractile differentiation

MicroRNAs (miRNAs) are small non-coding RNAs that can regulate gene expression by binding to the 3' untranslated region (UTR) of their target mRNA [10]. Over a thousand miRNAs have been identified and each of these miRNAs can have hundreds of mRNA targets. Thus miRNAs are thought to play a role in the control of most if not all cellular processes. Furthermore, miRNAs have been shown to be dysregulated in several vascular disease states and are promising targets for therapeutic intervention [7]. The biogenesis

of most miRNAs is dependent on the endoribonuclease Dicer, which cleaves the pre-miRNA into the short (~22nt) mature miRNA, which is incorporated into the RNA-induced silencing complex (RISC) [10]. To decipher the role of miRNAs for smooth muscle development and function we have used conditional Dicer knock-out (KO) mice. These were bred by crossing mice with a floxed Dicer allele [18] with mice that constitutively express SM22α-Cre at the early embryonic stage [31]. We found that deletion of Dicer in smooth muscle during embryonic development causes severe hemorrhage and 100% lethality of the embryos at E16.5-E17.5, which demonstrates that miRNAs play an essential role for smooth muscle development [6]. Furthermore, deletion of Dicer in smooth muscle results in reduced contractile function and differentiation and reduced wall thickness of the aorta. By crossing floxed Dicer mice with a tamoxifen inducible and smooth muscle specific Cre mouse [76] we were also able to clarify the role of miRNAs for smooth muscle function in adult mice. Similar to the effect in embryos, loss of Dicer in adult mice results in reduced smooth muscle function and reduced contractile differentiation at 10 weeks post tamoxifen treatment [5]. Since nearly all of the miRNAs in smooth muscle are down regulated following Dicer KO [60] it is likely that multiple pathways involved in the regulation of smooth muscle differentiation may be affected in these animals. Several studies have demonstrated the importance of specific miRNAs for the regulation of smooth muscle contractile differentiation including, but not limited to, miR-21, miR-221/222 and miR-143/145 [4,14,19-21,24,32,40,77].

The miR-143/145 cluster is by far the most widely studied since it is highly expressed in smooth muscle and has been shown to regulate smooth muscle fate and plasticity [19] and to play a role in vascular disease [17]. Knockout of miR-143/145 results in phenotypic modulation of vascular smooth muscle cells to a more synthetic and less contractile state as well as to reduced blood pressure [14,77]. In the work by Xin et al, miR-145 was shown to regulate the expression of genes that are involved in actin polymerization [77]. Furthermore, we demonstrated that deletion of Dicer causes a reduced actin polymerization in smooth muscle cells and that this effect is rescued by overexpression of miR-145 in

Dicer KO cells [6]. Inhibition of actin polymerization by Latrunculin B prevents this effect of miR-145, suggesting that actin polymerization is crucial for mir-145-induced contractile differentiation.

Mechanical stretch is an important stimulus in the regulation of smooth muscle phenotype and we thus set out to determine the role of miRNAs in the process of mechanotransduction by using Dicer KO portal veins in organ culture as described above. Although overall protein synthesis is increased in the Dicer KO portal veins, the stretch-induced expression of contractile smooth muscle markers is nearly abolished in the absence of miRNAs [70]. However, stretch-sensing per se is not affected since ERK phosphorylation in response to stretch is maintained in Dicer KO portal veins. The effect of Dicer KO is similar to what is observed in portal veins after inhibition of L-type calcium channels and we also found that L-type channel expression and function is decreased in Dicer KO portal veins. Multiple miRNAs may be responsible for the downregulation of L-type channels in Dicer KO portal veins but inhibition of miR-145 alone, in cultured cells, could reproduce the effect of Dicer KO on L-type calcium channel gene expression [70]. Like most miRNAs, miR-145 have multiple targets and several of these are known to regulate smooth muscle differentiation and function such as myocardin, Krüppel-like transcription factors, CamKIIδ and angiotensin converting enzyme (ACE) [4,14,17,19]. Interestingly, CamKIIδ was recently found to negatively regulate L-type calcium channel expression [59] and we found that the expression of CamKIIδ is significantly increased in Dicer KO portal veins [70]. Furthermore, inhibition of CamKII prevents the effect of miR-145 on L-type calcium channel expression and we suggest this as one possible mechanism for the effect of Dicer KO on stretch-induced contractile differentiation [69,70]. A role for increased expression of CamKIIδ in phenotype shift of vascular smooth muscle has been proposed [62]. The presently found miRNA-dependent mechanism for regulation of L-type channel expression is a novel mechanism that might fit into this scheme. Some of the pathways for stretch-dependent effects discussed here are shown schematically in Figure 1.

Stretch-induced expression of miRNAs in the portal vein

Although we demonstrated that miRNAs are essential for stretch-induced contractile differentiation and calcium signaling in the vascular wall, the effects of stretch on miRNA expression in the intact vascular smooth muscle had not been studied at the time. We thus used the portal vein model to investigate stretch-induced miRNA expression following 24 hours of organ culture [68]. Our experience is that many miRNAs have a quite slow turnover, and 24 hours may therefore be considered an early time point of the effect of stretch on miRNA expression. Different results may be obtained after longer periods of stretch. However, in our study we were interested in the possible relation between early, stretch-dependent, changes in miRNA expression at 24 hours and stretch-induced intracellular signaling and phenotype regulation after 5 days of stretch. A qPCR-based miRNA array, which includes 552 miRNAs, revealed a dramatic down regulation of the miR-144/451 cluster by 24 hours of stretch [68]. However, none of the more well-known smooth muscle enriched miRNAs were affected by mechanical stretch in our model at this time point.

The miR-144/451 cluster is known to play an important role in erythropoiesis [23] and recent studies have demonstrated that miR-451 is involved in the regulation of AMP-kinase in glioma cells and cardiomyocytes [16,28]. AMP-kinase is an important regulator of cell metabolism in most cell types and miR-451 regulates AMP-kinase activation by targeting MO25α, a scaffolding protein required for full activity of the upstream AMPK kinase, LKB1. Stretch of the portal vein induces AMP-kinase phosphorylation and an increased expression of the total level of AMP-kinase. In cultured smooth muscle cells, we found that miR-144 and miR-451 target AMPK and MO25, respectively. Although AMPK is most known for its regulation of cell metabolism, we could demonstrate that activation of AMPK by AICAR could promote smooth muscle differentiation in the portal vein. Thus, it is possible that stretch-

dependent down-regulation of the miR-144/451 cluster is involved in the regulation of stretch-dependent AMP-kinase activation and contractile differentiation of smooth muscle cells.

Perspectives

Although some underlying mechanisms of mechanotransduction in smooth muscle have now been elucidated, it is clear that many questions still remain to be answered. For example, multiple stretch sensors have been suggested, including integrins, ion channels and G-protein coupled receptors, but it is not clear how these sensors regulate stretch-induced contractile differentiation and remodeling in smooth muscle. Furthermore, the link between stretch-induced calcium influx and Rho-activation is not well defined and requires further investigation, as does the compartmentation of calcium signaling to support growth and differentiation, respectively, via different influx pathways. Most importantly however, we need to clarify if the mechanisms that are presented herein can also regulate remodeling and contractile differentiation in human arteries and veins. In a larger perspective, we need to understand in which situations contractile differentiation of smooth muscle cells is beneficial or detrimental for vascular disease. An increased contractility of smooth muscle may be negative if the therapeutic goal is to reduce blood pressure. On the other hand, maintaining smooth muscle cells in a contractile and quiescent state may confer protection against neointima formation. In the atherosclerotic plaque, smooth muscle migration and proliferation contribute to the occlusion of the vessel but also protect against plaque rupture by forming a fibrous cap. Thus, one future goal should be to identify specific mechanisms that are involved in separate vascular disease states in order to therapeutically target these mechanisms. The newly identified regulation of smooth muscle phenotype by non-coding RNAs is a promising approach in this regard.

Acknowledgement

This work was supported by the Swedish Research Council; the Swedish Heart and Lung Foundation, the Crafoord Foundation; the Royal Physiographic Society; the Åke Wiberg Foundation; the Jeansson Foundation; the Tore Nilson Foundation; the Greta and Johan Kock Foundation; the Magnus Bergvall Foundation and the Lars Hierta Memorial Foundation. A.B was supported by the European Union FP7 Marie Curie Initial Training Network Small Artery Remodeling (SmArt).

References

1. Aalkjaer C, Heagerty AM, Petersen KK, Swales JD, Mulvany MJ. Evidence for increased media thickness, increased neuronal amine uptake, and depressed excitation--contraction coupling in isolated resistance vessels from essential hypertensives. *Circ Res* **61**: 181-186, 1987.

2. Albinsson S, Hellstrand P. Integration of signal pathways for stretch-dependent growth and differentiation in vascular smooth muscle. *Am J Physiol Cell Physiol* **293**: C772-782, 2007.

3. Albinsson S, Nordström I, Hellstrand P. Stretch of the vascular wall induces smooth muscle differentiation by promoting actin polymerization. *J Biol Chem* **279**: 34849-34855, 2004.

4. Albinsson S, Sessa WC. Can microRNAs control vascular smooth muscle phenotypic modulation and the response to injury? *Physiol Genomics*, 2010.

5. Albinsson S, Skoura A, Yu J, Dilorenzo A, Fernandez-Hernando C, Offermanns S, Miano JM, Sessa WC. Smooth Muscle miRNAs Are Critical for Post-Natal Regulation of Blood Pressure and Vascular Function. *PLoS One* **6**: e18869, 2011.

6. Albinsson S, Suarez Y, Skoura A, Offermanns S, Miano JM, Sessa WC. MicroRNAs are necessary for vascular smooth muscle growth, differentiation, and function. *Arterioscler Thromb Vasc Biol* **30**: 1118-1126, 2010.

7. Albinsson S, Swärd K. Targeting smooth muscle microRNAs for therapeutic benefit in vascular disease. *Pharmacol Res*, 2013.

8. Bakker EN, van der Meulen ET, van den Berg BM, Everts V, Spaan JA, VanBavel E. Inward remodeling follows chronic vasoconstriction in isolated resistance arteries. *J Vasc Res* **39**: 12-20, 2002.

9. Barlow CA, Rose P, Pulver-Kaste RA, Lounsbury KM. Excitation-transcription coupling in smooth muscle. *J Physiol* **570**: 59-64, 2006.

10. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**: 281-297, 2004.

11. Bayliss WM. On the local reactions of the arterial wall to changes of internal pressure. *J Physiol* **28**: 220-231, 1902.

12. Birukov KG, Bardy N, Lehoux S, Merval R, Shirinsky VP, Tedgui A. Intraluminal pressure is essential for the maintenance of smooth muscle caldesmon and filamin content in aortic organ culture. *Arterioscler Thromb Vasc Biol* **18**: 922-927, 1998.

13. Birukov KG, Lehoux S, Birukova AA, Merval R, Tkachuk VA, Tedgui A. Increased Pressure Induces Sustained Protein Kinase C–Independent Herbimycin A–Sensitive Activation of Extracellular Signal–Related Kinase 1/2 in the Rabbit Aorta in Organ Culture. *Circ Res* **81**: 895-903, 1997.

14. Boettger T, Beetz N, Kostin S, Schneider J, Kruger M, Hein L, Braun T. Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster. *J Clin Invest* **119**: 2634-2647, 2009.

15. Bubb MR, Spector I, Beyer BB, Fosen KM. Effects of jasplakinolide on the kinetics of actin polymerization. An explanation for certain in vivo observations. *J Biol Chem* **275**: 5163-5170, 2000.

16. Chen H, Untiveros GM, McKee LA, Perez J, Li J, Antin PB, Konhilas JP. Micro-RNA-195 and -451 regulate the LKB1/AMPK signaling axis by targeting MO25. *PLoS One* **7**: e41574, 2012.

17. Cheng Y, Liu X, Yang J, Lin Y, Xu DZ, Lu Q, Deitch EA, Huo Y, Delphin ES, Zhang C. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. *Circ Res* **105**: 158-166, 2009.

18. Cobb BS, Nesterova TB, Thompson E, Hertweck A, O'Connor E, Godwin J, Wilson CB, Brockdorff N, Fisher AG, Smale ST, Merkenschlager M. T cell lineage choice and differentiation in the absence of the RNase III enzyme Dicer. *J Exp Med* **201**: 1367-1373, 2005.

19. Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, Lee TH, Miano JM, Ivey KN, Srivastava D. miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature* **460**: 705-710, 2009.

20. Davis BN, Hilyard AC, Lagna G, Hata A. SMAD proteins control DROSHA-mediated microRNA maturation. *Nature* **454**: 56-61, 2008.

21. Davis BN, Hilyard AC, Nguyen PH, Lagna G, Hata A. Induction of microRNA-221 by platelet-derived growth factor signaling is critical for modulation of vascular smooth muscle phenotype. *J Biol Chem* **284**: 3728-3738, 2009.

22. Davis MJ, Hill MA. Signaling mechanisms underlying the vascular myogenic response. *Physiol Rev* **79**: 387-423, 1999.

23. Dore LC, Amigo JD, Dos Santos CO, Zhang Z, Gai X, Tobias JW, Yu D, Klein AM, Dorman C, Wu W, Hardison RC, Paw BH, Weiss MJ. A GATA-1-regulated microRNA locus essential for erythropoiesis. *Proc Natl Acad Sci U S A* **105**: 3333-3338, 2008.

24. Elia L, Quintavalle M, Zhang J, Contu R, Cossu L, Latronico MV, Peterson KL, Indolfi C, Catalucci D, Chen J, Courtneidge SA, Condorelli G. The knockout of miR-143 and -145 alters smooth muscle cell maintenance and vascular homeostasis in mice: correlates with human disease. *Cell Death Differ*, 2009.

25. Enouri S, Monteith G, Johnson R. Characteristics of myogenic reactivity in isolated rat mesenteric veins. *Am J Physiol Regul Integr Comp Physiol* **300**: R470-478, 2011.

26. Fisher SA. Vascular smooth muscle phenotypic diversity and function. *Physiol Genomics* **42A**: 169-187, 2010.

27. Folkow B. Physiological aspects of primary hypertension. *Physiol Rev* **62**: 347-504, 1982.

28. Godlewski J, Nowicki MO, Bronisz A, Nuovo G, Palatini J, De Lay M, Van Brocklyn J, Ostrowski MC, Chiocca EA, Lawler SE. MicroRNA-451 regulates LKB1/AMPK signaling and allows adaptation to metabolic stress in glioma cells. *Mol Cell* **37**: 620-632, 2010.

29. Guilluy C, Bregeon J, Toumaniantz G, Rolli-Derkinderen M, Retailleau K, Loufrani L, Henrion D, Scalbert E, Bril A, Torres RM, Offermanns S, Pacaud P, Loirand G. The Rho exchange factor Arhgef1 mediates the effects of angiotensin II on vascular tone and blood pressure. *Nat Med* **16**: 183-190, 2010.

30. Hellstrand P, Albinsson S. Stretch-dependent growth and differentiation in vascular smooth muscle: role of the actin cytoskeleton. *Can J Physiol Pharmacol* **83**: 869-875, 2005.

31. Holtwick R, Gotthardt M, Skryabin B, Steinmetz M, Potthast R, Zetsche B, Hammer RE, Herz J, Kuhn M. Smooth muscle-selective deletion of guanylyl cyclase-A prevents the acute but not chronic effects of ANP on blood pressure. *Proc Natl Acad Sci U S A* **99**: 7142-7147, 2002.

32. Ji R, Cheng Y, Yue J, Yang J, Liu X, Chen H, Dean DB, Zhang C. MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. *Circ Res* **100**: 1579-1588, 2007.

33. Johansson B. Structural and functional changes in rat portal veins after experimental portal hypertension. *Acta Physiol Scand* **98**: 381-383, 1976.

34. Johansson B, Mellander S. Static and dynamic components in the vascular myogenic response to passive changes in length as revealed by electrical and mechanical recordings from the rat portal vein. *Circ Res* **36**: 76-83, 1975.

35. Kauffenstein G, Laher I, Matrougui K, Guerineau NC, Henrion D. Emerging role of G protein-coupled receptors in microvascular myogenic tone. *Cardiovasc Res* **95**: 223-232, 2012.

36. Knot HJ, Nelson MT. Regulation of arterial diameter and wall [Ca2+] in cerebral arteries of rat by membrane potential and intravascular pressure. *J Physiol* **508 (Pt 1)**: 199-209, 1998.

37. Korsgaard N, Aalkjaer C, Heagerty AM, Izzard AS, Mulvany MJ. Histology of subcutaneous small arteries from patients with essential hypertension. *Hypertension* **22**: 523-526, 1993.

38. Langton PD. Calcium channel currents recorded from isolated myocytes of rat basilar artery are stretch sensitive. *J Physiol* **471**: 1-11, 1993.

39. Lehoux S, Esposito B, Merval R, Tedgui A. Differential regulation of vascular focal adhesion kinase by steady stretch and pulsatility. *Circulation* **111**: 643-649, 2005.

40. Liu X, Cheng Y, Zhang S, Lin Y, Yang J, Zhang C. A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. *Circ Res* **104**: 476-487, 2009.

41. Malmqvist U, Arner A. Contractile properties during development of hypertrophy of the smooth muscle in the rat portal vein. *Acta Physiol Scand* **133**: 49-61, 1988.

42. Malmqvist U, Arner A. Isoform distribution and tissue contents of contractile and cytoskeletal proteins in hypertrophied smooth muscle from rat portal vein. *Circ Res* **66**: 832-845, 1990.

43. Martinez-Lemus LA, Hill MA, Bolz SS, Pohl U, Meininger GA. Acute mechanoadaptation of vascular smooth muscle cells in response to continuous arteriolar vasoconstriction: implications for functional remodeling. *FASEB J* **18**: 708-710, 2004.

44. Martinez-Lemus LA, Hill MA, Meininger GA. The plastic nature of the vascular wall: a continuum of remodeling events contributing to control of arteriolar diameter and structure. *Physiology (Bethesda)* **24**: 45-57, 2009.

45. Mathiassen ON, Buus NH, Sihm I, Thybo NK, Morn B, Schroeder AP, Thygesen K, Aalkjaer C, Lederballe O, Mulvany MJ, Christensen KL. Small artery structure is an independent predictor of cardiovascular events in essential hypertension. *J Hypertens* **25**: 1021-1026, 2007.

46. Mederos y Schnitzler M, Storch U, Gudermann T. AT1 receptors as mechanosensors. *Curr Opin Pharmacol* **11**: 112-116, 2011.

47. Miralles F, Posern G, Zaromytidou AI, Treisman R. Actin dynamics control SRF activity by regulation of its coactivator MAL. *Cell* **113**: 329-342, 2003.

48. Moreno-Dominguez A, Colinas O, El-Yazbi A, Walsh EJ, Hill MA, Walsh MP, Cole WC. Ca2+ sensitization due to myosin light chain phosphatase inhibition and cytoskeletal reorganization in the myogenic response of skeletal muscle resistance arteries. *J Physiol* **591**: 1235-1250, 2013.

49. Mulvany MJ. Small artery remodelling in hypertension: causes, consequences and therapeutic implications. *Med Biol Eng Comput* **46**: 461-467, 2008.

50. Nakamura S, Hayashi K, Iwasaki K, Fujioka T, Egusa H, Yatani H, Sobue K. Nuclear import mechanism for myocardin family members and their correlation with vascular smooth muscle cell phenotype. *J Biol Chem* **285**: 37314-37323, 2010.

51. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. *Physiol Rev* **84**: 767-801, 2004.

52. Pawlowski R, Rajakyla EK, Vartiainen MK, Treisman R. An actin-regulated importin alpha/beta-dependent extended bipartite NLS directs nuclear import of MRTF-A. *EMBO J* **29**: 3448-3458, 2010.

53. Ratz PH, Berg KM, Urban NH, Miner AS. Regulation of smooth muscle calcium sensitivity: KCl as a calcium-sensitizing stimulus. *Am J Physiol Cell Physiol* **288**: C769-783, 2005.

54. Ren J, Albinsson S, Hellstrand P. Distinct effects of voltage- and store-dependent calcium influx on stretch-induced differentiation and growth in vascular smooth muscle. *J Biol Chem* **285**: 31829-31839, 2010.

55. Retailleau K, Toutain B, Galmiche G, Fassot C, Sharif-Naeini R, Kauffenstein G, Mericskay M, Duprat F, Grimaud L, Merot J, Lardeux A, Pizard A, Baudrie V, Jeunemaitre X, Feil R, Gothert JR, Lacolley P, Henrion D, Li Z, Loufrani L. Selective involvement of serum response factor in pressure-induced myogenic tone in resistance arteries. *Arterioscler Thromb Vasc Biol* **33**: 339-346, 2013.

56. Rizzoni D, Porteri E, Boari GE, De Ciuceis C, Sleiman I, Muiesan ML, Castellano M, Miclini M, Agabiti-Rosei E. Prognostic significance of small-artery structure in hypertension. *Circulation* **108**: 2230-2235, 2003.

57. Rizzoni D, Porteri E, Guefi D, Piccoli A, Castellano M, Pasini G, Muiesan ML, Mulvany MJ, Rosei EA. Cellular hypertrophy in subcutaneous small arteries of patients with renovascular hypertension. *Hypertension* **35**: 931-935, 2000.

58. Rizzoni D, Porteri E, Guelfi D, Muiesan ML, Valentini U, Cimino A, Girelli A, Rodella L, Bianchi R, Sleiman I, Rosei EA. Structural alterations in subcutaneous small arteries of normotensive and hypertensive patients with non-insulin-dependent diabetes mellitus. *Circulation* **103**: 1238-1244, 2001.

59. Ronkainen JJ, Hanninen SL, Korhonen T, Koivumaki JT, Skoumal R, Rautio S, Ronkainen VP, Tavi P. Ca2+-calmodulin-dependent protein kinase II represses cardiac transcription of the L-type calcium channel alpha(1C)-subunit gene (Cacna1c) by DREAM translocation. *J Physiol* **589**: 2669-2686, 2011.

60. Sadegh MK, Ekman M, Rippe C, Uvelius B, Sward K, Albinsson S. Deletion of dicer in smooth muscle affects voiding pattern and reduces detrusor contractility and neuroeffector transmission. *PLoS One* **7**: e35882, 2012.

61. Sakurada S, Takuwa N, Sugimoto N, Wang Y, Seto M, Sasaki Y, Takuwa Y. Ca2+-dependent activation of Rho and Rho kinase in membrane depolarization-induced and receptor stimulation-induced vascular smooth muscle contraction. *Circ Res* **93**: 548-556, 2003.

62. Singer HA. Ca2+/calmodulin-dependent protein kinase II Function in Vascular Remodeling. *J Physiol*, **590**: 1349–1356, 2012.

63. Sotiropoulos A, Gineitis D, Copeland J, Treisman R. Signal-regulated activation of serum response factor is mediated by changes in actin dynamics. *Cell* **98**: 159-169, 1999.

64. Staiculescu MC, Galinanes EL, Zhao G, Ulloa U, Jin M, Beig MI, Meininger GA, Martinez-Lemus LA. Prolonged vasoconstriction of resistance arteries involves vascular smooth muscle actin polymerization leading to inward remodelling. *Cardiovasc Res* **98**: 428-436, 2013.

65. Sutter MC. The mesenteric-portal vein in research. *Pharmacol Rev* **42**: 287-325, 1990.

66. Swärd K, Mita M, Wilson DP, Deng JT, Susnjar M, Walsh MP. The role of RhoA and Rho-associated kinase in vascular smooth muscle contraction. *Curr Hypertens Rep* **5**: 66-72, 2003.

67. Thievent A, Connat JL. Cytoskeletal features in longitudinal and circular smooth muscles during development of the rat portal vein. *Cell Tissue Res* **279**: 199-208, 1995.

68. Turczynska KM, Bhattachariya A, Säll J, Goransson O, Sward K, Hellstrand P, Albinsson S. Stretch-Sensitive Down-Regulation of the miR-144/451 Cluster in Vascular Smooth Muscle and Its Role in AMP-Activated Protein Kinase Signaling. *PLoS One* **8**: e65135, 2013.

69. Turczynska KM, Hellstrand P, Swärd K, Albinsson S. Regulation of vascular smooth muscle mechanotransduction by microRNAs and L-type calcium channels. *Commun Integr Biol* **6**: e22278, 2013.

70. Turczynska KM, Sadegh MK, Hellstrand P, Swärd K, Albinsson S. MicroRNAs are essential for stretch-induced vascular smooth muscle contractile differentiation via microRNA (miR)-145-dependent expression of L-type calcium channels. *J Biol Chem* **287**: 19199-19206, 2012.

71. Uvelius B, Arner A, Johansson B. Structural and mechanical alterations in hypertrophic venous smooth muscle. *Acta Physiol Scand* **112**: 463-471, 1981.

72. Walsh MP, Cole WC. The role of actin filament dynamics in the myogenic response of cerebral resistance arteries. *J Cereb Blood Flow Metab* **33**: 1-12, 2013.

73. Wamhoff BR, Bowles DK, McDonald OG, Sinha S, Somlyo AP, Somlyo AV, Owens GK. L-type voltage-gated Ca2+ channels modulate expression of smooth muscle differentiation marker genes via a rho kinase/myocardin/SRF-dependent mechanism. *Circ Res* **95**: 406-414, 2004.

74. Wamhoff BR, Bowles DK, Owens GK. Excitation-transcription coupling in arterial smooth muscle. *Circ Res* **98**: 868-878, 2006.

75. Wang DZ, Li S, Hockemeyer D, Sutherland L, Wang Z, Schratt G, Richardson JA, Nordheim A, Olson EN. Potentiation of serum response factor activity by a family of myocardin-related transcription factors. *Proc Natl Acad Sci U S A* **99**: 14855-14860, 2002.

76. Wirth A, Benyo Z, Lukasova M, Leutgeb B, Wettschureck N, Gorbey S, Orsy P, Horvath B, Maser-Gluth C, Greiner E, Lemmer B, Schutz G, Gutkind JS, Offermanns S. G12-G13-LARG-mediated signaling in vascular smooth muscle is required for salt-induced hypertension. *Nat Med* **14**: 64-68, 2008.

77. Xin M, Small EM, Sutherland LB, Qi X, McAnally J, Plato CF, Richardson JA, Bassel-Duby R, Olson EN. MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. *Genes Dev* **23**: 2166-2178, 2009.

78. Ying Z, Giachini FR, Tostes RC, Webb RC. PYK2/PDZ-RhoGEF links Ca2+ signaling to RhoA. *Arterioscler Thromb Vasc Biol* **29**: 1657-1663, 2009.

79. Zeidan A, Nordström I, Albinsson S, Malmqvist U, Sward K, Hellstrand P. Stretch-induced contractile differentiation of vascular smooth muscle: sensitivity to actin polymerization inhibitors. *Am J Physiol Cell Physiol* **284**: C1387-1396, 2003.

80. Zeidan A, Nordström I, Dreja K, Malmqvist U, Hellstrand P. Stretch-Dependent Modulation of Contractility and Growth in Smooth Muscle of Rat Portal Vein. *Circ Res* **87**: 228-234, 2000.

Figure legend

Figure 1. Simplified scheme showing some of the stretch- and calcium-dependent pathways for smooth muscle cell (SMC) differentiation and growth discussed in this review. Stretch activates integrins (α/β) and associated focal adhesion proteins, such as focal adhesion kinase (FAK), Src and proline-rich tyrosine kinase-2 (PYK2) with downstream effects on Rho-dependent actin polymerization as well as on MAPK-ERK-dependent growth/proliferation. In addition, stretch causes membrane depolarization, possibly via stretch-dependent ion channels (not shown), which in turn activates voltage-dependent L-type calcium channels (LTCC). This also activates the Rho/Rho-kinase (ROCK) pathway, possibly (dotted line) via indirectly calcium-sensitive Rho- Guanine nucleotide exchange factor (GEF). Another calcium entry pathway is via non-voltage-dependent channels, here exemplified by store-operated channels (SOC), although several different classes of channel proteins may be involved. The resultant calcium inflow shows selectivity for regulating activity of the MAP-kinase (MAPK) cascade, possibly (dotted line) via interaction with PYK2 and/or multifunctional calcium-calmodulin dependent kinases (CamK). Rho/ROCK promotes polymerization of globular actin (G-actin) into filamentous actin (F-actin), partly via Lim-kinase (LIMK) mediated inhibition of the actin depolymerizing factor cofilin. This then drives the expression of smooth muscle proteins by promoting nuclear import of myocardin related transcription factor (MRTF), a co-activator of serum response factor (SRF)-dependent gene expression in synergy with myocardin (Myocd). MAPK-ERK activity stimulates nuclear import of ternary complex factors (TCF), acting as cofactors of SRF for expression of immediate-early genes, driving growth/proliferation. MicroRNAs may influence this scheme at several levels. Illustrated here is the effect of miR-145 to increase myocardin as well as LTCC expression, both of which effects contribute to increased smooth muscle gene expression and contractile differentiation. The figure is modified from [30,69].