Regulation of vascular smooth muscle mechanotransduction by microRNAs and L-type calcium channels

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The phenotype of smooth muscle cells is regulated by multiple environmental factors including mechanical forces. Mechanical stretch of mouse portal veins ex vivo has been shown to promote contractile differentiation by activation of the Rho-pathway, an effect that is dependent on the influx of calcium via L-type calcium channels. MicroRNAs have recently been demonstrated to play a significant role in the control of smooth muscle phenotype and in a recent report we investigated their role in vascular mechanosensing. By smooth muscle specific deletion of Dicer, we found that microRNAs are essential for smooth muscle differentiation in response to stretch by regulating CamKIIδ and L-type calcium channel expression. Furthermore, we suggest that loss of L-type calcium channels in Dicer KO is due to reduced expression of the smooth muscle-enriched microRNA, miR-145, which targets CamKIIδ. These results unveil a novel mechanism for miR-145 dependent regulation of smooth muscle phenotype.
of particular importance. In a recent study, we examined and a number of specific miRNAs have been identified to be involved in smooth muscle mechanosensing but the role of miRNAs in smooth muscle actin polymerization remains largely unknown.

Dicer 

KO portal veins exhibited a reduced expression of the pore forming α1C subunit of voltage dependent L-type calcium channels at both mRNA (Cacna1c) and protein (Cav1.2) levels. As mentioned earlier, inhibition of L-type calcium channels using verapamil or nifedipine is sufficient to prevent stretch-induced contractile differentiation. A key finding of our recent study was a close correlation between effects on force of Ca2+-channel inhibition and Dicer deletion, respectively. This suggests that Dicer deletion impairs force in smooth muscle in part via effects on L-type Ca2+ channels. The observed correlation is illustrated in Figure 2, where the effects of Dicer KO on responses to contractile agonists are seen to correlate with the effects of nifedipine in wild type vessels. The reduced Cacna1c expression in Dicer KO portal veins suggests a transcriptional effect on the L-type calcium channels mediated by miRNAs. Since miRNAs generally repress protein translation of their target we hypothesized that L-type calcium channels could be indirectly regulated in Dicer KO portal veins by a transcription factor or signaling molecule that inhibits L-type calcium channel expression and is upregulated in the absence of miRNAs. In a recent study by Ronkainen et al. CamKIIδ was shown to inhibit L-type calcium channel expression via the transcriptional inhibitor calsenilin/DREAM/KChIP3. Furthermore, CamKII KO mice display an increased expression of L-type calcium channels in cardiomyocytes. In addition to DREAM translocation, the effect of CamKII KO may depend on decreased nuclear translocation of the NFκB component p65, which suppresses transcription of Cacna1c. Since CamKIIδ is a confirmed target of miR-145 in vascular smooth muscle cells and is upregulated in Dicer KO portal veins, we hypothesized that miR-145 could regulate L-type calcium channels via CamKII. To test this hypothesis, we used isolated smooth muscle cells in culture transfected with miR-145 inhibitor. Interestingly, inhibition of miR-145 resulted in a reduction of L-type calcium channel mRNA expression, which closely correlated with the effect observed in Dicer KO portal vein. This indicates that the reduced expression of L-type calcium channel in Dicer KO smooth muscle is primarily caused by loss of miR-145. Furthermore, the effect of miR-145 on L-type calcium channel expression could be prevented by the CamKII inhibitor, KN93.

MiR-145 has previously been demonstrated to promote smooth muscle differentiation by targeting multiple factors involved in the regulation of smooth muscle phenotype including Krüppel-like factors, myocardin and angiotensin converting enzyme. Regulation of the L-type calcium channel by miR-145 therefore represents an additional mechanism by which miRNAs can control smooth muscle differentiation and contractile function (Fig. 3). We and others have shown that miR-145 is involved in smooth muscle actin polymerization but the role of L-type calcium influx in this process is not fully understood. Although miR-145 has been shown to directly target several factors involved in actin dynamics it is tempting to speculate that miR-145 promotes actin polymerization via increased expression of L-type calcium channels.
Although we find the portal vein to be a robust model to investigate stretch-induced effects in vascular smooth muscle, the effects of Dicer KO may differ between veins and arteries and even between different arterial beds. It is therefore important to investigate the role of miRNAs in pressure-induced effects in small resistance arteries as well. Regulation of L-type calcium channel expression by miRNAs, may play a role in other cell types including cardiomyocytes and skeletal muscle. Indeed, we recently reported that smooth muscle specific deletion of Dicer also results in reduced expression of L-type calcium channels in smooth muscle of the urinary bladder, indicating that miRNAs regulate these channels in multiple tissues.26

Disclosure of Potential Conflicts of Interest

There were no potential conflicts of interest to disclose.

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Figure 3. Contractile differentiation of vascular smooth muscle cells is promoted by mechanical stretch and miR-145. Regulation of L-type calcium expression (LTCC exp) via miR-145 and possibly other miRNAs plays an important role for stretch-induced differentiation. Stretch activates the Rho/Rho-kinase (ROCK), which promotes actin polymerization partly via inhibition of coflin. Myocardin related transcription factor (MRTF) is then released from monomeric actin (G-actin) and translocates to the nucleus where it, as a co-factor to serum response factor (SRF), promotes smooth muscle differentiation. MicroRNA-145 also regulates contractile differentiation via additional targets such as angiotensin converting enzyme (ACE), Kruppel-like transcription factors (KLF) 4 and 5 and a direct positive regulation of myocardin (Myocd). Furthermore, it is likely that several so far unknown miRNAs are involved in smooth muscle cell (SMC) contractile differentiation. FAK, focal adhesion kinase; ME2, myocyte enhancer factor-2.

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