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Smooth(ing) Muscle Differentiation by MicroRNAs

Ronald L. Neppl[1] and **Da-Zhi Wang**[1]

[1]Department of Cardiology, Children's Hospital Boston, Harvard Medical School, Boston, MA

In a recent report in *Nature*, Cordes et al. (2009) demonstrate that miR-143 and miR-145 modulate smooth muscle cell (SMC) plasticity in part by regulating key transcription factors involved in SMC fate determination.

Recent studies have established microRNAs (miRNAs) as a class of critical mediators involved in the regulation of cell proliferation and differentiation in cardiac (van Rooij et al., 2007 and Zhao et al., 2007), skeletal (Chen et al., 2006), and smooth (Cheng et al., 2009) muscles. In addition, miRNAs have also been demonstrated to play a role in the maintenance of embryonic stem cell (ESC) pluripotency (Xu et al., 2009). Cordes et al. (2009) now link the function of miRNAs to smooth muscle cell (SMC) fate determination and plasticity by showing that miR-143 and miR-145 regulate the proliferation and differentiation of vascular SMCs.

During the early embryonic stages of vasculogenesis, SMCs and their progenitors are highly proliferative and migratory. However, in adult blood vessels, SMCs become quiescent and express a repertoire of contractile, calcium regulatory, and signal transduction proteins necessary for the contractile function of fully differentiated SMCs (Owens et al., 2004). Further, SMCs, unlike cardiac and skeletal myocytes, are not terminally differentiated and are capable of regaining their highly proliferative and migratory characteristics under certain conditions such as vascular injury. Expression of nearly all SMC marker genes is known to be dependent upon one or more serum response elements (SRE, $CC(AT)_6GG$ or CArG boxes) in their promoters/enhancers. Serum response factor (SRF) is known to regulate growth response genes as well as muscle-specific genes through its interaction with the muscle cell-enriched SRF cofactor myocardin (Wang et al., 2001). Further, it is well documented that SRF cofactors, many of which are antagonistic in action, are mechanistically involved in regulating phenotypic switching of SMCs between proliferation and differentiation, thus providing a molecular explanation of cell fate maintenance and change at the transcriptional level (Owens et al., 2004 and Wang et al., 2004). However, the functional significance of miRNAs during SMC differentiation remains uncertain. In particular, whether a specific miRNA is both necessary and sufficient to induce and direct the differentiation of quiescent SMCs in vitro and in vivo remains to be determined.

The Srivastava lab previously reported that miR-143 expression is dramatically increased when ESCs were induced to differentiate into cardiomyocytes (Ivey et al., 2008). In their

Corresponding Author: Da-Zhi Wang, Department of Cardiology, Children's Hospital Boston, Enders 1260, 320 Longwood Avenue, Boston, MA 02115, USA.

most recent report, Cordes et al. (2009) convincingly demonstrate that miR-143 and miR-145, which are clustered together and initially transcribed as a single transcript, are highly expressed in the developing heart and in vascular SMCs. The cardiac and smooth muscle expression pattern of miR-143/145 parallels that of myocardin, which (not surprisingly) was found to be SRF/myocardin dependent. After the initial analysis of miR-143 and miR-145 expression during development, Cordes et al. (2009) examined miR-143 and miR-145 expression as a function of phenotypic switching in a carotid artery ligation model. In agreement with a prior study (Cheng et al., 2009), the authors demonstrate a reduction in miR-143/145 expression in the neointimal lesion after vascular injury. In support, Cordes et al. (2009) showed that lentiviral delivery of miR-145 in postinjured carotid arteries induced the expression of some, but not all, SMC marker genes. This observation is consistent with the view that miR-145 inhibits ESC self-renewal and proliferation while it concurrently promotes cellular differentiation (Xu et al., 2009), suggesting that miR-143/145 negatively regulate cell proliferation in this setting.

Gain-of-function and loss-of-function approaches were applied to fibroblasts and multipotent neural crest stem cells in vitro to demonstrate that miR-145 was both sufficient and necessary for SMC differentiation. One of the most striking observations revealed by Cordes et al. (2009) was that miR-145 actually enhanced myocardin-mediated SM gene expression and SMC conversion. This finding is in contrast to the generally accepted view that miRNAs degrade mRNA targets and/or repress protein translation. Searching for the molecular mechanism, the authors found that miR-145 increased, rather than decreased, luciferase activity in the presence of the *Myocd* 3′UTR. Additionally, miR-143 and miR-145 were found to inhibit luciferase activity in the presence of either the *Elk1* or *Klf4* 3′UTR. Together, these results suggest that miR-143/145 positively induces the differentiation of quiescent SMCs by simultaneously repressing Elk-1 and Klf4 expression while inducing the expression of myocardin. Complicating these results is the observation that the *Myocd* 3′UTR mediated a repression in luciferase activity in Cos cells but not in 293T cells. Although the underlying mechanism remains unclear, it will be interesting to test whether a similar repression exists in 10T1/2 fibroblasts, in which miR-145 was not able to induce smooth muscle differentiation, and in neural crest stem cells, in which miR-145 potently induced smooth muscle differentiation. Whether the activation of myocardin by miR-145 is constitutively or contextually dependent upon the *Myocd* 3′UTR is an important question that warrants further investigation. Furthermore, elucidation of the mechanism whereby miR-145 activates myocardin may be of great clinical significance, given the ability of ectopic miR-145 to inhibit neointimal formation in balloon-injured carotid arteries (Cheng et al., 2009).

Though multiple miRNAs can target a single mRNA for translational repression and/or degradation, a single miRNA can induce translational repression and/or degradation of multiple mRNAs. Intriguingly, the data presented by Cordes et al. (2009) would suggest rather that miR-143/145 both initiates and maintains the smooth muscle differentiation program through enhancement of myocardin expression and activity. The authors suggest that miR-145 promotes SMC differentiation “in part by increasing Myocd [myocardin] protein and functioning in a feed-forward reinforcement of its own expression by the SRF-

Myocd [myocardin] complex" whereas miR-143 represses Elk-1 and Klf4, SRF cofactors that inhibit smooth muscle differentiation. Interestingly, miR-145 expression is also under the control of Oct4, in that highly expressed Oct4 inhibits miR-145 transcription in pluripotent ESCs (Xu et al., 2009). Upon ESC differentiation, Oct4 and other pluripotent transcription factors are downregulated, leading to the expression of miR-145. miR-145, in turn, suppresses pluripotency and promotes cellular differentiation, in part, by repressing the level of Oct4, Sox2, and Klf4. Thus, we have learned that miRNAs and their transcriptional regulators can modulate each other's expression and hence transcriptional activity, in a positive feedback, feed-forward, or double-negative feedback mechanism. It is therefore speculated that we will see similar miRNA-dependent mechanisms in various biological systems that maintain precise cellular homeostasis during development, cell proliferation, differentiation, and apoptosis.

Although the study by Cordes et al. (2009) revealed some very interesting functions of miRNAs in SMCs, with the implication of potential miRNA-based cardiovascular and cancer therapeutics, one should keep in mind that many of the conclusions were based upon in vitro experimental systems or overexpression approaches. For example, if miR-145 is required for myocardin activity during smooth muscle differentiation in vitro, one would expect that miR-145 knockout mice will phenocopy myocardin knockout mice, as predicted by the model of Cordes et al. (2009). Similarly, one would also predict that miR-143/145 null animals would exhibit defects in ESC self-renewal, proliferation, and cell fate commitment, given the vital role of those miRNAs reported by Cordes et al. (2009) and Xu et al. (2009). The in vivo biological role of miR-143/145, though uncertain, may prove to be a pivotal regulatory factor in ESC self renewal, cell fate commitment, and maintenance of the quiescent SMC phenotype. Determination of the upstream regulatory signals (normal or pathological) that modulate both the magnitude and temporal control of miR-143/145 expression will greatly enhance our understanding of the SMC differentiation program.

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