See related article, pages 880–893

MicroRNA133a: A New Variable in Vascular Smooth Muscle Cell Phenotypic Switching

Joseph M. Miano, Eric M. Small

The human genome is replete with digital information, only 1.2% of which comprises protein-coding sequence. The nonprotein-coding sequence encompasses >1 million regulatory elements controlling gene expression and codes for such genomic processes as recombination, replication, splicing, transposition, and structural integration of the genome with the surrounding nucleoskeleton. Throughout such punctuated sequence information is a vast amount of transcribed nonprotein-coding RNA whose functions have only begun to be understood. These facts debunk the old notion that our genome is comprised largely of "junk DNA" and point to an ever-expanding role for genomic DNA sequence in various biological processes. For example, a portion of the nonprotein-coding RNA transcriptome encodes some 1000 microRNAs (miRs) whose activity relates primarily to the posttranscriptional regulation of the mRNA pool of a cell through targeted mRNA degradation.[1] Accordingly, miRs function to influence essentially all aspects of cellular biology by tailoring the proteome of a cell. Since the initial reporting of miRs in human cells,[2] there has been an explosive increase in the number of PubMed articles (Figure 1) related to these "molecular rheostats," and there are high hopes for applying knowledge gained through this body of work in the treatment of numerous diseases in which aberrant expression of miRs has been reported across body systems.[3]

Various cells of the cardiovascular system express a number of miRs that have been the subject of intense study over the past few years. These include the bicistronic miR1/133a and miR143/145 genes that are highly specific to cardiac myocytes and smooth muscle cells (SMC), respectively.[4] The miR1/133a gene is of particular interest because it is duplicated on separate chromosomes; miR1-1/133a-2 is transcribed in an intron of a hypothetical locus in humans, whereas miR1-2/133a-1 is found on the opposite strand of the MIB1 locus, encoding an E3 ubiquitin-protein ligase. Consistent with their abundant expression in cardiac and skeletal muscle, both miR1/133a genes are under the transcriptional control of several myogenic regulatory factors, including serum response factor (SRF) and myocyte enhancer factor 2.[5,6] Further work showed miR1/133a control cardiac muscle growth and differentiation through their repressive action on a number of validated target genes.[4] However, the expression and function of miR133a in SMC have, until now, been largely unexplored.

In this issue of *Circulation Research*, Torella et al[7] have demonstrated new functions and validated target genes for miR133a that contribute to SMC phenotypic states both in vitro and in vivo. Using a battery of sensitive assays, Torella et al first demonstrated measureable expression of miR133a (but not miR1) in SMC of the vessel wall, as well as dissociated SMC in culture. The fact that miR1 levels are very low in SMC suggests differential posttranscriptional processing of miR1 versus miR133a in SMC as compared to cardiac myocytes. Using acute gain-of-function and loss-of-function experiments, the authors went on to show an inverse relationship between expression of miR133a and vascular SMC proliferation. Moreover, growth stimulation of SMC with platelet-derived growth factor resulted in a decrease in miR133a expression. These findings are reminiscent of similar phenomena seen with miR145, indicating a network of miRs that maintains a quiescent SMC phenotype is subject to downregulation on cell-cycle entry. Torella et al next examined the effect of modulating miR133a levels on expression of several SMC differentiation genes. In contrast to miR145, which promotes expression of several SMC contractile genes,[8] miR133a inhibited the SMC markers, CNN1 and ACTA2. There was no change in mRNA expression of myocardin, the major transcriptional switch for SMC differentiation.[9] However, levels of SRF mRNA and protein were reduced with miR133a, a result consistent with previous work from Chen et al.[10] These results suggest that lower levels of SRF probably accounted for the reduced expression of CNN1 and ACTA2, both of which are known SRF target genes.[11] CNN1 and ACTA2 are expressed transiently in embryonic cardiac myocytes and do not show expression in adult cardiac myocytes unless the heart undergoes failure, and then these genes may be reactivated as part of the fetal gene program. In this context, Liu et al[12] showed a heart failure phenotype with elevated expression of CNN1 and ACTA2 in mice in which both miR133a alleles were genetically inactivated. In contrast to the attenuated expression of CNN1 and ACTA2 with miR133a, Torella et al observed increases in MYH11, which is not expressed in the developing heart and is the gold standard marker for SMC lineages.[13] How miR133a increased expression of the SRF-dependent MYH11 gene required some astute observations related to putative miR133a target transcripts.

A major bottleneck in miR research is the confident identification of target mRNAs and how such targeting

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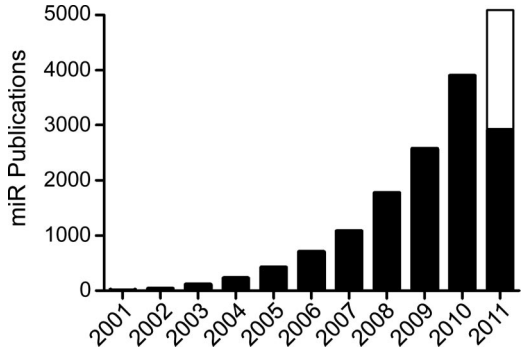

Figure 1. Number of articles published on microRNAs over the past 11 years. The white bar represents the predicted number of additional articles to be published in 2011.

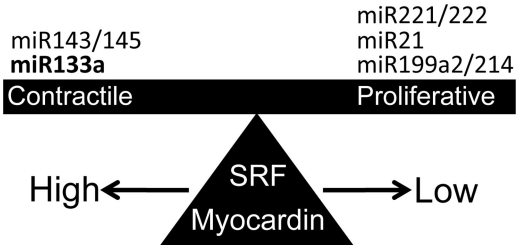

Figure 2. Known microRNAs involved in regulating smooth muscle cell (SMC) phenotype. The primary molecular switch for the SMC contractile phenotype is serum response factor (SRF)/myocardin. When levels of SRF/myocardin are elevated (*left*), the SMC contractile phenotype is promoted, in part, through increases in miR143/145 and miR133a. However, a reduction in SRF/myocardin, as occurs with growth stimulation or injury to the vessel wall, is associated with a proliferative SMC phenotype, decreases in miR143/145 and miR133a, and a new circuit of miR gene expression (*right*). The latter include the bicistronic miR221/222[20,21] and miR199a2/214[22] genes, as well as miR21.[23]

impacts biological processes. Several computer programs are available to assist investigators in this endeavor; however, each program uses slightly different criteria for scoring an mRNA transcript as a possible target, making the task for target mRNA identification challenging. Nevertheless, Torella et al noted two putative miR133a targets and went on to show miR133a-mediated repression of the SP1 zinc finger-containing transcription factor and muscle-restricted moesin, encoding an actin-binding protein.[7] Because elevated SP1 expression is associated with SMC hyperplasia in the vessel wall after injury,[14] Torella et al considered whether the inhibitory action of miR133a on SMC growth was linked to its effects on SP1. Indeed, expression of SP1 carrying a mutation in the miR133a binding site, located in the 3′ untranslated region, rescued the growth inhibition conferred by miR133a in SMC. Although miR133a was shown to reduce SMC migration, whether a mutant form of SP1 or moesin rescues this migration phenotype is presently unknown. However, the SP1 mutant was able to antagonize the increase in MYH11 observed with miR133a overexpression. This finding is consistent with work from the laboratory of Owens[15] showing repression of MYH11 through an SP1-binding GC-rich element in the upstream promoter region.

The in vitro findings of Torella et al were then extended to an in vivo model of vascular SMC hyperplasia and lesion development (balloon injury of rat carotid artery). Levels of miR133a were reduced after injury, which is similar to the expression changes reported for miR143/145.[8,16] In contrast, miR221, SP1, and moesin were all increased after injury. Adenoviral transfer of miR133a to the vessel wall attenuated proliferating SMC and reduced the increases seen in SP1 and moesin protein expression, thus confirming the in vitro findings in an animal model of SMC hyperplasia. More importantly, miR133a reduced and antimiR133a augmented neointimal formation 14 days after balloon injury. These results establish a pathophysiologically relevant role for miR133a in minimizing SMC phenotypic adaptation and resultant lesion formation.

The detailed work of Torella et al provides new insight into a miR previously thought to be limited in function to striated muscle growth and differentiation. That miR133a and miR143/145 both require SRF for expression and show overlapping functions in controlling SMC phenotype, highlights redundant miR-dependent pathways for the maintenance of a contractile SMC phenotype. These miRs are counterbalanced by other miRs on phenotypic switching of SMC (Figure 2). Therefore, what emerges is a complex web of feedback and feed-forward circuits involving transcription factors, their target miRs, and the miR target mRNAs themselves whose encoded proteins perform biological functions related to cellular phenotypic states. The work of Torella et al provokes as many questions as those answered in the series of elegant experiments presented. First, we still have limited knowledge regarding the full repertoire of mRNAs targeted by miRs in vascular SMC. Next-generation sequencing after either miR overexpression or Argonaute pull-down will likely provide further insight into how miRs maintain vascular SMC homeostasis. Second, it will be interesting to ascertain the relative expression levels of each miR133a transcript (133a-1 vs 133a-2) and understand why levels of miR1 are so low in SMC despite its assumed presence in the primary miR transcript. Delineating the potential roles of miR1 and miR133a during formation of the heart and SMC lineages may provide further insight into the factors governing reactivation of the fetal gene programs associated with cardiac and vascular remodeling. Finally, there is mounting evidence that a combinatorial microRNA code exists, similar to that observed with transcriptional regulators, with multiple seemingly unrelated miRs regulating the same transcript or a common biological process.[17] Thus, it will be informative to generate compound mutants of miR143/145 and miR133a to determine whether the absence of the latter will evoke more dramatic vascular phenotypes than those previously reported for miR143/145.[18,19]

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