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. 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, 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.

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. 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 miR133a genes are under the transcriptional control of several myogenic regulatory factors, including serum response factor (SRF) and myocyte enhancer factor 2. Further work showed miR1/133a control cardiac muscle growth and differentiation through their repressive action on a number of validated target genes. However, the expression and function of miR133a in SMC have, until now, been largely unexplored.

In this issue of Circulation Research, Torella et al 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, miR133a inhibited the SMC markers, CNN1 and ACTA2. There was no change in mRNA expression of myocardin, the major transcriptional switch for SMC differentiation. However, levels of SRF mRNA and protein were reduced with miR133a, a result consistent with previous work from Chen et al. 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. 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 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. 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
overlapping functions in controlling SMC phenotype, high-
muscle growth and differentiation. That miR133a and
results establish a pathophysiologically relevant role for
neointimal formation 14 days after balloon injury. These
importantly, miR133a reduced and antimiR133a augmented
findings in an animal model of SMC hyperplasia. More
moesin protein expression, thus confirming the in vitro
proliferating SMC and reduced the increases seen in SP1 and
Adenoviral transfer of miR133a to the vessel wall attenuated
miR221, SP1, and moesin were all increased after injury.

This finding is consistent with work from the laboratory of
Owens15 showing repression of MYH11 through an SP1-
creasing the same transcript or a common biological process.17
regulators, with multiple seemingly unrelated miRs regulat-
associated with cardiac and vascular remodeling. Finally,
the factors governing reactivation of the fetal gene programs
potential roles of miR1 and miR133a during formation of the
nance of a contractile SMC phenotype. These miRs are
counterbalanced by other miRs on phenotypic switching of
S MC (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
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 regulat-
ing 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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Joseph M. Miano and Eric M. Small

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