

Long Noncoding RNAs and Angiogenesis

Regulatory Information for Chromatin Remodeling

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Accumulating evidence supports the notion that the mammalian genome is pervasively transcribed. A large proportion of the transcripts do not encode a protein and are thus regarded as noncoding RNAs. Based on their length, they can be divided into small RNAs (<200 nucleotides) and long noncoding RNAs (lncRNAs; >200 nucleotides).^{1,2} lncRNAs can be encoded in the same or opposite strand to protein-coding DNA and can be either within or separate to protein-coding genes. They can be exported to the cytoplasm or remain in the nucleus. Although their function is not fully understood, lncRNAs have been reported to mediate the expression of other genes, affect the organization of the nucleus, and modify other RNAs.^{1,2}

In this issue of *Circulation*, Leisegang and colleagues³ identify a novel lncRNA that regulates angiogenesis. Previous work by this group highlighted the role of a histone demethylase, the protein JARID1B, in maintaining the angiogenic capacity of human umbilical vein endothelial cells.⁴ In the present study, the authors performed a transcriptomic screen following knockdown of JARID1B in human umbilical vein endothelial cells. They discovered a novel lncRNA, named MANTIS, to be strongly upregulated. MANTIS is located in the antisense strand of an intronic region of the gene for Annexin A4, a calcium and phospholipid binding protein. MANTIS is a nuclear lncRNA that is enriched in endothelial cells but also expressed in other cell types. Reducing MANTIS levels led to impaired endothelial sprouting, tube formation, and attenuated endothelial migration. It is notable that human umbilical vein endothelial cells lost their ability to orientate themselves in the direction of flow. On silencing MANTIS, gene expression profiling revealed downregulation of angiogenesis-related genes.

Brahma-like gene 1 (BRG1) was identified as a direct interaction partner of MANTIS, implying a role of MANTIS in the formation of the switch/sucrose non-fermentable (SWI/SNF) complex (Figure). The SWI/SNF complex is one of the best characterized chromatin remodeling complexes. It uses energy released from ATP hydrolysis to alter the shape of chromatin, making DNA accessible for transcription initiation and thus promoting gene expression.⁵ Different catalytic subunits that confer this ATPase activity can be recruited to the complex. In the cardiovascular system, Brahma or BRG1 are particularly important and mutually exclusive.⁶ Binding of lncRNAs to specific ATPase subunits of the SWI/SNF complex has emerged as a common regulatory mechanism.⁷⁻¹⁰ Recruitment of specific lncRNAs might confer uniqueness to the ubiquitous SWI/SNF complex and allows it to target a distinct group of genes in different cell types (Figure).

In the case of MANTIS, binding to BRG1 was shown to stabilize the BRG1 interaction with the chromatin remodeling factor BAF155. BAF155 affects the ATPase activity of BRG1. It is intriguing that this finding was shown to occur in the transcription initia-

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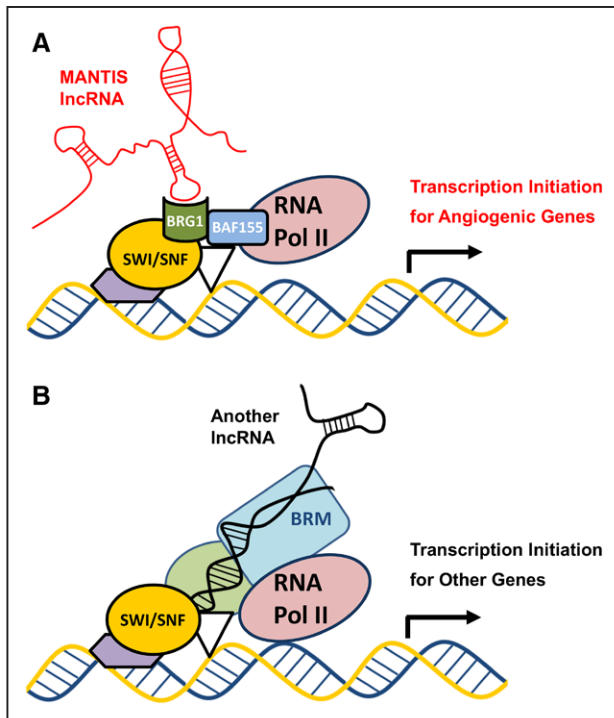


Figure. Interaction of long noncoding RNAs (lncRNAs) with the ATPase catalytic subunits confers specificity to the switch/sucrose nonfermentable (SWI/SNF) chromatin remodeling complex.

A, The proposed model of interaction for MANTIS in the SWI/SNF complex. Direct binding of MANTIS to the ATPase catalytic subunit BRG1 of the complex strengthens the Brahma-like gene 1 (BRG1) interaction with BAF155 and facilitates nucleosome remodeling and transcription initiation in a subset of angiogenic genes. **B**, A hypothetical lncRNA in the SWI/SNF complex may interact with the ATPase catalytic subunit Brahma (BRM) and recruit different cofactors to facilitate SWI/SNF complex-mediated nucleosome sliding and transcription initiation for a distinct set of genes. Thus, distinct groups of genes might get targeted by the same ubiquitous protein complexes because of differences in lncRNA expression in different cell types.

tion sites of transcription factors involved in angiogenesis. Hence, by inducing an open chromatin conformation, MANTIS was proposed to maintain endothelial angiogenic potential. Further domain mapping will define the precise nature of the interactions between MANTIS and BRG1. Several questions remain unanswered. How is MANTIS recruited to the promoter of the angiogenic genes it regulates? Does it bind to DNA directly? What is the mechanism of action for MANTIS in regulating other potential targets? What is the role of MANTIS in other cell types?

Full-length MANTIS is only partially conserved beyond primates, but lncRNA structure may be better conserved. The current assumption is that, in common with proteins, the secondary structure of lncRNAs, rather than their precise sequence, mediates function.¹ Direct evidence, however, is still sparse. In this study,

Leisegang et al³ provide evidence that an Alu element in MANTIS has a similar effect as the full-length lncRNA. Whereas MANTIS knockdown reduced endothelial sprouting, overexpression of a 450-nucleotide Alu element in MANTIS partially rescued this response. In addition, overexpression of the MANTIS Alu element was sufficient to increase the interaction between BRG1 and BAF155. A short interspersed nucleotide element (SINE B1) was found within the intron of Annexin A4 in mice and rats. An animal model of pulmonary arterial hypertension demonstrated downregulation of this SINE B1 that is accompanied by a trend toward reduced expression of angiogenic transcription factors.

Links between structure and function are emerging, but a database correlating RNA elements and motifs to functions is missing. Experimental validation of structural predictions in another lncRNA, HOTAIR, using chemical probing methods (eg, selective 2'-hydroxyl acylation analysed by primer extension or dimethyl sulfate) revealed independent structural modules and evolutionary conserved elements.¹¹ These approaches can provide a detailed understanding of the native structure of individual lncRNAs and offer insights into their functional roles.¹² Gene editing technologies such as CRISPR/Cas9 offer an opportunity to study the functional contributions of specific motifs in lncRNAs.¹³ In combination with homology directed repair, the CRISPR/Cas9 platform can be exploited to produce mutations in distinct elements. This elegant approach alleviates the need to knock down the entire lncRNA and overcomes the limitations of deleting large genomic fragments that may also harbor regulatory elements. A combined use of selective 2'-hydroxyl acylation analysed by primer extension and CRISPR/Cas9 technology was recently used to delineate the mechanism of function of Braveheart, a lncRNA implicated in cardiovascular lineage commitment in mice.¹⁴ Experimental determination of the secondary structure of Braveheart led to the identification of a short loop-like structure, an asymmetrical G-rich internal loop. CRISPR/Cas9-mediated deletion of this looped region demonstrated its critical role in cardiomyocyte differentiation. Mouse embryonic stem cells harboring a truncated lncRNA can no longer commit to cardiovascular lineage. Although attractive, such workflows can be technically challenging and have their limitations. Most chemical probing is performed on in vitro transcripts. Defining the RNA structure in vivo when RNA molecules undergo active unfolding and binding to proteins will require further refinement of the techniques and may also depend on RNA abundance.¹⁵ Genome engineering to obtain lncRNA genetic mutants would also require the use of cell lines with high proliferative capacity. Thus, primary cells such as human umbilical vein endothelial cells may not be the best option.

With the advances in quantitative RNA structural analysis, it becomes clear that RNA molecules, just like proteins, consist of domains and structural elements that govern their function. Establishing the link between RNA secondary structure and its function will be essential to understand the contribution of lncRNAs to diseases and harness potential opportunities for the development of RNA-based therapeutic approaches.

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FOOTNOTES

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