Editorial

LINCing MALAT1 and Angiogenesis

Thomas Thum, Jan Fiedler

Our understanding of the complex molecular interplay of various aspects of endothelial biology increased dramatically during the past years. In that context, noncoding RNAs (ncRNAs) such as microRNAs (miRs) have been shown to play crucial roles in the control of endothelial cell biology. For example, the endothelial-enriched, 22-nucleotide miR-126 was shown to sustain vascular homeostasis and blood flow. Several underlying molecular pathways for many miRs are now better understood, and first approaches have been taken to target certain miRs therapeutically in small and large animals, paving the way for potential first clinical approaches. Next to miRs, the Encyclopedia of DNA Elements (ENCODE) consortium (http://encodeproject.org/ENCODE/) and others have identified many additional ncRNA species with regulatory roles in the genome. A new example of those is long ncRNAs (lncRNAs), which are a class of heterogeneous RNAs comprising lengths >200 nucleotides. lncRNAs are localized in the nucleus or cytoplasm and are involved in different downstream mechanisms such as chromatin remodeling, protein scaffolding, or even translational control. In lncRNA-based mediation of intracellular signaling is poorly understood because of the many potential mechanistic properties of this RNA subclass. Only few reports have proven the participation of specific lncRNAs in the cardiovascular system. A stem cell–based study highlighted the importance of a lncRNA called Braveheart to guide cardiac development. A knockout strategy showed a functional role of lncRNA Fendrr to control chromatin modifications and thereby developmental signaling in the heart. Another study recently examined the interactions between the lncRNA cardiac hypertrophy related factor (CHRF) and miR-489; here, a hypertrophic response was mediated by sponging of miR-489 via CHRF in cardiomyocytes, implicating another modus operandi of lncRNA biology. Recently, the novel lncRNA Sencr was described to be smooth muscle cell– and endothelial cell–enriched. RNA sequencing revealed Sencr abundance in both cell types and its participation in smooth muscle cell biology via the regulation of differentiation factors such as myocardin. Scientific reports describing a single IncRNA–dependent mechanism in endothelial cells are not available. In this issue of Circulation Research, Michalik et al report an interesting new function of the endothelial-enriched IncRNA metastasis-associated lung adenocarcinoma transcript 1 (Malat1) to sustain endothelial cell proliferation. Applying the innovative RNA deep sequencing, Michalik et al discovered 5 highly enriched and, at least between the human and the mouse, conserved ncRNAs in endothelial cells. Subcellular RNA localization experiments showed high abundance of the IncRNA Malat1 in the nucleus, and interestingly, hypoxic stimuli triggered enhanced Malat1 expression in vitro. Effects of siRNA-mediated Malat1 knockdown were then studied in more detail to decipher the endothelial cell function. Firstly, Malat1 deficiency led to enhanced sprouting and migration of endothelial cells in a spheroid model and wound-healing assay. However, endothelial sprouts derived from Malat1-knockdown cells exhibited an incomplete extension of growing sprouts, indicating a defective proliferative potential of stalk cells. In line, endogenous Malat1 repression reduced cell number and lowered the S-phase population. In addition, further measurements pointed to increased apoptosis in Malat1-deficient cells. Taken together, the loss of Malat1 induced a characteristic phenotype switching from a proliferative toward a more migratory pattern. In addition to siRNA technology, Michalik et al also used the novel LNA GapmeR technology to target nuclear Malat1. Specific GapmeR application also triggered the induction of migratory potential, whereas endothelial cell proliferation was hampered by cell cycle blockade. Michalik et al next translated the findings into a mouse Malat1-knockout model. At baseline, Malat1 deficiency did not cause obvious developmental effects or disorders in the adult. In the current study, neovascularization in the developing mouse retina of Malat1-knockouts was analyzed in more detail. The loss of Malat1 halted vascular cell proliferation and thus caused a reduced vascular network in comparison to wild-type littermates. Next to the analysis of vascular development, Malat1 repression was tested therapeutically after ischemic disease in a mouse model of hindlimb ischemia. In vivo application of antisense GapmeR lowered Malat1 expression sufficiently and thereby impaired neovascularization as well as blood flow recovery at the injured site. Collectively, in vivo findings support the hypothesis that the loss of Malat1 is detrimental for endothelial cell proliferation. Several studies were also performed for the proof of mechanistic insight related to endothelial Malat1. Transcriptome profiling of MALAT1-deficient endothelial cells revealed the dysregulation of several cell cycle–related factors, for example, cyclins or kinases. Specifically, Malat1 repression lowered the expression of endothelial cyclins CCNA2 and CCNB1/2, all of which are key players in the
S-phase of cell cycle; in contrast, inhibitory factors such as protein (Cdc42/Rac)-activated kinase (p21) or the cyclin-dependent kinase inhibitor p27Kip1 were upregulated. The herein proposed Malat1 function is thus mechanistically linked to endothelial cell cycle progression regulating the endothelial cell turnover. Any evidence for Malat1 impacting on post-transcriptional splicing events as previously suggested was not found in endothelial cells. The current study highlights a novel IncRNA-based mechanism to control angiogenesis and is, therefore, of broad interest for future discoveries in the field of ncRNAs (Figure). However, some open issues and limitations remain. Malat1 was found in a RNA sequencing approach beside other highly abundant IncRNAs, which have not been analyzed in the current study. For instance, Malat1 silencing might affect the expression of other IncRNAs, miRs, or mRNAs that then are responsible for the observed phenotypic effects in endothelial cells. Future phenotypic analysis for IncRNA gain and loss of function would help to understand the importance of the other identified IncRNAs. In addition, the function of Malat1 has only been studied in a loss-of-function setup so far. Specific endothelial overexpression studies may also clarify the function of this IncRNA in endothelial biology. For instance, one could speculate that the overexpression of Malat1 may lead to increased endothelial proliferation but may have detrimental effects on sprouting behavior. Functional Malat1 overexpression studies could also help to identify the potential underlying molecular mechanisms related to, for example, chromatin remodeling as previously proposed for Malat1. Beside the use of venous endothelial cells for in vitro analysis of Malat1 function, an interesting aspect could be to study Malat1 function in arterial endothelial cells as well as in other cardiovascular cell types such as cardiomyocytes, smooth muscle cells, inflammatory cells, or cardiac fibroblasts. The transcriptional regulation of Malat1 is also unclear and needs to be addressed specifically in endothelial cells. Because Malat1 is upregulated on hypoxia, it can be assumed that hypoxia-related transcription factors are in control of MALAT1 transcription. In vivo endothelial-specific Malat1-knockout studies are needed in future to better understand the biological roles of IncRNAs. The systemic application of GapmeR antisense nucleotides to repress Malat1 after hindlimb ischemia needs to be validated carefully. Pharmacokinetics and pharmacodynamics for such novel chemistry should be clarified in this mouse model, and more target site–specific delivery approaches need to be developed. Translational aspects could then benefit from these findings to apply Malat1-based therapies in human disease. Also, it has been shown that genetic variants can affect the expression of ncRNAs such as AnrIL, and thus it would be interesting to investigate the influence of genetic variants on Malat1 expression. To answer whether Malat1 is also either a risk marker or a circulating biomarker in vascular diseases remains to be elucidated. In prostate cancer, for instance, Malat1 has been already demonstrated as a novel biomarker, and a recent study could also highlight the biomarker potential of IncRNA LIPCAR in heart failure.

The functional discovery of novel IncRNAs in cardiovascular disease has just begun. In conclusion, Michalik et al report an important study LINCing the endothelial IncRNA Malat1 to angiogenesis and pioneered first IncRNA-mediated effects in endothelial biology.

**Sources of Funding**

We have financial support from IFB-Tx (BMBF 01EO0802; T. Thum), Deutsche Forschungsgemeinschaft (TH 903/11-1; T. Thum), and Fondation Leducq (T. Thum).

**Disclosures**

None.

**References**


Key Words: Editorials ■ angiogenesis modulating agents ■ RNA, long noncoding
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Circ Res. 2014;114:1366-1368
doi: 10.1161/CIRCRESAHA.114.303896

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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World Wide Web at:
http://circres.ahajournals.org/content/114/9/1366

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