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Review

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Long Non-Coding RNAs as Regulators of Angiogenesis: A Mini-Review

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Abstract

While only about 5-10% mammalian transcripts act as mRNAs, the vast majority of them do not have protein-coding capability, of which, a class of non-coding RNAs with a length of over 200 nucleotides are defined as long non-coding RNAs (lncRNAs). It has been shown that lncRNAs interact with RNA, DNA, or proteins through diverse mechanisms to regulate gene expression, thereby controlling a wide range of biological processes. Angiogenesis, a process of new blood vessel formation from pre-existing ones, occurs under both physiological and pathological conditions. It is involved in many diseases. In this article, lncRNA regulatory roles in angiogenesis and their therapeutic potentials were reviewed and discussed.

Keywords: Long non-coding RNAs; Angiogenesis; Endothelial cells; Cell proliferation; Tube formation

Introduction

Transcripts of eukaryotic genomes differ in size and protein-coding capability. Genome-wide studies have revealed that only 5-10% mammalian transcripts function as mRNAs while the vast majority of them do not have protein-coding capability [1-3]. Based on the size, a class of non-coding RNAs with a length of over 200 nucleotides are defined as long non-coding RNAs (lncRNAs) [4, 5]. It is known lncRNAs regulate gene expression through a variety of mechanisms involving chromatin remodeling, the regulation of splicing and the control of microRNA function [5-8]. Studies have shown dysregulation of lncRNAs in many disease states, implication of lncRNAs in disease pathogenesis [9-15].

Angiogenesis is a process of new vessel formation from pre-existing ones, which is controlled by multiple angiogenic molecules and signaling pathways. It occurs under both physiological and pathological conditions. Angiogenesis is involved in wounding healing, tumor growth and metastasis, inflammation and many other disorders. Targeting abnormal angiogenesis is an important therapeutic strategy for various diseases [16-18]. In this article, several recently emerged angiogenic lncRNAs and their potentials as therapeutic targets were reviewed and discussed.

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LncRNA Maternally Expressed Gene 3 (MEG3)

Encoded by the MEG3, LncRNA MEG3 is a tumor suppressor [19]. Recently, several lines of evidence suggest a role for MEG3 in angiogenesis [20-23]. It is shown that the embryonic brain of MEG3$^{-/-}$ mice has remarkably elevated VEGF-A and VEGF-receptor 1 mRNA levels compared with that of wildtype littermates, indicating greater angiogenic activity in the brain after MEG3 knockout [20]. Indeed, MEG3-null embryos have a higher cortical microvessel density [20]. Increased expression of MEG3 in senescent human umbilical vein endothelial cells (HUVECs) is detected by RNA deep sequencing technology, and MEG3 elevation is associated with reduced HUVEC sprouting activity [21]. When MEG3 expression is suppressed, the impaired angiogenic activity of senescent HUVECs is restored. The inhibitory effect of MEG3 in angiogenesis has been further validated in a murine hind-limb ischemic model, as significantly increased new vessels and markedly improved blood flow are observed in the ischemic hind-limb after MEG3 silencing [21]. In line with these findings, Qiu et al have reported that knockdown of MEG3 in RF/6A endothelial cells promotes cell proliferation, migration and tube formation, and the mechanistic study suggests MEG3 impedes RF/6A cell angiogenic activity by blocking the activation of the PI3K-Akt signaling pathway [22]. More recently, another group demonstrates that MEG3 overexpression significantly suppresses endothelial proliferation and *in vitro* angiogenesis, whereas knockdown of MEG3 has the opposite effect [23]. These data indicate MEG3 is a negative regulator of angiogenesis.

LncRNA Metastasis-Associated Lung Adenocarcinoma Transcript 1 (MALAT1)

The MALAT1, an LncRNA that was originally described to be associated with metastasis of lung cancers [14], exhibits pro-angiogenic properties [10, 24-28]. MALAT1 deficiency results

in impaired HUVEC proliferation, which is in accord with *in vivo* findings, as the number of proliferating endothelial cells and the vessel density in the retina of MALAT1$^{-/-}$ mice were significantly lower than those of wildtype controls [24]. Li et al have shown that suppression of MALAT1 markedly compromises the ability of HUVECs to form capillary-like structures [25]. MALAT1 knockdown also leads to decreased proliferation of retinal RF/6A endothelial cells through the regulation of the p38/MAPK signaling pathway [26]. In human umbilical cord mesenchymal stem cells (SMCs), MALAT1 up-regulates VEGF expression, and the conditioned medium from SMCs overexpressing MALAT1 enhances HUVEC tube formation [10]. Tumor angiogenesis plays a pivotal role in tumor growth and metastasis. LncRNA MALAT1 promoting tumor angiogenesis has also been reported [25, 27, 28]. Two groups have found lncRNA MALAT1 promotes tumor angiogenesis by mediating the production of fibroblast growth factor-2 (FGF-2). Huang et al reported that LncRNA MALAT1 up-regulated FGF-2 in tumor-associated macrophages, thereby boosting angiogenesis and furthering thyroid cancer cell migration and invasion [27]. Augmented expression of LncRNA MALAT1 has been detected in human neuroblastoma cells under hypoxic conditions, which is associated with dramatically increased production of FGF-2 from neuroblastoma cells, leading to robust angiogenesis contributing to tumor growth [28].

Other Angiogenic LncRNAs

LncRNA MANTIS has been shown to be elevated in endothelial cells isolated from glioblastoma but reduced in pulmonary artery endothelial cells from lungs of patients with end stage idiopathic pulmonary arterial hypertension [29]. Knockdown of MANTIS in endothelial cells leads to attenuated cell migration, proliferation and tube formation. Thus, MANTIS positively maintains endothelial angiogenic capacity [29]. LncRNA IGF2AS expression is augmented in myocardial microvascular endothelial (mMVE) cells isolated from rats with type 2 diabetes. Inhibition of lncRNA IGF2AS in mMVE cells increases cell proliferation through up-regulating insulin-like growth factor 2 and VEGF [30]. LncRNA MIAT has emerged as an angiogenic activator, as repression of MIAT compromises the proliferative ability of endothelial cells [31]. High glucose can induce the expression of MIAT in multiple endothelial cells including human microvascular endothelial cells, HUVECs and retinal endothelial cells (RF/6A), suggesting MIAT might be involved in diabetes mellitus-induced microvascular dysfunction [31]. SENCR is a vascular-enriched lncRNA and its expression is diminished in endothelial cells isolated from patients with critical limb ischemia or premature coronary artery disease [32]. Overexpression of SENCR in HUVECs stimulates cell migration and promotes tube formation through upregulation of proangiogenic chemokines CCL5 and CX3CL1 [32]. Two important pro-angiogenic lncRNAs LINC00323-003 and MIR503HG are induced in endothelial cells by hypoxia. Silencing LINC00323-003 or MIR503HG results in defective HUVEC proliferation. However, HUVECs deficient in LINC00323-003 or MIR503HG differ in their ability to form capillary-like structures. Knockdown of LINC00323-003 leads to reduced tube formation in HUVECs, while MIR503HG deficiency does not affect tube formation significantly [33].

Angiogenic LncRNAs as Therapeutic Targets

The research field of angiogenic lncRNAs is evolving. The mechanisms governing lncRNA angiogenic actions remain largely elusive, impeding the investigation of angiogenic lncRNAs as therapeutic targets. Indeed, there are only a limited number of studies that have explored angiogenic lncRNAs for therapeutic purposes. MEG3 knockdown has been examined for the treatment of stroke in a rat ischemic stroke model. *In vivo* application of lentiviral particles expressing hairpin RNA to suppress MEG3 increases microvessel density in the ischemic region and reduces brain lesion [34]. The Gapmer antisense oligonucleotide can specifically and effectively cleave target RNAs including lncRNAs *in vivo* and appears as a promising therapeutic agent [35]. The locked nucleic acid Gapmer directed against MALAT1 has been therapeutically tested in a mouse model of hind-limb ischemia. Intraperitoneal injection of the Gapmers is able to inhibit MALAT1 expression in both control and ischemic muscle tissues, and MALAT1 repression significantly reduces capillary density and blood flow in the ischemic muscles [24]. These data suggest angiogenesis can be enhanced or blocked by modulating lncRNA expression, which could be potentially applied for the treatment of angiogenesis-related diseases.

Conclusions

LncRNAs plays an important role in angiogenesis. Targeting lncRNAs as a novel therapeutic approach for angiogenesis-related diseases seems promising. However, numerous issues are to be solved such as how to avoid off-site effect of therapeutic RNA molecules, how to efficiently deliver *in vivo* therapeutic molecules, and how to minimize the adverse effects, which warrants further studies.

Conflicts of Interest

None.

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