

Review Article

Long noncoding RNAs, emerging and versatile regulators of tumor-induced angiogenesis

Jing Zhao^{1,2}, Li Li¹, Zhong-Ying Han³, Zheng-Xin Wang¹, Lun-Xiu Qin^{1,2}

¹Department of General Surgery, Huashan Hospital, Fudan University, Shanghai 200040, China; ²Cancer Metastasis Institute, Fudan University, Shanghai 200040, China; ³Department of Hepatology, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai 200011, China

Received May 18, 2019; Accepted June 7, 2019; Epub July 1, 2019; Published July 15, 2019

Abstract: Angiogenesis is an essential step in maintaining tumor growth and facilitating metastasis. The regulatory mechanisms of tumor-induced angiogenesis are extremely complicated, and include sophisticated crosstalk between tumors and surrounding microenvironment cells, oncogenic signaling pathway activation and aberrant expression of angiogenesis-related genes. Recently, emerging evidence demonstrated that long noncoding RNAs (lncRNAs) play crucial roles in angiogenesis. However, there are lack of reports to review the progression in this scientific field. Here, we focus on and summarize the latest findings of lncRNA in angiogenesis in various cancers. Firstly, we introduced how lncRNAs in tumor cells to modulate the cellular signaling axis, interact with proteins and serve as competitive endogenous RNAs (ceRNAs) to alter target gene expression, by which induce endothelial cell to form capillaries. Then, we recapitulated the essential functions of lncRNA in endothelial cells, and how lncRNAs in tumor-associated macrophages to mediate angiogenesis. Next, the angiogenesis mechanism of tumor-derived lncRNAs via exosomes were collectively described. At last, the effects of lncRNAs on vasculogenic mimicry were summarized, which showed that malignant tumor cells acquire dedifferentiated and endothelial properties to form vessel-like structures by themselves. This review provides new insights into the complexity of angiogenesis, and suggests that lncRNAs may become promising biomarkers and targets for enhancing the efficacy of anti-angiogenesis therapy in cancer.

Keywords: Angiogenesis, lncRNA, signaling pathway, ceRNA, tumor microenvironment

Introduction

Angiogenesis is one of the most important hallmarks of cancer [1]. During the process of tumor growth, new vasculature is needed to supply ample nutrients and remove metabolic wastes. Tumor-induced angiogenesis frequently displays disordered morphology and is characterized by precocious, tortuous, permeable and dysfunctional vascular structure [1]. These atypical vasculature networks create a hypoxic microenvironment within tumors, which activates hypoxia-induced factors (HIFs) to drive the expression of numerous oncogenes and induce immunosuppression. Additionally, the high permeability and leakage of the neovasculature system facilitate the direct entry of tumor cells into the bloodstream to trigger metastasis. Moreover, this disabled vasculature makes

it difficult to deliver drugs efficiently to tumors and results in therapeutic resistance [2]. Therefore, angiogenesis-related factors have become attractive targets for the development of anti-cancer drugs, such as FDA-approved bevacizumab (anti-VEGF) and ramucirumab (anti-VEGFR2) [3]. Though these anti-angiogenic drugs may improve progression-free survival in some patients, the long-term response is disappointing [3]. Extremely intricate mechanisms beyond these well-documented coding genes regulate the angiogenesis process. Further exploration and a better understanding of these mechanisms are required to develop more effective strategies for cancer therapy.

Over the past decades, the development of high-throughput sequencing technology has revealed that more than 70% of the human

The regulatory mechanisms of lncRNAs in tumor angiogenesis

genome is pervasively transcribed, but only less than 2% of the transcripts are translated into proteins; most of human transcriptome are noncoding RNAs (ncRNAs) [4]. Emerging evidence has demonstrated that instead of being “transcriptional junk”, such as miRNA, siRNA, piRNA, circRNA, snoRNA and the attractive long noncoding RNAs, play essential roles in manipulating gene expression [5]. Long noncoding RNAs (lncRNAs) are collectively defined as transcripts with more than 200 nucleotides that have limited potential to encode proteins; lncRNAs are the primary class of noncoding family [6]. lncRNAs may display diverse functions and participate widely in developmental, physiological and pathophysiological processes, including cancer progression. Mechanistically, lncRNAs can associate with DNA, RNA and proteins to modulate local or global gene expression in a *cis* or *trans* manner [7]. For instance, lncRNA HOTAIR can serve as scaffold to recruit various protein complexes to specific genomic regions, thereby reprogramming the chromosomal state to affect gene expression [8]. Additionally, lncRNA may function as a competing endogenous RNA (ceRNA) to sequester miRNA from its targets, and antagonize the repressive effects of miRNAs on mRNAs [9]. Recently, many lncRNAs have been found to participate in tumor-induced angiogenesis by triggering oncogenic signaling pathways, binding to various proteins and miRNAs, encapsulating into exosome for delivery, or reprogramming tumor microenvironment. These findings provide new insights into the complexity of angiogenesis.

In this review, we systematically summarized the functions and underlying mechanisms of lncRNAs in tumor-associated angiogenesis, and proposed that lncRNAs have great potential to be novel biomarkers and targets in cancer therapy.

Aberrant lncRNA expression in cancers drive angiogenesis via multiple manners

lncRNAs trigger angiogenesis through activating oncogenic signaling pathways in tumor cells

The activation of oncogenic signaling pathways, such as STAT3, NF- κ B, AKT, mTOR and WNT, plays important roles in driving angiogenesis of tumor. Emerging studies demonstrate that lncRNAs can alter the activation of these path-

ways to regulate angiogenesis as summarized in **Figure 1**.

Our recent study revealed that lncRNA PVT1 promotes angiogenesis by inducing the STAT3/VEGFA axis in gastric cancer. PVT1 is upregulated in gastric cancer tissues and cells, and is associated with high microvessel density and shorter survival time. Further investigation showed that PVT1 can form a complex with STAT3 in the nucleus, which protects STAT3 from polyubiquitination and proteasome-mediated degradation. Reciprocally, STAT3 can occupy the PVT1 promoter to increase PVT1 transcription. The positive feedback loop between PVT1 and STAT3 sustainably increases VEGFA expression to induce angiogenesis [10]. In non-small cell lung cancer (NSCLC), lncRNA TNK2-AS1 also mediates angiogenesis via the STAT3 signaling pathway. Similar to PVT1, TNK2-AS1 binds to and stabilizes STAT3 to enhance VEGFA expression for neovascularization, while STAT3 triggers TNK2-AS1 transcription in turn [11].

LINC01410 is another critical lncRNA involved in the angiogenesis of gastric cancer. Unlike the abovementioned mechanism, LINC01410 acts as a ceRNA that interacts with and depletes miR-532-5p, which increases the expression of the miR-532-5p target NCF2, and subsequently activates the NF- κ B pathway by increasing p65 protein levels in the nucleus. Interestingly, NCF2 can also stimulate LINC01410 expression via the NF- κ B pathway. As a result, the constitutive activation of the LINC01410/miR-532-5p/NCF2/NF- κ B feedback loop aggravates the malignant progression of gastric cancer [12].

In hepatocellular carcinoma (HCC), Lin et al. demonstrated that lncRNA UBE2CP3 expression is upregulated in HCC tissues with higher vessel density. In a co-culture system of cancer cells and human umbilical vein endothelial cells (HUVECs), dysregulated UBE2CP3 expression in the cancer cells can alter endothelial cell proliferation, migration and tube formation. Consistently, gaining UBE2CP3 expression may enhance the arteriole formation and vessel density of xenografts *in vivo*. A mechanistic study revealed that UBE2CP3 can activate the ERK/HIF-1 α /p70S6K signaling cascade to augment VEGFA levels and drive angiogenesis in HCC [13].

The regulatory mechanisms of lncRNAs in tumor angiogenesis

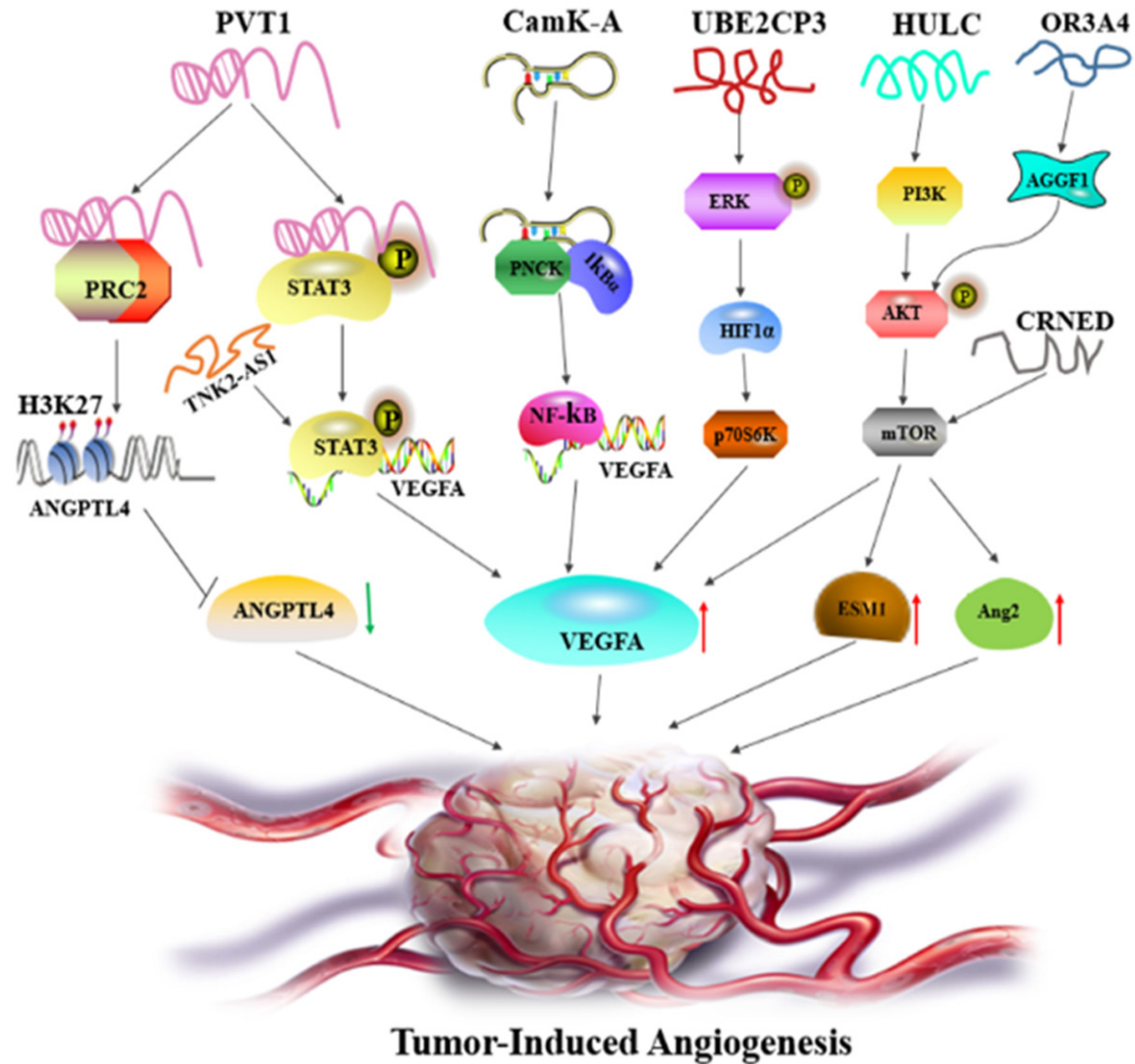


Figure 1. lncRNAs regulate angiogenesis through modulating multiple signaling pathways. lncRNA PVT1 binds to and activates STAT3 pathway to drive VEGFA expression, or interacts with PRC2 complex to induce H3K37 chromosomal modification to repress ANGPTL4 expression. lncRNA TNK-AS1 exerts similar mechanism with PVT1 to elicit STAT3/VEGFA axis. lncRNA CamK-A associate with PNCK and degrade I κ B α , thus stimulate NF- κ B pathway to increase VEGFA expression. UBE2CP3 triggers ERK/HIF-1 α /p70S6K cascade to elevate VEGFA levels. HULC regulates ESM1 expression via the PI3K/AKT/mTOR pathway. lncRNA OR3A4 mediates angiogenesis by the AGGF1/AKT/mTOR axis. lncRNA CRNDE induce VEGFA and Ang-2 expression through activating mTOR pathway. The red arrows indicate the upregulation and the green arrow represents the downregulation.

Several studies indicate that some lncRNAs can alter the activation of the AKT/mTOR pathway to govern angiogenesis in various cancers. For instance, the oncogenic lncRNA HULC is overexpressed and positively correlated with the expressing of proangiogenic factors VEGF and ESM-1, as well as with high microvessel density in glioma tissues. HULC silencing can decrease proliferation, migration, invasion, and capillary tube formation in glioma cells. The authors also found that ESM-1 is responsible for HULC-mediated angiogenesis, and that

HULC regulates ESM-1 expression via the PI3K/AKT/mTOR signal pathway [14]. Analogously, lncRNA OR3A4 overexpression can mediate proliferation, migration and angiogenesis via the AGGF1/AKT/mTOR pathway in HCC [15]. In hepatoblastoma, upregulation of lncRNA CRNDE is markedly linked to VEGF, ESM-1 and CD34 expression. Silencing CRNDE has anti-proliferative and anti-angiogenic effects via blocking the mTOR/P70S6K signaling. Rapamycin, an inhibitor of the mTOR pathway, can successfully decrease CRNDE-mediated VEGFA and Ang-2

The regulatory mechanisms of lncRNAs in tumor angiogenesis

expression [16]. In osteosarcoma, lncRNA MALAT1 exerts a proangiogenic effect via activating the mTOR/HIF-1 α pathway. Mutually, activated mTOR/HIF-1 α induces MALAT1 expression to sustain oncogenic functions [17]. In lung adenocarcinoma, lncRNA LOC100132354 enables the activation of the VEGFA/VEGFR2/RAF/MEK/ERK signaling pathway to enhance angiogenesis [18].

Several lncRNAs that are not proangiogenic, such as GAS5 and MEG3, have the capacity to suppress angiogenesis. In colorectal cancer (CRC), GAS5 can inactivate the Wnt/ β -catenin pathway to block angiogenesis and metastasis [19]. MEG3 is expressed at low levels in breast cancer. MEG3 overexpression can inhibit cell proliferation and invasion, as well as the expression of angiogenesis-related factors, such as VEGFA/B, PGF, bFGF, SDF-1, TGF- β and angiogenin. MEG3 suppresses the capillary tube formation of endothelial cells through inactivating AKT signaling [20].

lncRNAs mediate angiogenesis via binding to various proteins within tumor cells

lncRNAs can associate directly with proteins, which may affect protein secretion, stability, enzymatic activity, guide proteins to specific genomic regions, or decoy transcriptional factors away from target gene promoters [7]. lncRNAs can interact with various proteins to mediate angiogenesis within tumors.

MVIH is the first identified lncRNA accounting for tumor-induced angiogenesis. MVIH is substantially overexpressed in HCC specimens with high microvascular invasion and tumor node metastasis, and these patients frequently suffer from shorter recurrence-free survival and overall survival time. MVIH can associate with the PKG1 protein and inhibit PKG1 secretion to induce angiogenesis in HCC [21].

In cervical cancer, lnc-CCDST acts as a scaffold to enhance the interaction of the E3 ubiquitin ligase MDM2 with the oncogenic protein DHX9. The lnc-CCDST-dependent interplay facilitates DHX9 degradation by MDM2 and inhibits angiogenesis. However, in HPV-infected cervical cancer, the virus-encoded E6 and E7 proteins diminish lnc-CCST expression and elevate DHX9 abundance to induce malignant behaviors [22].

In gastric cancer, the upregulation of lncRNA OR3A4 is associated closely with invasion and lymphatic and distal metastasis. Mechanisti-

cally, OR3A4 can trigger a variety of gene expression and form complexes with the PDLIM2, MACC1, NTN4 and GNB2L1 proteins, thus promoting angiogenesis, tumor growth and metastasis both *in vitro* and *in vivo* [23]. In contrast to the oncogenic function of OR3A4, NBAT1 is a tumor suppressing lncRNA in gastric cancer, which can inhibit proliferation, migration, invasion and capillary tube formation. Low expression levels of NBAT1 significantly correlate with advanced tumor stage, lymph node metastasis and poor prognosis. NBAT1 interacts with Sox9 to mediate its proteasome-dependent proteolysis. Sox9 degradation leads to a reduction in NBAT1, as Sox9 can bind to the NBAT1 promoter to drive its expression. The negative feedback between NBAT1 and Sox9 continuously aggravates gastric cancer progression [24].

lncRNA PVT1 has proangiogenic properties in more cancer types than gastric cancer. Yu et al. demonstrated that lncRNA PVT1 can control angiogenesis, proliferation and apoptosis in cholangiocarcinoma (CCA) via reprogramming the chromatin state. PVT1 binds to EZH2, the crucial component of PRC2 complexes, and induces H3K27me3 epigenetic modifications within the ANGPTL4 promoter, thereby decreasing ANGPTL4 expression to promote tumorigenesis [25].

There are some oncogenic lncRNAs, such as MALAT1 and HOTAIR, which also drive angiogenesis through pleiotropic regulatory patterns. For example, MALAT1 can induce angiogenesis by affecting Sox2 expression in pancreatic cancer [26], while MALAT1 was found to promote vasculature formation via increasing FGF2 secretion in neuroblastoma cells under hypoxic conditions [27]. In nasopharyngeal carcinoma (NPC), HOTAIR knockdown attenuates tumor growth and angiogenesis both *in vitro* and *in vivo*. A subsequent study revealed that HOTAIR can bind directly to the specific DNA motif of VEGFA promoter to modify the genome structure and increase VEGFA transcription. On the other hand, HOTAIR can regulate GRP78 protein levels to indirectly affect VEGFA expression [28].

lncRNAs affect angiogenesis through cross-talk with miRNAs and mRNAs in tumor cells

In addition to interactions with proteins, intricate crosstalk exists among lncRNAs, miRNAs

The regulatory mechanisms of lncRNAs in tumor angiogenesis

and mRNAs. lncRNA may function as ceRNA, which sponges miRNA via complementary miRNA binding sites, and this sponging function antagonizes miRNA availability to mRNAs and attenuates the suppressive roles of miRNA on its targets [29]. The mechanisms of ceRNA have been investigated widely in tumor angiogenesis.

In colorectal cancer (CRC), lncRNA-ZFAS1 is markedly upregulated in cancer tissues and is associated with shorter survival time. Gain- and loss- of ZFAS1 expression have a great impact on invasion, migration and angiogenesis. The transcriptional factor SP1 can be recruited within ZFAS1 promoter to initiate its expression. A mechanistic study demonstrated that ZFAS1 acts as miRNA sponge to sequester miR-150-5p away from its target VEGFA in an AGO2-dependent manner. ZFAS1-mediated VEGFA elevation activates VEGFR2 and the downstream AKT/mTOR pathway to exacerbate cancer progression [30].

Glioblastoma is a typically vessel-enriched malignant brain tumor. lncRNA TUG1 upregulation is partly responsible for the aggressive phenotype. Depletion of TUG1 notably inhibits tumor-induced endothelial cell proliferation, migration, capillary tube formation and spheroid-based angiogenesis *in vitro*, as well as retards tumor growth and decreases microvessel density *in vivo*. TUG1 can directly bind to and repress miR-299 expression. As a result, the miR-299 target VEGFA is upregulated by TUG1 and contributes to angiogenesis in glioblastoma [31]. In addition, Dong et al. reported that high lncRNA TUG1 expression can lead to angiogenesis in hepatoblastoma through ceRNA function. TUG1 may titrate and decrease miR-34a-5p levels, thereby elevating the expression of its target gene VEGFA. VEGFA antibody and miR-34a-5p treatment can block TUG1-induced tumor growth and angiogenesis [32].

lncRNA XIST overexpression not only induces angiogenesis in glioma cells but also modulates the biological properties of glioma endothelial cells (GECs). Cheng et al. found that lncRNA XIST displays significant elevation in glioma samples and that XIST knockdown can repress metastasis and angiogenesis both *in vitro* and *in vivo*. A subsequent study revealed that miR-429 can target and silence XIST expression, while XIST can function as a ceRNA to sponge miR-429 in turn. The expression lev-

els of lncRNA XIST and miR-429 are inversely correlated in glioma [33]. Intriguingly, Yu et al. demonstrated that lncRNA XIST is overexpressed in glioma endothelial cells (GECs) cocultured with glioma cell line U118. Silencing XIST in GECs can effectively increase the permeability of blood-tumor barrier and reduce angiogenesis. A deep investigation showed that XIST binds to miR-137, and they reciprocally inhibit each other's expression. The tight junction-related proteins ZO-2 and FOXC1 are direct targets of miR-137. XIST regulates blood-tumor barrier permeability and angiogenesis via sequestering miR-137 to increase ZO-2 and FOXC1 expression [34].

In bladder cancer (BC), lncRNA H19 and DNMT3B are highly co-expressed in tissue samples and cells. H19 promotes the rearrangement of cytoskeleton, epithelial-to-mesenchymal transition (EMT), tumorigenesis, angiogenesis and metastasis both *in vivo* and *in vitro*. This study also verified that H19 can bind to and co-localize with miR-29b, which relieves the silencing effect of miR-29b on DNMT3B and promotes bladder cancer development [35]. Recently, the same group found that lncRNA RP11-79H23.3 and the tumor suppressor gene PTEN are remarkably downregulated in BC tissues, and this downregulation can inhibit tumorigenesis, angiogenesis and metastasis [36]. Similar to H19, lncRNA RP11-79H23.3 functions as a ceRNA of miR-107 and represses the effect of miR-107 on PTEN. The elevation in PTEN levels consequently blocks PI3K/AKT signaling pathway activity to suppress BC progression [36].

In pancreatic ductal adenocarcinoma (PDAC), linc00511 serves as a ceRNA to titrate miR-29-3P and subsequently increases the expression of miR-29 target VEGFA to induce angiogenesis. High levels of linc00511 are associated with metastasis and poor prognosis in PDAC [37].

In HCC, lncRNA HULC can act as ceRNA to sequester miR-107, which increases the expression of miR-107 target E2F1. As a result, the transcriptional factor E2F1 binds to the SPHK1 promoter and elevates SPHK1 expression to induce angiogenesis [38].

Taken together, lncRNAs can exert diverse mechanisms to stimulate tumor cells to release angiogenic factors, and these growth factors

The regulatory mechanisms of lncRNAs in tumor angiogenesis

induce endothelial cells to form capillaries network within tumors. As we described above, there exist many feedback regulation between aberrant lncRNA expression and oncogenic signaling pathway activation, such as the reciprocal interplay loop between PVT1 and STAT3, TNK2-AS1 and STAT3, as well as LINC1410 and NF- κ B. These positive feedback loops represent essentially biological switches to consecutively sustain oncogenic properties including angiogenesis, which may be critical intervention targets for cancer therapy.

It should be realized that there are some limits in these present studies. Firstly, the activation of STAT3, NF- κ B, AKT, mTOR and WNT pathways may initiate a lot of downstream gene expression, some of which are responsible for activating tumor microenvironment cells. Tumor angiogenesis need the crosstalk among tumor, endothelial and mesenchymal cells. However, most of present studies were directly carried out with co-culture system between tumor cells and endothelial cells *in vitro*, and took immune deficient mice as the *in vivo* model, which can't fully reflect the effects of tumor immune microenvironment on angiogenesis, and the regulatory roles of other cell types such as myeloid cells or fibroblasts on angiogenesis. Secondly, the ceRNA function of lncRNA has been widely studied in angiogenesis. It is noteworthy that lncRNA has different miRNA response elements, and each miRNA has various targets. The interaction among different miRNAs and their targets may synergistically contribute to angiogenesis, but there are lack of further studies. In addition, although a number lncRNAs have been identified as oncogenes to promote the secretion of angiogenic factor from tumor cells into the tumor microenvironment, the underlying mechanisms of how endothelial cells respond to the dysregulation of lncRNAs in tumors are largely unknown, which need to be elucidated in future studies.

lncRNAs are essential for maintaining endothelial cell functions

lncRNAs also play important roles in endothelial cells to sustain specialized endothelial cell phenotypes and functions, including new blood vessel formation. Recently, the functions and underlying mechanisms of some lncRNAs in endothelial cells have been identified.

lncRNA STEEL is an endothelial cell-enriched lncRNA that can increase tube formation capacity. STEEL can transcriptionally increase eNOS and KLF2 expression via activating the epigenetic status of H3K4me3 in their promoters. In addition, STEEL complexes with PARP1 and recruits PARP1 to the KLF2 promoter, which partially accounts for STEEL-mediated KLF2 upregulation. Conversely, eNOS and KLF2 exert feedback inhibition on lncRNA STEEL [39].

In tumor-associated endothelial cells, tumor cells may alter the lncRNA profile in endothelial cells to modulate angiogenesis. Glioma has been extensively reported to control the biological behaviors of glioma vascular endothelial cells through the lncRNA/miRNA axis. Zheng et al. found that when glioma vascular endothelial cells are stimulated with glioma-conditioned medium, PVT1 is highly expressed and increases cell proliferation, migration and angiogenesis in glioma vascular endothelial cells. A subsequent investigation elucidated that PVT1 acts as a ceRNA to sequester miR-186 and blocks the silencing effect of miR-186 on its targets Atg7 and Beclin1, by which increasing Atg7 and Beclin1 expression to enhance protective autophagy [40]. H19 is expressed highly in the microvessels of glioma tissues and endothelial cells cultured with glioma-conditioned medium. Further study indicated that H19 can regulate VASH2 expression to mediate angiogenesis by binding directly to and inhibiting miR-29a in endothelial cells [41]. MCM3AP-AS1 is another lncRNA, that is upregulated in glioma-associated endothelial cells (GECs). Silencing MCM3AP-AS1 reduces GEC cell viability, migration and tube formation potential. MCM3AP-AS1 can increase KLF5 levels by decreasing its negative regulator miR-211. Then, KLF5 is enriched within the promoter region of AGGF1 to initiate AGGF1 expression, thereby triggering the PI3K/AKT and ERK1/2 signaling pathways to enhance glioma angiogenesis [42].

In addition to those in GECs, lncRNAs in vascular endothelial cells have been closely investigated. lncRNA PVT1 upregulation in vascular endothelial cells can trigger proliferation, migration and capillary tube formation as well. Mechanistically, PVT1 binds to and degrades miR-26b to increase the expression levels of CTGF and ANGPT2 in vascular endothelial cells [43]. Another study in human umbilical vein

The regulatory mechanisms of lncRNAs in tumor angiogenesis

endothelial cells (HUVECs) showed that DNA methylation controls lncRNA MEG3 expression under normoxic conditions, whereas HIF-1 α is responsible for MEG3 transcription in hypoxic treatment. MEG3 knock-down diminishes VEGFR2 expression and reduces angiogenesis by exclusively blocking the VEGFA/VEGFR2 pathway [44].

Collectively, the stimuli from tumor cells can alter lncRNA expression profiles in endothelial cells. These deregulated lncRNAs have great impact on biological properties of vascular endothelial cells through various mechanisms, and result in angiogenesis. Now, endothelial-derived lncRNAs have not been extensively studied, and the functions of aberrant lncRNA expression in endothelial cells are still unclear. Meanwhile, what are the determining factors in tumor stimuli, and how these factors to change lncRNA profiles in endothelial cells need deeply investigated as well.

Dysregulation of lncRNAs in tumor-associated macrophages stimulates angiogenesis

Tumor immune microenvironment plays pivotal roles in cancer development. Tumor-associated macrophages (TAMs) are the major component of tumor microenvironment, which can recruit endothelial cell to form capillaries within tumors, induce immune evasion and metastasis [45]. Several studies reported that dysregulation of lncRNAs may endow macrophage with TAM properties and promote angiogenesis.

It has been established that TAMs commonly share many properties with M2 macrophages in that they both modulate tumor growth, angiogenesis and immune escape. Cao et al. used IL-4 and IL-13 induction to construct cell-based M2 polarization models and observed the importance of lncRNA-MM2P in determining M2 differentiation; the lncRNA-MM2P expression levels increase during M2 polarization but decrease in M1 macrophages [46]. This study also showed that silencing lncRNA-MM2P expression in macrophages markedly blocks cytokine-mediated M2 polarization and inhibits M2-induced angiogenesis both *in vitro* and *in vivo* by inactivating the STAT6 signaling pathway [46]. Recently, Sang et al. identified a crucial lncRNA, CamK-A, that enables the remodel of cancer cell metabolic and immune microenvironments. lncRNA CamK-A can bind directly to the calmodulin-dependent kinase PNCK to

phosphorylate and degrade I κ B- α , which in turn stimulates the NF- κ B pathway in a calmodulin-dependent manner. Consequently, the NF- κ B signaling axis transactivates a number of cytokines, such as VEGF, IL-6, IL-8 and metabolism-related genes, which results in macrophage recruitment, angiogenesis and tumor progression [47].

In thyroid cancer, Huang et al. enriched CD14 positive monocytes of human peripheral blood mononuclear cells (PBMCs) and treated them with LPS, IL-4 and conditioned medium of cancer cell line FTC133 to induce M1, M2 and TAM models respectively. TAMs have properties similar to those of M2, in which IL-10 and FGF2 are increased, whereas TNF- α and IL-12 secretion are decreased. When lncRNA MALAT1 is knocked down in TAMs, the condition medium from the TAMs can suppress the proliferation, migration and invasion of tumor cells, as well as reduce angiogenesis in endothelial cells. These results show that MALAT1 in TAMs mediates angiogenesis through influencing FGF2 secretion [48].

TAMs is believed to play critical roles in mediating immunosuppression. lncRNAs are implicated in the polarization process of macrophage. The number of TAMs infiltrated into tumors is closely related to malignant cancer progression. There are several means to construct TAMs cell models *in vitro*, including IL-4/IL-13-induced M2 polarization, and cancer cell conditioned medium-treated macrophages and PBMCs. But these cell-based TAM models *in vitro* are greatly different from the TAMs infiltrated within tumors *in vivo*. Therefore, isolation and purification of the infiltrated TAMs from solid tumors may provide more convincing evidence. The comparison of lncRNA signature among cell/PBMC-based and infiltrated TAMs is helpful to identify the specific lncRNAs for TAM polarization. Additionally, how lncRNAs in tumor cells educate tumor microenvironment cells to become TAMs, cancer-associated fibroblasts and endothelial cells, which still need further exploration.

Tumor-derived lncRNAs can be delivered into endothelial cells via exosomes to promote angiogenesis

Exosomes are a subset of extracellular vesicles with diameters of 30-150 nm, which serve as an important communication system. Exo-

The regulatory mechanisms of lncRNAs in tumor angiogenesis

somes can load a variety of molecules including DNA, RNA, protein, lncRNA, miRNA and circRNA, carrying these molecules to neighboring cells. Tumor cells can release millions of exosomes to reprogram tumor microenvironment to aggravate tumor progression [49]. Tumor-derived exosomes play crucial roles in inducing angiogenesis [50]. Several studies have demonstrated that exosomal lncRNAs from tumor cells can be taken up by endothelial cell to modulate angiogenesis.

Linc-POU3F3 is highly expressed in glioma tissues and cells. When exosomes from glioma cells are co-incubated with human brain microvascular endothelial cells (HBMECs), the exosomes are internalized by the HBMECs and linc-POU3F3 expression is increased within endothelial cells. The glioma-derived exosomes can markedly promote migration, proliferation, tubular-like structure formation *in vitro* and arteriole formation *in vivo* by increasing the levels of angiogenesis-related proteins in HBMECs [51]. Exosomes from another glioma cell line, U87, that has high linc-CCAT2 expression, can transmit linc-CCAT2 to endothelial cells to induce angiogenic phenotypes both *in vitro* and *in vivo*, as well as exert anti-apoptosis effects via hypoxia. Linc-CCAT2 upregulates VEGFA, TGF- β and Bcl-2, but downregulates Bax and caspase-3 expression to inhibit hypoxia-mediated apoptosis in endothelial cells [52]. HOTAIR also has the capacity to enhance angiogenesis through increasing VEGFA in glioma cells. Further studies showed that HOTAIR can be encapsulated into glioma cell-derived extracellular vesicles for delivery to endothelial cells [53].

In HCC, exosomes isolated from CD90⁺ liver cancer stem-like cells can stimulate endothelial cells to form capillary networks and enhance the adhesion of CD90⁺ cells to monolayer endothelial cells. LncRNA profiling revealed that lncRNA H19 is enclosed into CD90⁺ cell-derived exosomes and mediates the expression of pro-angiogenic factors, including VEGF, VEGFR1 and ICAM1 in endothelial cells [54].

In epithelial ovarian cancer (EOC), lncRNA MALAT1 is transported to endothelial cells through cancer cell-secreted exosomes, and then stimulates proangiogenic behaviors through modulating angiogenesis-relevant genes in endothelial cells. Furthermore, serum exosomal

al MALAT1 levels are tightly correlated with advanced and metastatic outcomes, and they act as an independent predictive factor for overall survival in EOC [55].

In addition to exosome loading, lncRNAs can affect tumor-derived exosome production. During colorectal carcinoma (CRC) development, adenomatous polyposis coli (APC) gene mutations are a critical event. Wang et al. identified an APC-activated lncRNA-APC1 that can weaken tumor growth, metastasis and angiogenesis. A mechanistic study revealed that lncRNA-APC1 associates directly with Rab5b mRNA and decreases its mRNA stability, thereby reducing CRC-derived exosome production. When APC mutations occur in CRC cells, they may release more exosomes to activate MAPK pathway in endothelial cells to induce angiogenesis [56].

Exosome is an efficient carrier for intercellular information communication. Tumor-derived exosomes may contain specific proteins and RNA repertoires including numerous lncRNAs. The processes of exosome loading, secretion and uptake are precisely regulated. How the specific lncRNAs are sorted into exosomes and how the exosome are specifically uptake by vascular endothelial cells, these detailed mechanisms are warranted to figure out.

lncRNAs remodel cancer cell to form vasculogenic mimicry to aggravate cancer development

Angiogenesis is not the only means of tumor vasculature development. Vasculogenic mimicry (VM) is another common neovascularization process in tumor development, which was first found in melanoma in 1999 [57]. VM refers to highly plastic tumor cells that may dedifferentiate and obtain endothelial properties, which allow aggressive tumor cells to form vessel-like structures by themselves [57]. Similar to angiogenesis, VM channels contain plasma and red blood cells and have the capacity to transport oxygen and nutrients [58]. In addition, VM networks enable detached tumor cells to enter the blood stream directly, thereby facilitating tumor metastasis to distant organs. Hence, VM density frequently correlates with a high risk of metastasis and poor prognosis [59]. Recently, several lncRNAs have been identified to be involved in tumor VM formation.

The regulatory mechanisms of lncRNAs in tumor angiogenesis

In gastric cancer, lncRNA MALAT1 is upregulated and closely associated with VM density and endothelial vessels. MALAT1 was found to regulate the expression of the VE-cadherin/ β -catenin complex, ERK/MMPs and FAK/paxillin signaling pathways. Consistently, p-ERK inhibitors can effectively inhibit gastric cancer cell from forming VM networks [60]. MALAT1 is also involved in ER β -mediated VM formation in non-small cell lung cancer (NSCLC), but the mechanism is different from that in gastric cancer. In NSCLC, ER β occupies the estrogen response elements within the MALAT1 promoter to activate its transcription directly. Then, the upregulation of MALAT1 decreases miR145-5p expression and increases the protein levels of its target NEDD9, thus promoting VM formation [61]. In lung adenocarcinoma, LINC00312 can induce migration, invasion and VM via forming a complex with the transcriptional factor YBX1. LINC00312 upregulation is positively related to VM in lung adenocarcinoma specimens [62].

A previous study reported that cancer stem cells (CSCs) are closely linked to the VM process, as tumor cells capable of VM resemble CSCs with highly undifferentiated and plastic properties [63]. In HCC, lncRNA n339260 is overexpressed in cancer stem-like cells and is associated with VM appearance and shorter survival time in mice xenograft model. lncRNA n339260 can induce VE-cadherin expression, which is a well-established molecular marker of VM [64].

In osteosarcoma, Ren et al. reported that lncRNA n340532 has the capacity to stimulate VM. Knockdown of lncRNA n340532 can suppress VM formation *in vitro* and reduce VM density in orthotopic graft *in vivo*. But the mechanism has not been interpreted [65].

Glioma is not only characterized as highly angiogenic malignance but also enriched with VM networks. HOXA-AS2 is upregulated and positively correlated with VM density in glioma specimens. Further study revealed that HOXA-AS2 can bind to miR-373 and diminish its expression. As a result, the target of miR-373, EGFR, is increased, which activates the PI3K pathway to increase VE-cadherin and MMP2/9 expression and leads to VM formation [66]. Similarly, LINC00339 is found to regulate VM in glioma as well. Mechanistically, LINC00339 functions as ceRNA to prevent miR-539-5p

from its target Twist1. The upregulation of Twist1 enhances its transcriptional activity to the promoters of MMP2/14, which can degrade extracellular matrix and facilitate VM formation. LINC00339 depletion and miR-539-5p introduction can significantly shrink tumor growth and reduce VM density *in vivo* xenografts [67].

VM networks have been observed in multiple types of highly aggressive tumors, and regarded as an important factor for the failure of anti-angiogenesis treatment. The malignant tumor cells form extracellular matrix (ECM)-rich vasculogenic-like channels, which can be stained by periodic acid-schiff (PAS). In the present studies, VM densities in tumor tissues were determined by dual staining for endothelial marker CD31 or CD34 and PAS. The lumens with positive CD31 or CD34 staining are endothelial vessels, whereas capillaries with positive PAS but negative CD31 or CD34 staining are defined as VM. Increasing evidence suggests that the properties of cancer stem cells and epithelial mesenchymal transition may contribute to VM formation. How lncRNAs direct the undifferentiated and plastic tumor-cell phenotype need to study in-depth.

Conclusion

In this review, we summarized the versatile mechanisms of lncRNAs in tumor-induced angiogenesis as listed in **Table 1**, and indicated the complexity of lncRNA-mediated angiogenesis in cancer development. Notably, the same lncRNA, such as PVT1, MALAT1, HULC and TUG1, may act via different mechanisms to trigger angiogenesis. In gastric cancer, we found PVT1 facilitates angiogenesis via eliciting the STAT3/VEGFA axis [10]. In cholangiocarcinoma, PVT1 promotes capillary formation through epigenetically silencing ANGPTL4 expression [25]. In addition to cancer cells, PVT1 affects the vasculatures capacity of glioma microvascular endothelial cells by sponging miR-186 to induce protective autophagy [40]. PVT1 can also enhance the tube network formation of vascular endothelial cells by targeting miR-26b to activate CTGF and ANGPT2 [43]. lncRNA MALAT1 acts via diverse mechanisms to control angiogenesis by activating mTOR/HIF-1 α signal pathway in osteosarcoma [17], enhancing Sox2 expression in pancreatic cancer [26], and increasing FGF2 secretion in neuroblastoma [27]. Moreover, MALAT1 can directly remod-

The regulatory mechanisms of lncRNAs in tumor angiogenesis

Table 1. The versatile mechanisms of lncRNAs in tumor-induced angiogenesis

LncRNAs regulate angiogenesis by proteins			
LncRNA Expression	Regulatory Target	Cancer types	Reference
PVT1	↑ STAT3/VEGFA	Gastric cancer	[10]
PVT1	↑ PRC2	Cholangiocarcinoma	[25]
MALAT1	↑ mTOR/HIF-1 α	Osteosarcoma	[17]
MALAT1	↑ Sox2	Pancreatic cancer	[26]
MALAT1	↑ FGF2	Neuroblastoma	[27]
MALAT1	↑ FGF2 secretion	Thyroid cancer	[48]
MALAT1	↑ VE-cadherin/ β -catenin, ERK/MMPs, and FAK/paxillin	Gastric cancer	[60]
lncRNA-MM2P	↑ STAT6 activation	Osteosarcoma	[46]
TNK2-AS1	↑ STAT3/VEGFA	Non-small cell lung cancer	[11]
LOC100132354	↑ VEGFA/VEGFR2/Raf/MEK/ERK	Lung adenocarcinoma	[18]
UBE2CP3	↑ ERK/HIF-1 α /p70S6K/VEGFA	Hepatocellular carcinoma	[13]
OR3A4	↑ AGGF1/AKT/mTOR	Hepatocellular carcinoma	[15]
MVIH	↑ PKG1	Hepatocellular carcinoma	[21]
LINC00312	↑ YBX1	Hepatocellular carcinoma	[62]
n339260	↑ VE-cadherin	Hepatocellular carcinoma	[64]
H19	↑ VEGF, VEGFR1 and ICAM1	Hepatocellular carcinoma	[54]
CRNDE	↑ mTOR/P70S6K/VEGFA and ESM1	Hepatoblastoma	[16]
HULC	↑ PI3K/Akt/mTOR/ESM-1	Glioma	[14]
linc-CCAT2	↑ VEGFA, TGF- β , Bcl-2, Bax, caspase-3	Glioma	[52]
HOTAIR	↑ VEGFA	Glioma	[53]
OR3A4	↑ PDLIM2, MACC1, NTN4 and GNB2L1	Gastric cancer	[23]
HOTAIR	↑ VEGFA promoter/GRP78	Nasopharyngeal carcinoma	[28]
CamK-A	↑ PNCK/I κ B- α /NF- κ B/VEGF, IL-6 and IL-8	Breast cancer	[47]
GAS5	↓ Wnt/ β -catenin	Colorectal cancer	[19]
lncRNA-APC1	↓ Rab5b	Colorectal cancer	[56]
MEG3	↓ AKT/VEGF	Breast cancer	[20]
lnc-CCDST	↓ MDM2/DHX9	Cervical cancer	[22]
NBAT1	↓ Sox9	Gastric cancer	[24]
LncRNAs regulate angiogenesis via ceRNA function			
LncRNA Expression	miRNA and target	Cancer types	Reference
LINC01410	↑ miR-532-5p/NCF2/NF- κ B	Gastric cancer	[12]
TUG1	↑ miR-299/VEGFA	Glioblastoma	[31]
TUG1	↑ miR-34a-5p/VEGFA	Hepatoblastoma	[32]
lncRNA XIST	↑ miR-429	Glioma	[33]
HOXA-AS2	↑ miR-373/EGFR/PI3K/VE-cadherin/MMPs	Glioma	[66]
LINC00339	↑ miR-539-3p/Twist1/MMPs	Glioma	[67]
linc00511	↑ miR-29-3P/VEGFA	Pancreatic cancer	[37]
HULC	↑ miR-107/E2F1/SPHK1	Hepatocellular carcinoma	[38]
ZFAS1	↑ miR-150-5p/VEGFA	Colorectal cancer	[30]
MALAT1	↑ miR145-5p/NEDD9	Non-small cell lung cancer	[61]
H19	↑ miR-29b/DNMT3B	Bladder cancer	[35]
RP11-79H23.3	↓ miR-107/PTEN	Bladder cancer	[36]
LncRNAs regulate angiogenesis via maintaining endothelial cells function			
LncRNA Expression	miRNA and target	Endothelia cell types	Reference
STEEL	↑ STEEL/PARP1/KLF2 negative feedback	Endothelial cells	[39]
PVT1	↑ miR-186/Atg7 and Beclin1	Glioma endothelial cells	[40]
H19	↑ miR-29a/VASH2	Glioma endothelial cells	[41]
MCM3AP-AS1	↑ miR-211/KLF5/AGGF1/PI3K/AKT/ERK	Glioma endothelial cells	[42]
lncRNA XIST	↑ miR-137/ZO-2 and FOXC1	Glioma endothelia cells	[34]
PVT1	↑ miR26b/CTGF and ANGPT2	Vein endothelial cells	[43]
MEG3	↑ VEGFA/VEGFR2	Vein endothelial cells	[44]

The regulatory mechanisms of lncRNAs in tumor angiogenesis

el cancer cells to induce vasculogenic mimicry through impacting the ERK/MMPs and FAK/paxillin axis [60], or by decoying miR-145-5p to elevate NEDD9 expression [61]. These independent studies demonstrate that lncRNAs are multifunctional regulators, and that distinct mechanisms of controlling angiogenesis may depend on different cellular backgrounds or stimulated contexts. Investigations of these regulatory mechanisms are helpful to develop effective and precise approaches for cancer treatment.

To the best of our known, although anti-angiogenesis therapy has greatly improved progression-free survival, the overall survival time remains unsatisfactory because of the lack of proper biomarkers to identify sensitive responders [3]. VEGFA alone is inadequate to predict the effects of bevacizumab (anti-VEGF) treatment [68]. Some lncRNAs, such as PVT1 and MALAT1, are very stable in physiological environments and can be detected successfully in serum and exosomes; thus, these lncRNAs have potential diagnostic and prognostic value [55, 69]. Nowadays, lncRNA PCA3 has been approved as early diagnostic biomarker for prostate cancer by FDA [70]. Combining lncRNAs and proteins might be a feasible strategy to monitor the efficacy and predict the prognosis of anti-angiogenesis therapy. Additionally, the regulatory signaling pathways of lncRNAs in angiogenesis may be promising therapeutic targets. The clinical application of lncRNAs merits further investigations in the future.

Acknowledgements

This study was supported by the Natural Science Foundation of China (81672378, 81201521 and 81873874) and the National Key Project for Infectious Disease of China (2017ZX10203205-002-004 and 2017ZX10203-205-003-003).

Disclosure of conflict of interest

None.

Address correspondence to: Zheng-Xin Wang and Lun-Xiu Qin, Department of General Surgery, Huashan Hospital, Fudan University, 12 Urumqi Road (M), Shanghai 200040, China. E-mail: wangzhengxin@huashan.org.cn (ZXW); qinlx@fudan.edu.cn (LXQ)

References

- [1] Hanahan D and Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144: 646-674.
- [2] Folkman J. Role of angiogenesis in tumor growth and metastasis. *Semin Oncol* 2002; 29: 15-18.
- [3] Jayson GC, Kerbel R, Ellis LM and Harris AL. Antiangiogenic therapy in oncology: current status and future directions. *Lancet* 2016; 388: 518-529.
- [4] ENCODE Project Consortium, Birney E, Stamatoyannopoulos JA, Dutta A, Guigó R, Gingeras TR, Margulies EH, Weng Z, Snyder M, Dermitzakis ET, Thurman RE, Kuehn MS, Taylor CM, Neph S, Koch CM, Asthana S, Malhotra A, Adzhubei I, Greenbaum JA, Andrews RM, Flicek P, Boyle PJ, Cao H, Carter NP, Clelland GK, Davis S, Day N, Dhami P, Dillon SC, Dorschner MO, Fiegler H, Giresi PG, Goldy J, Hawrylycz M, Haydock A, Humbert R, James KD, Johnson BE, Johnson EM, Frum TT, Rosenzweig ER, Karmani N, Lee K, Lefebvre GC, Navas PA, Neri F, Parker SC, Sabo PJ, Sandstrom R, Shafer A, Vetrie D, Weaver M, Wilcox S, Yu M, Collins FS, Dekker J, Lieb JD, Tullius TD, Crawford GE, Sunyaev S, Noble WS, Dunham I, Denoeud F, Raymond A, Kapranov P, Rozowsky J, Zheng D, Castelo R, Frankish A, Harrow J, Ghosh S, Sandelin A, Hofacker IL, Baertsch R, Keefe D, Dike S, Cheng J, Hirsch HA, Sekinger EA, Lagarde J, Abril JF, Shahab A, Flamm C, Fried C, Hacker-müller J, Hertel J, Lindemeyer M, Missal K, Tanzer A, Washietl S, Korb J, Emanuelsson O, Pedersen JS, Holroyd N, Taylor R, Swarbreck D, Matthews N, Dickson MC, Thomas DJ, Weirauch MT, Gilbert J, Drenkow J, Bell I, Zhao X, Srinivasan KG, Sung WK, Ooi HS, Chiu KP, Foisac S, Alioto T, Brent M, Pachter L, Tress ML, Valencia A, Choo SW, Choo CY, Ucla C, Manzano C, Wyss C, Cheung E, Clark TG, Brown JB, Ganesh M, Patel S, Tammana H, Chrast J, Henriksen CN, Kai C, Kawai J, Nagalakshmi U, Wu J, Lian Z, Lian J, Newburger P, Zhang X, Bickel P, Mattick JS, Carninci P, Hayashizaki Y, Weissman S, Hubbard T, Myers RM, Rogers J, Stadler PF, Lowe TM, Wei CL, Ruan Y, Struhl K, Gerstein M, Antonarakis SE, Fu Y, Green ED, Karaöz U, Siepel A, Taylor J, Liefer LA, Wetterstrand KA, Good PJ, Feingold EA, Guyer MS, Cooper GM, Asimenos G, Dewey CN, Hou M, Nikolaev S, Montoya-Burgos JI, Löytynoja A, Whelan S, Pardi F, Massingham T, Huang H, Zhang NR, Holmes I, Mullikin JC, Ureta-Vidal A, Paten B, Srinivasan M, Church D, Rosenbloom K, Kent WJ, Stone EA; NISC Comparative Sequencing Program; Baylor College of Medicine Human Genome Sequencing Center; Washington Uni-

The regulatory mechanisms of lncRNAs in tumor angiogenesis

- versity Genome Sequencing Center; Broad Institute; Children's Hospital Oakland Research Institute, Batzoglu S, Goldman N, Hardison RC, Haussler D, Miller W, Sidow A, Trinklein ND, Zhang ZD, Barrera L, Stuart R, King DC, Ameer A, Enroth S, Bieda MC, Kim J, Bhinge AA, Jiang N, Liu J, Yao F, Vega VB, Lee CW, Ng P, Shahab A, Yang A, Moqtaderi Z, Zhu Z, Xu X, Squazzo S, Oberley MJ, Inman D, Singer MA, Richmond TA, Munn KJ, Rada-Iglesias A, Wallerman O, Komorowski J, Fowler JC, Couttet P, Bruce AW, Dovey OM, Ellis PD, Langford CF, Nix DA, Euskirchen G, Hartman S, Urban AE, Kraus P, Van Calcar S, Heintzman N, Kim TH, Wang K, Qu C, Hon G, Luna R, Glass CK, Rosenfeld MG, Aldred SF, Cooper SJ, Halees A, Lin JM, Shulha HP, Zhang X, Xu M, Haidar JN, Yu Y, Ruan Y, Iyer VR, Green RD, Wadelius C, Farnham PJ, Ren B, Harte RA, Hinrichs AS, Trumbower H, Clawson H, Hillman-Jackson J, Zweig AS, Smith K, Thakapallayil A, Barber G, Kuhn RM, Karolchik D, Armengol L, Bird CP, de Bakker PI, Kern AD, Lopez-Bigas N, Martin JD, Stranger BE, Woodroffe A, Davydov E, Dimas A, Eyras E, Hallgrimsdóttir IB, Huppert J, Zody MC, Abecasis GR, Estivill X, Bouffard GG, Guan X, Hansen NF, Idol JR, Maduro VV, Maskeri B, McDowell JC, Park M, Thomas PJ, Young AC, Blakesley RW, Muzny DM, Sodergren E, Wheeler DA, Worley KC, Jiang H, Weinstock GM, Gibbs RA, Graves T, Fulton R, Mardis ER, Wilson RK, Clamp M, Cuff J, Gnerre S, Jaffe DB, Chang JL, Lindblad-Toh K, Lander ES, Koriabine M, Nefedov M, Osoegawa K, Yoshinaga Y, Zhu B, de Jong PJ. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. *Nature* 2007; 447: 799-816.
- [5] Nagano T and Fraser P. No-nonsense functions for long noncoding RNAs. *Cell* 2011; 145: 178-181.
- [6] Gutschner T and Diederichs S. The hallmarks of cancer: a long non-coding RNA point of view. *RNA Biol* 2012; 9: 703-719.
- [7] Kopp F and Mendell JT. Functional classification and experimental dissection of long non-coding RNAs. *Cell* 2018; 172: 393-407.
- [8] Gupta RA, Shah N, Wang KC, Kim J, Horlings HM, Wong DJ, Tsai MC, Hung T, Argani P, Rinn JL, Wang Y, Brzoska P, Kong B, Li R, West RB, van de Vijver MJ, Sukumar S and Chang HY. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. *Nature* 2010; 464: 1071-1076.
- [9] Salmena L, Poliseno L, Tay Y, Kats L and Pandolfi PP. A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? *Cell* 2011; 146: 353-358.
- [10] Zhao J, Du P, Cui P, Qin Y, Hu C, Wu J, Zhou Z, Zhang W, Qin L and Huang G. lncRNA PVT1 promotes angiogenesis via activating the STAT3/VEGFA axis in gastric cancer. *Oncogene* 2018; 37: 4094-4109.
- [11] Wang Y, Han D, Pan L and Sun J. The positive feedback between lncRNA TNK2-AS1 and STAT3 enhances angiogenesis in non-small cell lung cancer. *Biochem Biophys Res Commun* 2018; 507: 185-192.
- [12] Zhang JX, Chen ZH, Chen DL, Tian XP, Wang CY, Zhou ZW, Gao Y, Xu Y, Chen C, Zheng ZS, Weng HW, Ye S, Kuang M, Xie D and Peng S. LINC01410-miR-532-NCF2-NF-kB feedback loop promotes gastric cancer angiogenesis and metastasis. *Oncogene* 2018; 37: 2660-2675.
- [13] Lin J, Cao S, Wang Y, Hu Y, Liu H, Li J, Chen J, Li P, Liu J, Wang Q and Zheng L. Long non-coding RNA UBE2CP3 enhances HCC cell secretion of VEGFA and promotes angiogenesis by activating ERK1/2/HIF-1alpha/VEGFA signalling in hepatocellular carcinoma. *J Exp Clin Cancer Res* 2018; 37: 113.
- [14] Zhu Y, Zhang X, Qi L, Cai Y, Yang P, Xuan G and Jiang Y. HULC long noncoding RNA silencing suppresses angiogenesis by regulating ESM-1 via the PI3K/Akt/mTOR signaling pathway in human gliomas. *Oncotarget* 2016; 7: 14429-14440.
- [15] Li W, Fu Q, Man W, Guo H and Yang P. lncRNA OR3A4 participates in the angiogenesis of hepatocellular carcinoma through modulating AGGF1/akt/mTOR pathway. *Eur J Pharmacol* 2019; 849: 106-114.
- [16] Dong R, Liu XQ, Zhang BB, Liu BH, Zheng S and Dong KR. Long non-coding RNA-CRND1: a novel regulator of tumor growth and angiogenesis in hepatoblastoma. *Oncotarget* 2017; 8: 42087-42097.
- [17] Zhang ZC, Tang C, Dong Y, Zhang J, Yuan T, Tao SC and Li XL. Targeting the long noncoding RNA MALAT1 blocks the pro-angiogenic effects of osteosarcoma and suppresses tumour growth. *Int J Biol Sci* 2017; 13: 1398-1408.
- [18] Wang Y, Zhang F, Wang J, Hu L, Jiang F, Chen J, Chen J and Wang L. lncRNA LOC100132354 promotes angiogenesis through VEGFA/VEGFR2 signaling pathway in lung adenocarcinoma. *Cancer Manag Res* 2018; 10: 4257-4266.
- [19] Song J, Shu H, Zhang L and Xiong J. Long non-coding RNA GAS5 inhibits angiogenesis and metastasis of colorectal cancer through the Wnt/beta-catenin signaling pathway. *J Cell Biochem* 2019; [Epub ahead of print].
- [20] Zhang CY, Yu MS, Li X, Zhang Z, Han CR and Yan B. Overexpression of long non-coding RNA MEG3 suppresses breast cancer cell proliferation, invasion, and angiogenesis through AKT pathway. *Tumour Biol* 2017; 39: 1010428317701311.

The regulatory mechanisms of lncRNAs in tumor angiogenesis

- [21] Yuan SX, Yang F, Yang Y, Tao QF, Zhang J, Huang G, Yang Y, Wang RY, Yang S, Huo XS, Zhang L, Wang F, Sun SH and Zhou WP. Long noncoding RNA associated with microvascular invasion in hepatocellular carcinoma promotes angiogenesis and serves as a predictor for hepatocellular carcinoma patients' poor recurrence-free survival after hepatectomy. *Hepatology* 2012; 56: 2231-2241.
- [22] Ding X, Jia X, Wang C, Xu J, Gao SJ and Lu C. A DHX9-lncRNA-MDM2 interaction regulates cell invasion and angiogenesis of cervical cancer. *Cell Death Differ* 2018; [Epub ahead of print].
- [23] Guo X, Yang Z, Zhi Q, Wang D, Guo L, Li G, Miao R, Shi Y and Kuang Y. Long noncoding RNA OR3A4 promotes metastasis and tumorigenicity in gastric cancer. *Oncotarget* 2016; 7: 30276-30294.
- [24] Yan J, Huang W, Huang X, Xiang W, Ye C and Liu J. A negative feedback loop between long non-coding RNA NBAT1 and Sox9 inhibits the malignant progression of gastric cancer cells. *Biosci Rep* 2018; 38.
- [25] Yu Y, Zhang M, Liu J, Xu B, Yang J, Wang N, Yan S, Wang F, He X, Ji G, Li Q and Miao L. Long non-coding RNA PVT1 promotes cell proliferation and migration by silencing ANGPTL4 expression in cholangiocarcinoma. *Mol Ther Nucleic Acids* 2018; 13: 503-513.
- [26] Jiao F, Hu H, Han T, Yuan C, Wang L, Jin Z, Guo Z and Wang L. Long noncoding RNA MALAT-1 enhances stem cell-like phenotypes in pancreatic cancer cells. *Int J Mol Sci* 2015; 16: 6677-6693.
- [27] Tee AE, Liu B, Song R, Li J, Pasquier E, Cheung BB, Jiang C, Marshall GM, Haber M, Norris MD, Fletcher JI, Dinger ME and Liu T. The long non-coding RNA MALAT1 promotes tumor-driven angiogenesis by up-regulating pro-angiogenic gene expression. *Oncotarget* 2016; 7: 8663-8675.
- [28] Fu WM, Lu YF, Hu BG, Liang WC, Zhu X, Yang HD, Li G and Zhang JF. Long noncoding RNA Hotair mediated angiogenesis in nasopharyngeal carcinoma by direct and indirect signaling pathways. *Oncotarget* 2016; 7: 4712-4723.
- [29] Karreth FA and Pandolfi PP. ceRNA cross-talk in cancer: when ce-bling rivalries go awry. *Cancer Discov* 2013; 3: 1113-1121.
- [30] Chen X, Zeng K, Xu M, Hu X, Liu X, Xu T, He B, Pan Y, Sun H and Wang S. SP1-induced lncRNA-ZFAS1 contributes to colorectal cancer progression via the miR-150-5p/VEGFA axis. *Cell Death Dis* 2018; 9: 982.
- [31] Cai H, Liu X, Zheng J, Xue Y, Ma J, Li Z, Xi Z, Li Z, Bao M and Liu Y. Long non-coding RNA taurine upregulated 1 enhances tumor-induced angiogenesis through inhibiting microRNA-299 in human glioblastoma. *Oncogene* 2017; 36: 318-331.
- [32] Dong R, Liu GB, Liu BH, Chen G, Li K, Zheng S and Dong KR. Targeting long non-coding RNA-TUG1 inhibits tumor growth and angiogenesis in hepatoblastoma. *Cell Death Dis* 2016; 7: e2278.
- [33] Cheng Z, Li Z, Ma K, Li X, Tian N, Duan J, Xiao X and Wang Y. Long non-coding RNA XIST promotes glioma tumorigenicity and angiogenesis by acting as a molecular sponge of miR-429. *J Cancer* 2017; 8: 4106-4116.
- [34] Yu H, Xue Y, Wang P, Liu X, Ma J, Zheng J, Li Z, Li Z, Cai H and Liu Y. Knockdown of long non-coding RNA XIST increases blood-tumor barrier permeability and inhibits glioma angiogenesis by targeting miR-137. *Oncogenesis* 2017; 6: e303.
- [35] Lv M, Zhong Z, Huang M, Tian Q, Jiang R and Chen J. lncRNA H19 regulates epithelial-mesenchymal transition and metastasis of bladder cancer by miR-29b-3p as competing endogenous RNA. *Biochim Biophys Acta Mol Cell Res* 2017; 1864: 1887-1899.
- [36] Chi H, Yang R, Zheng X, Zhang L, Jiang R and Chen J. lncRNA RP11-79H23.3 functions as a competing endogenous RNA to regulate PTEN expression through sponging hsa-miR-107 in the development of bladder cancer. *Int J Mol Sci* 2018; 19.
- [37] Zhao X, Liu Y, Li Z, Zheng S, Wang Z, Li W, Bi Z, Li L, Jiang Y, Luo Y, Lin Q, Fu Z and Rufu C. Linc00511 acts as a competing endogenous RNA to regulate VEGFA expression through sponging hsa-miR-29b-3p in pancreatic ductal adenocarcinoma. *J Cell Mol Med* 2018; 22: 655-667.
- [38] Lu Z, Xiao Z, Liu F, Cui M, Li W, Yang Z, Li J, Ye L and Zhang X. Long non-coding RNA HULC promotes tumor angiogenesis in liver cancer by up-regulating sphingosine kinase 1 (SPHK1). *Oncotarget* 2016; 7: 241-254.
- [39] Man HSJ, Sukumar AN, Lam GC, Turgeon PJ, Yan MS, Ku KH, Dubinsky MK, Ho JJD, Wang JJ, Das S, Mitchell N, Oettgen P, Sefton MV and Marsden PA. Angiogenic patterning by STEEL, an endothelial-enriched long noncoding RNA. *Proc Natl Acad Sci U S A* 2018; 115: 2401-2406.
- [40] Ma Y, Wang P, Xue Y, Qu C, Zheng J, Liu X, Ma J and Liu Y. PVT1 affects growth of glioma microvascular endothelial cells by negatively regulating miR-186. *Tumour Biol* 2017; 39: 1010428317694326.
- [41] Jia P, Cai H, Liu X, Chen J, Ma J, Wang P, Liu Y, Zheng J and Xue Y. Long non-coding RNA H19 regulates glioma angiogenesis and the biological behavior of glioma-associated endothelial cells by inhibiting microRNA-29a. *Cancer Lett* 2016; 381: 359-369.
- [42] Yang C, Zheng J, Xue Y, Yu H, Liu X, Ma J, Liu L, Wang P, Li Z, Cai H and Liu Y. The effect of MC-

The regulatory mechanisms of lncRNAs in tumor angiogenesis

- M3AP-AS1/miR-211/KLF5/AGGF1 axis regulating glioblastoma angiogenesis. *Front Mol Neurosci* 2017; 10: 437.
- [43] Zheng J, Hu L, Cheng J, Xu J, Zhong Z, Yang Y and Yuan Z. lncRNA PVT1 promotes the angiogenesis of vascular endothelial cell by targeting miR26b to activate CTGF/ANGPT2. *Int J Mol Med* 2018; 42: 489-496.
- [44] Ruan W, Zhao F, Zhao S, Zhang L, Shi L and Pang T. Knockdown of long noncoding RNA MEG3 impairs VEGF-stimulated endothelial sprouting angiogenesis via modulating VEGFR2 expression in human umbilical vein endothelial cells. *Gene* 2018; 649: 32-39.
- [45] De Palma M and Lewis CE. Macrophage regulation of tumor responses to anticancer therapies. *Cancer Cell* 2013; 23: 277-286.
- [46] Cao J, Dong R, Jiang L, Gong Y, Yuan M, You J, Meng W, Chen Z, Zhang N, Weng Q, Zhu H, He Q, Ying M and Yang B. lncRNA-MM2P identified as a Modulator of Macrophage M2 Polarization. *Cancer Immunol Res* 2018; 7: 292-305.
- [47] Sang LJ, Ju HQ, Liu GP, Tian T, Ma GL, Lu YX, Liu ZX, Pan RL, Li RH, Piao HL, Marks JR, Yang LJ, Yan Q, Wang W, Shao J, Zhou Y, Zhou T and Lin A. lncRNA CamK-A regulates Ca²⁺-signaling-mediated tumor microenvironment remodeling. *Mol Cell* 2018; 72: 71-83, e77.
- [48] Huang JK, Ma L, Song WH, Lu BY, Huang YB, Dong HM, Ma XK, Zhu ZZ and Zhou R. lncRNA-MALAT1 promotes angiogenesis of thyroid cancer by modulating tumor-associated macrophage FGF2 protein secretion. *J Cell Biochem* 2017; 118: 4821-4830.
- [49] Tkach M and Thery C. Communication by extracellular vesicles: where we are and where we need to go. *Cell* 2016; 164: 1226-1232.
- [50] Ludwig N and Whiteside TL. Potential roles of tumor-derived exosomes in angiogenesis. *Expert Opin Ther Targets* 2018; 22: 409-417.
- [51] Lang HL, Hu GW, Chen Y, Liu Y, Tu W, Lu YM, Wu L and Xu GH. Glioma cells promote angiogenesis through the release of exosomes containing long non-coding RNA POU3F3. *Eur Rev Med Pharmacol Sci* 2017; 21: 959-972.
- [52] Lang HL, Hu GW, Zhang B, Kuang W, Chen Y, Wu L and Xu GH. Glioma cells enhance angiogenesis and inhibit endothelial cell apoptosis through the release of exosomes that contain long non-coding RNA CCAT2. *Oncol Rep* 2017; 38: 785-798.
- [53] Ma X, Li Z, Li T, Zhu L, Li Z and Tian N. Long non-coding RNA HOTAIR enhances angiogenesis by induction of VEGFA expression in glioma cells and transmission to endothelial cells via glioma cell derived-extracellular vesicles. *Am J Transl Res* 2017; 9: 5012-5021.
- [54] Conigliaro A, Costa V, Lo Dico A, Saieva L, Buccheri S, Dieli F, Manno M, Raccosta S, Mancone C, Tripodi M, De Leo G and Alessandro R. CD90+ liver cancer cells modulate endothelial cell phenotype through the release of exosomes containing H19 lncRNA. *Mol Cancer* 2015; 14: 155.
- [55] Qiu JJ, Lin XJ, Tang XY, Zheng TT, Lin YY and Hua KQ. Exosomal metastas is associated lung adenocarcinoma transcript 1 promotes angiogenesis and predicts poor prognosis in epithelial ovarian cancer. *Int J Biol Sci* 2018; 14: 1960-1973.
- [56] Wang FW, Cao CH, Han K, Zhao YX, Cai MY, Xiang ZC, Zhang JX, Chen JW, Zhong LP, Huang Y, Zhou SF, Jin XH, Guan XY, Xu RH and Xie D. APC-activated long non-coding RNA inhibits colorectal carcinoma pathogenesis through reducing exosome production. *J Clin Invest* 2019; 129: 727-743.
- [57] Maniotis AJ, Folberg R, Hess A, Seftor EA, Gardner LM, Pe'er J, Trent JM, Meltzer PS and Hendrix MJ. Vascular channel formation by human melanoma cells in vivo and in vitro: vasculogenic mimicry. *Am J Pathol* 1999; 155: 739-752.
- [58] Krishna Priya S, Nagare RP, Sneha VS, Sidhanth C, Bindhya S, Manasa P and Ganesan TS. Tumour angiogenesis-Origin of blood vessels. *Int J Cancer* 2016; 139: 729-735.
- [59] Paulis YW, Soetekouw PM, Verheul HM, Tjan-Heijnen VC and Griffioen AW. Signalling pathways in vasculogenic mimicry. *Biochim Biophys Acta* 2010; 1806: 18-28.
- [60] Li Y, Wu Z, Yuan J, Sun L, Lin L, Huang N, Bin J, Liao Y and Liao W. Long non-coding RNA MALAT1 promotes gastric cancer tumorigenicity and metastasis by regulating vasculogenic mimicry and angiogenesis. *Cancer Lett* 2017; 395: 31-44.
- [61] Yu W, Ding J, He M, Chen Y, Wang R, Han Z, Xing EZ, Zhang C and Yeh S. Estrogen receptor beta promotes the vasculogenic mimicry (VM) and cell invasion via altering the lncRNA-MALAT1/miR-145-5p/NEDD9 signals in lung cancer. *Oncogene* 2019; 38: 1225-1238.
- [62] Peng Z, Wang J, Shan B, Li B, Peng W, Dong Y, Shi W, Zhao W, He D, Duan M, Cheng Y, Zhang C and Duan C. The long noncoding RNA LINC00312 induces lung adenocarcinoma migration and vasculogenic mimicry through directly binding YBX1. *Mol Cancer* 2018; 17: 167.
- [63] Yao XH, Ping YF and Bian XW. Contribution of cancer stem cells to tumor vasculogenic mimicry. *Protein Cell* 2011; 2: 266-272.
- [64] Zhao X, Sun B, Liu T, Shao B, Sun R, Zhu D, Zhang Y, Gu Q, Dong X, Liu F, Zhao N, Zhang D, Li Y, Meng J, Gong W, Zheng Y and Zheng X. Long noncoding RNA n339260 promotes vasculogenic mimicry and cancer stem cell devel-

The regulatory mechanisms of lncRNAs in tumor angiogenesis

- opment in hepatocellular carcinoma. *Cancer Sci* 2018; 109: 3197-3208.
- [65] Ren K, Ni Y, Li X, Wang C, Chang Q, Li Y, Gao Z, Wu S, Shi X, Song J, Yao N and Zhou J. Expression profiling of long noncoding RNAs associated with vasculogenic mimicry in osteosarcoma. *J Cell Biochem* 2019; 120: 12473-12488.
- [66] Gao Y, Yu H, Liu Y, Liu X, Zheng J, Ma J, Gong W, Chen J, Zhao L, Tian Y and Xue Y. Long non-coding RNA HOXA-AS2 regulates malignant glioma behaviors and vasculogenic mimicry formation via the MiR-373/EGFR Axis. *Cell Physiol Biochem* 2018; 45: 131-147.
- [67] Guo J, Cai H, Liu X, Zheng J, Liu Y, Gong W, Chen J, Xi Z and Xue Y. Long non-coding RNA LINC00339 stimulates glioma vasculogenic mimicry formation by regulating the miR-539-5p/TWIST1/MMPs axis. *Mol Ther Nucleic Acids* 2018; 10: 170-186.
- [68] Sennino B and McDonald DM. Controlling escape from angiogenesis inhibitors. *Nat Rev Cancer* 2012; 12: 699-709.
- [69] Yuan CL, Li H, Zhu L, Liu Z, Zhou J and Shu Y. Aberrant expression of long noncoding RNA PVT1 and its diagnostic and prognostic significance in patients with gastric cancer. *Neoplasma* 2016; 63: 442-449.
- [70] Fujita K and Nonomura N. Urinary biomarkers of prostate cancer. *Int J Urol* 2018; 25: 770-779.