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Review

Long non-coding RNAs: A crucial part of the vasculature puzzle

Ankita Garg[a], Shashi Kumar Gupta[a], Thomas Thum[a,b,c,*]

[a] Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School, Hannover, Germany
[b] REBIRTH Excellence Cluster, Hannover Medical School, Hannover, Germany
[c] National Heart and Lung Institute, Imperial College London, London, UK

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ABSTRACT

With the advent of empowering sequencing techniques over the past two decades, the scientific community has uncovered many underlying secrets of our genome. Non-coding transcripts covering a staggering 98% of our genome strongly suggest their involvement in diverse cellular pathways. A special class of non-coding RNAs (ncRNAs) namely long non-coding RNAs (lncRNAs) has garnered tremendous attention considering its implications in multiple developmental and pathophysiological processes. Accumulating evidence has established lncRNAs as robust regulators of pathways ranging from embryonic cell development to ultimate diseased phenotype. Here, in particular, we summarize the lncRNAs actively participating in the development of our vasculature and the ones which function as drivers or modulators of fatal vascular diseases.

1. Introduction

Our growing understanding of vascular biology has highlighted the invaluable contribution of the circulatory system in health and disease. Besides maintaining an adequate blood flow under physiological conditions, our vasculature is also prone to numerous fatal diseases including the likes of peripheral artery disease (PAD), abdominal aortic aneurysm (AAA), atherosclerosis and cardiovascular diseases triggered due to extravascular stimuli. According to WHO statistics, these ailments have secured top ranks as the causes of deaths worldwide (http://www.who.int/mediacentre/factsheets/fs317/en/). Considering the immense importance of vascular biology, many researchers are actively studying the mechanisms behind these vascular diseases to eventually reach a therapeutic intervention.

Over the past decade, the scientific community has acknowledged the role of transcripts from so-called “Junk DNA” as exceedingly important regulatory molecules, named as non-coding RNAs (ncRNAs) [1]. The size-based subdivision of ncRNAs identified two classes: (1) small ncRNAs with length < 200 nucleotides, and (2) long ncRNAs (lncRNAs) with length > 200 nucleotides [2]. The sky-high interest in the class of lncRNAs can be attributed to their dysregulation under various pathophysiological states [3–5] as well as to the recent discoveries establishing their role in biological processes such as transcription, post-transcriptional regulation (RNA splicing), translation, metabolic regulation, and cellular development [6]. Recently, several lncRNAs have been shown to be involved in cardiovascular development and diseases. Our group identified *CHAST* (Cardiac hypertrophy associated transcript) to be directly connected with cardiac hypertrophy [7]. Levels of *CHAST* were upregulated under a pressure overload-induced cardiac hypertrophic condition in mice and its overexpression also led to induction of cardiac hypertrophy both *in vivo* and *in vitro*. Furthermore, we demonstrated that the GapmeR mediated silencing of *CHAST* can ameliorate hypertrophic phenotype in TAC mice model. In general, GapmeRs are small LNA™-DNA molecules (15–16 nucleotides in length, DNA core flanked by LNA™) designed specific to its intended target sequence. These highly stable and nuclease resistance GapmeRs bind to their target RNA where the DNA core acts as a substrate for RNase H, ultimately leading to the cleavage of bound target RNA [8]. Moreover, highlighting the relevance of *CHAST* as a target for translational medicine, the authors observed an increased

Abbreviations: ACAT2, Acetyl-Coenzyme A acetyltransferase 2; Akt, Protein Kinase B; API5, Apoptosis inhibitor-5; BCL2L12, B-Cell Lymphoma 2 Like 12; CCL2, Chemokine (C-C motif) ligand 2; CCL5, Chemokine (C-C motif) ligand 5; CERS1, Ceramide Synthase 1; CX3CL1, Chemokine (C-X3-C motif) ligand 1; EZH2, Enhancer of Zeste homologue2; FGF-2, Fibroblast growth factor-2; HAS2, Hyaluronan synthase 2; ICAM-1, Intercellular Adhesion Molecule 1; IL-1α, Interleukin 1 alpha; LARP1, La-related protein 1; LNA, Locked Nucleic Acids; MAPK, Mitogen-activated protein kinase; MDK, Midkine; MDM2, Mouse double minute 2 homolog; MESP1, Mesoderm posterior 1; MIXL1, Mix-like 1; MMP2, Matrix metalloproteinase-2; NAT8L, N-Acetyltransferase 8 Like; NFAT, Nuclear factor of activated T-cells; NF-kB, Nuclear factor kappa-light-chain-enhancer of activated B cells; NOTCH1, Neurogenic locus notch homolog protein 1; NUPR1, Nuclear Protein 1; PCNA, Proliferating cell nuclear antigen; PDGF, Platelet-derived growth factor; PI3K, Phosphoinositide 3-kinase; PTEN, Phosphatase and tensin homolog; PTN, Pleiotrophin; TAC, Transverse aortic constriction; TGF-β, Transforming growth factor beta; CNN1, Calponin 1; TIA1, T-cell intracellular antigen 1; TNFα, Tumor necrosis factor alpha; VCAM-1, Vascular Cell Adhesion Molecule 1; VEGF, Vascular endothelial growth factor

* Corresponding author at: Institute of Molecular and Translational Therapeutic Strategies, OE 8886, Hannover Medical School, Carl-Neuberg Strasse 1, 30625 Hannover, Germany.
E-mail address: Thum.Thomas@mh-hannover.de (T. Thum).

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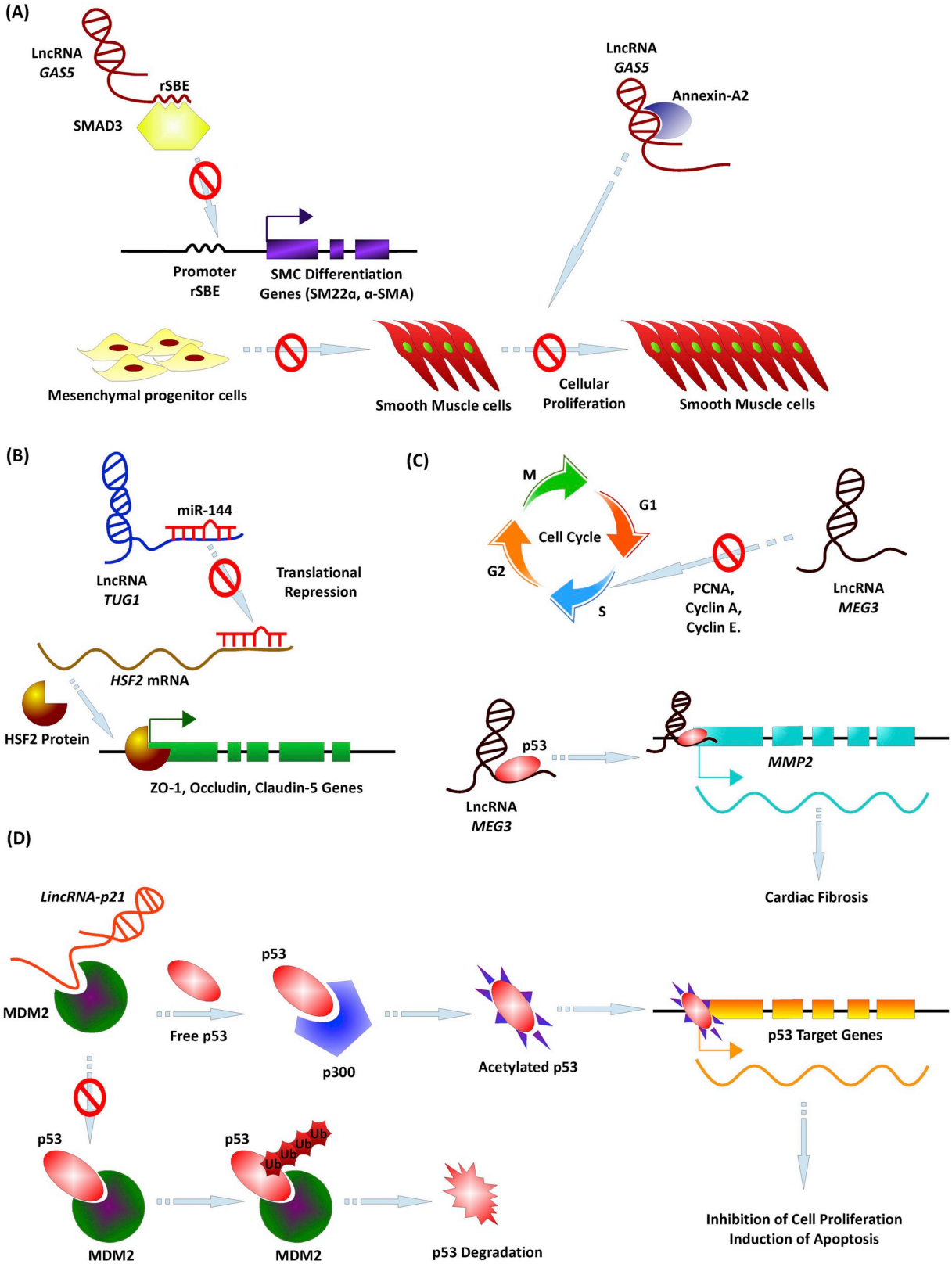

Fig. 1. Schematic overview of the mechanisms followed by various lncRNAs in Vasculature. (A) LncRNA *GAS5*-SMAD3 interaction prevents SMC differentiation by inhibiting SMAD3 binding to the promoters of differentiation genes while *GAS5*-Annexin A2 cross-talk reduces cellular proliferation. (B) LncRNA *TUG1* working as a miRNA sponge, quenching miR-144 which releases its downstream target *HSF2*. (C) LncRNA *MEG3* inhibits cell cycle progression by affecting the levels of PCNA, Cyclin A and Cyclin E while its binding to p53 directs the complex to *MMP2* gene promoter. (D) *LincRNA-p21* binding to MDM2 prevents the MDM2 mediated degradation of p53 and increases p53-p300 interaction. The p300 mediated acetylation of p53 promotes transcriptional activity of p53 and thus induces p53 target genes.

CHAST expression in patients with aortic stenosis while the overexpression of a conserved human *CHAST* in murine HL-1 cells leads to increase in the cell size [7]. Other lncRNAs involved in cardiac hypertrophy are nicely summarized by Chatterjee S et al. highlighting the potential therapeutic role of lncRNAs in cardiovascular diseases [9].

Of notable interest is the crosstalk between lncRNAs and vasculature. Several pieces of evidence point towards lncRNAs being key players in regulating the development of vessel lining and recruitment of immune cells such as macrophages at the site of injury and inflammation [10]. In this review, we summarize the contribution of these novel lncRNAs in vascular biology and their significance as therapeutic targets in numerous vascular diseases with cardiovascular diseases as the central focus.

2. LncRNAs and cellular functions

The importance of vascular development lies in the fact that it is one of the first organs to develop during embryogenesis [11]. Interestingly, our vasculature becomes functional in its early development phase and from this stage onwards, the blood vessels and capillaries maintain an active circulation while developing itself at the same time. A typical blood vessel wall is constituted of two main types of cells namely, smooth muscle cells and endothelial cells. And how lncRNAs are modulating cellular functions as well as vascular development in a cell specific manner is discussed below:

2.1. Smooth-muscle cells

Smooth muscle cells (SMCs) are the major contributors of the medial region of blood vessels and are responsible for relaxation and contraction of vessels regulating the blood pressure and flow [12]. Recently, many researchers showed interest in lncRNAs mediated regulation of SMCs by performing high-throughput lncRNAs analysis using RNA sequencing (RNA-Seq), microarray and quantitative real-time PCR to identify novel lncRNA candidates which are key players in both physiological and pathophysiological settings. For example, *SENCR* (smooth muscle and endothelial cell enriched migration/differentiation-associated Long Non-Coding RNA), a cytoplasmic lncRNA, was first identified by RNA-seq of human coronary artery smooth muscle cells [13]. Further investigation into its role in SMCs identified its involvement in modulating contractile and migratory genes. Bell et al. showed that *SENCR* knockdown reduces the expression of myocardin, a key SMC contractile gene, while migratory genes *MDK* and *PTN* were induced. *In vitro* experiments of cell migration such as scratch assay and Boyden chamber assay further established *SENCR* as a cell migration inhibitor, thereby promoting the contractile phenotype in SMCs [13]. Similarly, another cytoplasmic lncRNA called *MYOSLID* (Myocardin-induced Smooth muscle LncRNA, Inducer of Differentiation) was found to promote contractile phenotype of vascular SMCs [14]. *MYOSLID* was induced with TGF-β, and activator of SMC differentiation while reduced in de-differentiated human arteriovenous fistula patients. Depletion of *MYOSLID* decreases the expression of contractile genes (*CNN1* and *ACAT2*) and disrupts F-actin assembly. *MYOSLID* promotes TGF-β induced SMAD2 phosphorylation and activates TGF-β pathway and its own expression, thus forming a positive feedback loop. Whether *MYOSLID* directly interacts with SMAD2 to promote its phosphorylation remains to be explored [14].

LncRNAs, being an integral part of the non-coding force, have their functionality residing in the sequence specificity towards their interactive partners. LncRNA *GAS5* (growth arrest–specific 5), first discovered in the year 1988 from mouse embryonic fibroblast cells, was found to be involved in diverse physiological procedures such as cellular proliferation, apoptosis and growth arrest [15–17]. *GAS5* regulates SMC differentiation by a direct physical interaction with Smad3 protein *via* the multiple RNA Smad-binding elements (rSBEs) present in its lncRNA sequence. This competitive interaction inhibits Smad3 from binding to the Smad-binding elements present in TGF-β target gene promoters leading to a decrease in the transcription of SMC marker genes (SM22α, α-SMA) thereby suppressing TGF-β/Smad3-mediated SMC differentiation (Fig. 1) [18]. A previous report by Li et al. found a link between the level of *GAS5* expression and cell proliferation of the human saphenous vein smooth muscle cells (HSVSMCs) *via* a Ca^{2+}-dependent RNA-binding protein, Annexin A2 [19]. The gain and loss of function studies established a negative co-relation between *GAS5* and cellular behaviors such as cell proliferation, migration, and cell cycle while Annexin A2 has a positive corelation with cell proliferation. The key role of Annexin A2 in mediating the *GAS5* functions was found by RNA pull down assay which identified a direct binding between the two. The silmultaneous inhibition of *GAS5* and Annexin A2 reduced the proliferation as compared to *GAS5* inhibition alone showing that Annexin A2 mediates the effect of *GAS5* on cell proliferation (Fig. 1) [19].

Another lncRNA *TUG1* (taurine up-regulated gene 1) was found to be essential for EZH2 mediated methylation of α-actin and thus promoting cortex F-actin polymerization in synthetic vascular smooth muscle cells (VSMCs) [20]. The involvement of EZH2 in cytoskeleton formation was confirmed by immunoprecipitation assays which showed an interaction between EZH2, αactin and F-actin. Furthermore, *TUG1* silencing hampered the interaction between EZH2, and α-actin thereby decreasing the EZH2 mediated lysine methylation of α-actin. The authors showed that since methylation status of α-actin is crucial for F-actin polymerization, the *TUG1*/EZH2/α-actin RNA-protein complex is of high importance in cortex cytoskeleton formation [20].

Apart from direct interaction with proteins, lncRNAs could also function as transcriptional regulators. *SMILR* (Smooth muscle-induced lncRNA enhances replication) expression was found to be increased in human saphenous VSMCs upon PDGF and IL-1α stimulation, capable of inducing vasculoproliferative phenotype [21]. The authors observed that *SMILR* imparts a positive effect on cell proliferation which can be attributed to its regulation of the proximal gene *HAS2, a gene encoding an enzyme involved in the formation of atherosclerotic lesions*. *SMILR* knockdown by RNAi decreases the transcript levels of *HAS2* which consolidate the proposed function of *SMILR* as a pro-atherosclerotic lncRNA. They further validated the above effect in the unstable atherosclerotic plaques from patients undergoing endarterectomy following an acute and symptomatic neurovascular event where *SMILR* was found to be upregulated [21].

2.2. Endothelial cells

Vascular endothelial cells (ECs) line the inner surface of blood vessels *i.e.* intima and are directly in contact with the circulating blood. They feature as the barrier between the surrounding tissues and blood regulating the passage of nutrients and signaling molecules [22]. Over the past one decade, many reports linked the mechanisms of vascular development to lncRNAs. In 2009, Li et al. identified an antisense transcript of gene *tie-1* (tyrosine kinase containing immunoglobulin and epidermal growth factor homology domain-1), *tie-1 AS*, in embryonic zebrafish. The lncRNA *tie-1 AS* was found to reduce the transcript levels of *tie-1* by specifically binding to it which in turn decreases the protein levels of Tie-1. Since Tie-1 is important for the maintenance of endothelial cell networks, the *tie-1/tie-1 AS* interaction leads to the mislocalization of endothelial cell junctions and causes hindrance in proper vessel formation [23]. Another lncRNA known as *NRON* (noncoding repressor of NFAT) was also found to be involved in tube formation where its shRNA mediated silencing induced proliferative, migratory and tube forming capability in human umbilical vein endothelial cells (HUVECs) while the *NRON* overexpressing HUVECs showed opposite phenotype [24]. Interestingly, *NRON* has also been identified as a circulating biomarker for the prediction of heart failure as the plasma levels of *NRON* transcript were found to be significantly higher in patients with heart failure as compared to control group [25].

Yan et al. established the critical role of *MIAT* (myocardial

infarction–associated transcript) in governing retina's microvascular functions under diabetic conditions. They observed that the levels of *MIAT* in the retinas of streptozotocin (STZ)-induced diabetic rats were significantly high while the *in vitro* experiments with RF/6A (a type of retinal ECs) under high glucose treatment also confirmed the induction of *MIAT* [26]. In human microvascular endothelial cells (HMVECs), the silencing of *MIAT* leads to the decrease in endothelial cell proliferation and cell viability while increasing apoptotic rate while in STZ-induced diabetic rats, *MIAT* knockdown improves visual functions by reducing diabetes induced pathological endothelial proliferation and migration, vascular leakage and inflammation. By functioning as a competitive endogenous RNA (ceRNA) for miR-150-5p, *MIAT* sponges miR-150-5p and releases its downstream target VEGF, a positive regulator of angiogenesis, to positively regulate cell proliferation and viability [26]. Interestingly, lncRNA *Meg3* (maternally expressed gene 3) was also found to be involved in diabetes related microvascular dysfunction as Qiu et al. illustrated its downregulation in endothelial cells under high glucose and oxidative stress as well as in the retinas of mice under STZ-induced diabetes [27]. Loss of function studies established that *Meg3* suppression worsens the retinal vascular leakage in mice while inducing the expression of inflammatory proteins such as VEGF, TNF-α, IL-1, IL-6, and CCL2. *In vitro* experiments showed that *Meg3* knockdown increases the endothelial cell proliferation by regulating PI3K/Akt signaling, an important pathway for glycogen metabolism as well as proliferation, where it increased the phosphorylation of PI3K and Akt while keeping their total expression level undisturbed [27]. In addition to regulating cellular proliferation in ECs, *MEG3* also contributes to the process of angiogenesis, a crucial aspect of vascular development [28]. Recently, He et al. demonstrated that lentivirus mediated *MEG3* overexpression showed reduced angiogenesis as analyzed by capillary like network formation assay in HUVECs while its knockdown had the reverse effect. They could also confirm the negative impact of *MEG3* overexpression on cell proliferation as discussed above. Mechanistically, *MEG3* was found to be sponging miR-9, a known positive regulator of angiogenesis, to reduce angiogenesis and proliferation. However, simultaneous overexpression miR-9 with *MEG3* could not reverse the effects of *MEG3* completely indicating the contribution of more interactive partners which require further attention [28].

One of the new entrants in the class of angiogenesis regulator is lncRNA *NONHSAT073641* which shares 92% sequence similarity with an important neurological development protein Platelet-activating factor acetylhydrolase 1B1 (PAFAH1B1) as found by cDNA sequence comparisons [29]. A moderately high expression of PAFAH1B1 especially in our vasculature pointed towards the potential participation of PAFAH1B1 and *NONHSAT073641* in the processes of vascular development. Hence, by performing gain and loss of function studies, Josipovic et al. showed that both PAFAH1B1 and *NONHSAT073641* improve tube formation, sprout number and length in HUVECs. The involvement of Matrix Gla Protein (*MGP*), a key promoter of angiogenesis, with PAFAH1B1 in controlling angiogenesis was observed by microarray analysis and was further confirmed *via* reduced tube formation, sprouting capacity and migration in HUVECs upon *MGP* knockdown. Moreover, PAFAH1B1 was found to be preserving the histone 3 lysine 4 trimethylation (H3K4me3) and facilitating the binding of RNA Polymerase II at *MGP* promoter thereby inducing the expression of *MGP*. Even though this study does not discuss in detail regarding the functionality of lncRNA *NONHSAT073641*, it does highlight it as a promising lncRNA driving vascular development requiring further attention [29].

MALAT1 (Metastasis-associated lung adenocarcinoma transcript 1) is an extremely popular lncRNA among researchers which has been valued as an important prognostic marker in various types of cancer [30]. However, its significance in vascular biology gained attraction more recently in 2014 with Michalik et al. elucidating its role in maintaining endothelial cell proliferation. With the loss of function studies using *MALAT1* specific siRNAs and GapmeRs, the authors showed that the repression of *MALAT1* induces a switch from proliferative to migratory phenotype in HUVECs under the stimulation of VEGF by blocking the cell cycle progression in S phase. They further confirm the same in *in vivo* mouse model and illustrate that GapmeR mediated *MALAT1* repression hinders neovascularization and thus impairs blood flow to the site of injury post hind limb ischemia [31].

In addition to the role of *SENCR* in SMCs migration and contraction, it also works as a crucial regulator of endothelial cell development and function [32]. Boulberdaa et al. explain that *SENCR* is very important for the commitment of human embryonic stem cells towards endothelial cells lineage by using specific differentiation protocols where they observed an increase in levels of mesodermal genes (*MESP1*, *MIXL1*, *BRACHYURY*) as well as in mesodermal progenitor $CD326^{low}CD56^{high}$ population upon *SENCR* overexpression. In terms of endothelial functions, *SENCR* was found to induce cellular processes such as proliferation, migration and tube formation in HUVECs *via* the upregulation of pro-angiogenic genes (*CCL5* and *CX3CL1*) pointing towards its involvement in many diverse pathways [32]. With respect to the above mentioned processes of vascular development, *Dll4-AS* is also quite an interesting lncRNA which originates as a natural antisense transcript of its host gene Delta-like 4 (*Dll4*), a known arterial endothelial specific NOTCH1 receptor ligand functioning as an angiogenic regulator [33]. *Dll4-AS* overexpression and suppression in MS1 cells were found to be affecting the levels of *Dll4* linearly both at transcript and protein level indicating that *Dll4-AS* can regulate *Dll4*. In line with the functions of *Dll4*, *Dll4-AS* also decreased cell proliferation, migration and sprouting capacity in both MS1 and EOMA cells (endothelial cell types) as observed after *Dll4-AS* depletion experiments, suggesting that *Dll4-AS* mediates the functions of *Dll4*. With this study, the authors point out the importance of antisense lncRNAs in regulating the levels as well as the functions of their host genes driving diverse developmental pathways [33].

3. LncRNAs and vascular diseases

The association between the non-coding transcripts and vascular diseases has garnered significant attention over the years considering that ncRNAs act as regulatory entities in many important pathways. Here, in particular, we try to explore the role of lncRNAs in driving various vascular diseases further highlighting disease characteristics.

3.1. Pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH), which is being considered as one of the fatal conditions affecting our lung vascular system, involves the buildup of pulmonary artery obstruction *via* remodeling of endothelial and smooth muscle cells in addition to other physiological changes [34]. Interestingly, many researchers worldwide are working towards further establishing lncRNAs as regulators of PAH. For instance, Sun et al. discussed the role of *MEG3* as a potential therapeutic target for PAH and showed that there is decreased levels of *MEG3* in PAH patient tissues as well as in hypoxic pulmonary artery SMCs (PASMCs) [35]. *MEG3* knockdown with siRNAs increased cellular proliferation, migration and cell cycle progression in PASMCs aggravating vascular resistance. In terms of mechanisms, *MEG3* inhibition increased cell cycle progression *via* PCNA, Cyclin A, and Cyclin E induction both in normal and hypoxic conditions while the positive effect on cellular proliferation was attributed to the decrease in the levels of p53 upon *MEG3* knockdown (Fig. 1) [35]. Our group has also managed to successfully explain the interaction between *Meg3* and p53 in cardiac fibroblasts where *Meg3* was found to be working as a regulator of *MMP-2* (matrix metalloproteinase-2) in a p53 dependent manner [36]. We showed that the GapmeR mediated inhibition of *Meg3* leads to a blockage in *MMP-2* induction *via* decreased p53 activity, as binding of p53 to the promoter region of *MMP2* is essential for its transcriptional regulation, thereby ameliorating cardiac fibrosis and diastolic

dysfunction (Fig. 1) [36]. Continuing again with the connection between PAH and *Meg3*, Zhu et al. confirmed the findings of Sun et al. However, they illustrated a different modus operandi where *Meg3* by the sponging action on miR-21 upregulated its direct target *PTEN* and decreased cell proliferation and migration in hypoxic PASMCs [37].

Previously described lncRNA *MALAT1* was found to be involved in hypoxia-induced pulmonary hypertension by Brock et al., where they demonstrate that the expression level of *MALAT1* is positively affected by hypoxia *via* hypoxia-inducible factor 1α (HIF1α) in both human pulmonary artery smooth muscle cells (HPASMC) and endothelial cells (HPAEC) [38]. In line with the previous publications, they also found a significant reduction in the proliferation and migration of HPASMCs upon GapmeR mediated *MALAT1* silencing which was attributed to the modulation of Cyclin-dependent kinase (CDK) inhibitors by *MALAT1*. Hence, inhibition of *MALAT1* can lead to improvement in cardiac hypertrophy in hypoxia-induced pulmonary hypertensive mice [38].

GAS5 lncRNA described to play a critical role in SMCs differentiation also regulates hypertension-induced vascular remodeling by altering endothelial biology. Levels of *GAS5* in Spontaneously hypertensive rats (SHR) retinas, caudal arteries, renal arteries, thoracic arteries and carotid arteries is significantly low as compared to the control where the shRNA mediated *GAS5* knockdown worsens the hypertension induced capillary leakage by disorganized proliferation of endothelium and abnormal endothelial activation [39]. The underlying positive effect of *GAS5* under hypertensive conditions was found to be via modulation of β-Catenin signaling pathway where *GAS5* knockdown mediates the nuclear translocation of β-Catenin and thus activates expression of its downstream target genes c-Myc, cyclin D1, and peroxisome proliferator–activated receptor (PPAR) δ, which are responsible for controlling endothelial growth, permeability as well as cellular functions [39]. These reports point out that even a single lncRNA can participate in many diverse pathways to carry out their physiological and pathophysiological functions.

3.2. Atherosclerosis

The root cause behind coronary artery disease, stroke and peripheral artery disease is the accumulation of plaque inside the arteries reducing their effective blood flow area. This vascular condition of atherosclerosis is the most commonly found disorder and its modulation by lncRNAs has brought in significant attention over the past few years. For instance, in the year 2014 Wu et al. identified the role of *LincRNA-p21* in modulating atherosclerosis. By analyzing the aortic atherosclerotic plaques from ApoE$^{-/-}$ (atherosclerosis animal model) mice, they found that the levels of *LincRNA-p21* are significantly lower as compared to wild type [40]. *In vitro* experiments showed that *LincRNA-p21* inhibition increases cell proliferation and decreases apoptotic rate. In order to have an insight into the mechanism, a gene array analysis was performed upon *LincRNA-p21* silencing which highlighted the involvement of p53 signaling pathway genes [40]. Transcriptional activity of p53 is controlled by opposing functions of MDM2 (mouse double minute 2), an ubiquitin-ligase and p300, an acetyltransferase. MDM2 binds to p53 and mediates its degradation *via* ubiquitin-proteasome pathway while p300 acetylates p53 and activates its transcriptional activity [41,42]. *LincRNA-p21* act as an inhibitor of MDM2 by directly binding to its RING domain and inhibiting its interaction with p53 thereby making p53 available to interact with p300. This increase in transcriptional activity of p53 leads to its increased recruitment at the promoter or enhancer regions of its target genes inhibiting vascular smooth muscle cell proliferation and driving apoptosis (Fig. 1) [40]. Similar to *in vitro* results, *in vivo* inhibition of *LincRNA-p21* in mouse carotid artery injury model increased neointimal hyperplasia by activating proliferation and decreased apoptosis. Furthermore, levels of *LincRNA-p21* were found to be decreased in patients with Coronary Heart Disease further consolidating the therapeutic importance of *LincRNA-p21* in the treatment against atherosclerosis [40].

Recent publication by Han et al. discussed about an interesting side of upstream regulation of *LincRNA-p21* using natural compounds where they demonstrate that Oleanolic acid (OA), a plant-based natural compound, is capable of increasing the levels of *LincRNA-p21* which leads to reduction of cell proliferation and NF-κB signaling in VSMCs. They further show that the shRNA mediated *LincRNA-p21* suppression increased cell proliferation even in the presence of OA confirming the importance of *LincRNA-p21* in modulating atherosclerosis [43].

Besides regulating endothelium properties, exosomal *GAS5* secreted by endothelial cells was found to be taken up by VSMCs thereby regulating their proliferation and migration. Another study by Chen et al. also found exosomal *GAS5* to mediate cross-talk between monocyte and endothelial cells. They explored the role of *GAS5* in mediating atherosclerosis using THP-1 (monocyte cell line) and HUVECs treated with oxidized low density lipoprotein (oxLDL) as a model for atherosclerosis [44]. They observed that shRNA mediated *GAS5* knockdown reduces the apoptosis in oxLDL treated THP-1 cells while its lentivirus mediated overexpression has the opposite effect. In an interesting experiment, the authors treated HUVECs with exosomes isolated from THP-1 cells with inhibition and overexpression of *GAS5* and found reduced and heightened apoptosis in endothelial cells respectively [44]. Based on *in vitro* results, *GAS5* was concluded to promote atherosclerosis by promoting apoptosis of monocytes and endothelial cells. On contrary, previously mentioned publication from Want et al. have found that *GAS5* inhibition, increases cellular adhesion markers like VCAM-1, ICAM and E-Selectin which could promote monocyte adhesion and thus the plaque formation ([39]). Of note, atherosclerosis is a complex and multi-cellular disease involving lipid accumulation, endothelial activation, monocyte/macrophage infiltration, extracellular matrix deposition by SMCs and plaque rupture at later stages [45]. Thus with presence of *GAS5* in monocyte, SMCs, and ECs, *in vivo* experiments are needed to clarify the pro/anti atherosclerotic function of *GAS5*.

Similar to *GAS5*, lncRNA *H19* was also observed to be involved in atherosclerosis. Pan et al. analyzed the serum samples of 42 atherosclerotic patients and found that levels of *H19* are significantly higher as compared to healthy controls depicting its diagnostic potential [46]. *In vitro* experiments by overexpressing *H19* in HUVECs further depicted an increase and decrease in cell proliferation and cell apoptosis respectively. In terms of mechanism, the authors suggest the involvement of MAPK and NF-kB signaling pathway as they observed a significant increase in p38 and p65 levels upon *H19* overexpression which are known participants of MAPK and NF-kB signaling pathway [46].

Now that we are elaborating the implications of lncRNAs as circulating entities, it is logical to mention *SENCR* and *MIAT*, involved in smooth muscle and endothelial cell biology, functioning as circulating lncRNAs present in body fluid like serum. The analysis of serum from 48 type 2 diabetes patients revealed that the serum levels of *SENCR* and *MIAT* can directly predict the incidence of left ventricular remodeling in type 2 diabetes patients as their presence was in direct correlation with left ventricular mass volume (LVMV) ratio highlighting the biomarker potential of lncRNAs in cardiovascular complications [47]. Another lncRNA *LIPCAR* (long intergenic non-coding RNA predicting cardiac remodeling), even though not directly related to the vasculature, was also found to be of importance, considering its association with left ventricular diastolic function making it a reliable predictor of left ventricular dysfunction [47]. Previously, circulating levels of *LIPCAR* was reported to be increased in patients with chronic heart failure and associated with higher risk of cardiovascular death [48]. These studies highlight the diagnostic and prognostic potential of *LIPCAR* and urge further studies to identify the cell source of increased *LIPCAR* in circulation. Overall, these reports highlight the multifarious possibilities in which lncRNAs can be implied to regulate a diseased phenotype such as atherosclerosis and type 2 diabetes.

3.3. Aging

Vascular cell senescence is one of the important drivers of age-associated cardiovascular diseases. In the year 2015, Bianchessi et al. highlighted the impact of mitochondrial lncRNA *ASncmtRNA-2* (Antisense mitochondrial noncoding RNA-2) in establishing replicative senescence (RS) specifically in ECs [49]. The authors incorporated aortas of old mice as an apt model to study aging and found an increased expression of lncRNA *ASncmtRNA-2*. MiRNAs, hsa-miR-4485, and hsa-miR-1973, homologous to the *ASncmtRNA-2's* double strand region, were also found to be induced under RS conditions similar to their precursor lncRNA. Furthermore, the study shows an increased number of ECs arrested in G2/M phase upon *ASncmtRNA-2* overexpression illustrating the importance of *ASncmtRNA-2* in regulating RS. Even though the observations are not discussed mechanistically, the authors hypothesize the involvement of hsa-miR-4485, and hsa-miR-1973 in *ASncmtRNA-2* functions [49]. The concept of RS was further incorporated by Dimmeler group to explain the role of *MEG3* in governing endothelial cell aging. They observed that senescent HUVECs at passage 16 to18 showed higher levels of *MEG3* as compared to early age cells at passage 3 to 4 [50]. This finding was further consolidated by an increase in angiogenic sprouting of aged HUVECs upon GapmeR mediated *MEG3* inhibition as well as by *in vivo MEG3* silencing experiments which showed improvement in ischemic hind limb blood circulation. These results strongly put forward the idea of *MEG3*'s involvement in aging; however in-depth mechanistic studies are still lacking [50].

3.4. Inflammation

Since inflammation plays a major role in causing endothelial dysfunction, many researchers are trying to associate the regulatory properties of lncRNAs with inflammation. One such report by Huang et al. talks about lncRNA *TGFB2-OT1* (TGFB2 overlapping transcript 1) as a regulator of an important miRNAs signaling pathway leading to autophagy and inflammation [51]. By using lipopolysaccharide and oxLDL as inflammation inducers, the authors found an induction in the levels of *TGFB2-OT1* due to increase in the expression of NUPR1 and TIA1 which work as upstream regulators of *TGFB2-OT1*. The microarray analysis, performed to find *TGFB2-OT1* interacting miRNAs, identified *MIR3960, MIR4488* and *MIR4459*, regulated by *TGFB2-OT1 via* binding to them as a ceRNA. This lncRNA mediated miRNA regulation further modulates the levels of their downstream targets *CERS1*, *NAT8L*, and *LARP1*, which are reportedly involved in pathways leading to autophagy and inflammation [51].

3.5. Apoptosis

Apart from autophagy and inflammation, cell apoptosis is also considered as one of the markers for the diseased phenotype. Few recent reports talk about the function of lncRNAs in controlling cellular apoptosis especially in vascular diseases, for example, lncRNA *LOC100129973* was found to be a suppressor of cellular apoptosis in HUVECs [52]. Lu et al. used 6-amino-2,3-dihydro-3-hydroxymethyl-1,4-benzoxazine (ABO), a known apoptosis inhibitor, to suppress the apoptosis caused due to serum and FGF-2 starvation and analyzed the differentially expressed transcripts by microarray. LncRNA *LOC100129973*, showing the highest upregulation in ABO treated samples, was demonstrated to sponge the miR-4707-5p and miR-4767 by direct binding, thereby increasing the levels of their target apoptotic inhibitors API5 and BCL2L12 [52]. Another report in the same year demonstrated the involvement of lncRNA *TUG1* in modulating Tanshinol mediated repression of endothelial apoptosis in the atherosclerotic model. Tanshinol treatment leads to the downregulation of *TUG1* mRNA which improves the levels of miR-26a, as *TUG1* itself represses miR-26a by direct binding, and further ameliorate aortic atherosclerotic lesions in the aorta [53]. One more apt example of lncRNA mediating apoptosis would be *LincRNA-p21* which induces cellular apoptosis. It behaves as an endogenous sponge for miR-130b, a known promoter of cell proliferation, thereby inhibiting cell proliferation and triggering apoptosis in SVEC4 cell line [54].

3.6. Vasculature and cancer

The interplay between cancerous tumors and vasculature is of significance to researchers keeping in view that blood vessels are the means to deliver chemotherapeutic drugs to the tumor site. However, the role of lncRNAs in such situations is seen as a new genre requiring more attention. A recent publication by Cai et al. discussed the role of LncRNA *TUG1*, a highly expressed lncRNA in glioma vascular endothelial cells, in modulating the blood-tumor barrier (BTB) in glioma tissues [55]. They observed an improvement in BTB permeability while the levels of endothelial tight junction proteins ZO-1, occludin, and claudin-5 were decreased upon knockdown of *TUG1*. Mechanistically, *TUG1* was found to be binding to miR-144, releasing miR-144's target Heat shock transcription factor 2 (HSF2) from miRNA mediated repression and making HSF2 available for interacting with the promoters of ZO-1, occludin, and claudin-5, thereby increasing their protein levels but in turn impairing the BTB permeability (Fig. 1) [55]. Another report in the same year discussed the modulation of ECs phenotype *via* the exosomes comprising of lncRNAs released by cancerous cells. Conigliaro et al. showed that Hepatocellular carcinoma (HCC) CD90 + cells are capable of releasing exosomes of average size 50 nm. HUVECs treated with these exosomes showed improvement in tube formation capacity and cell-cell adhesion as indicated by increased ICAM-1 levels [56]. LncRNA expression analysis showed that lncRNA *H19* was enriched by 10 folds in exosomes from CD90 + cells as compared to the exosomes from parental Huh7 cells. The exosome-mediated effects of *H19* were further confirmed by transient overexpression of *lncH19* in HUVECs where the authors observed that *H19* has the ability to induce angiogenesis and cell-cell adhesion [56]. Moreover, a recent publication by Jia et al. discussed the mechanism behind pro-angiogenic effects of lncRNA *H19* in glioma-induced ECs [57]. Their findings established that *H19* inhibits miR-29c by directly binding to it and relieves its downstream target vasohibin 2 (VASH2) which functions as an angiogenic factor. They further show that knockdown of *H19* along with miR-29c overexpression can be used to repress the angiogenesis in glioma tumors [57]. Therefore, these studies give a new insight into the lncRNA mediated interaction between our vasculature and cancer.

4. Concluding remarks

From being considered as a part of “Junk DNA” to becoming powerful regulators of important cellular pathways, lncRNAs have come a long way. Interestingly, our vasculature also falls into the expansive area of lncRNAs regulation. Starting from the very nascent stages of embryonic development, stem cell differentiation and lineage commitment, lncRNAs such as *SENCR* and *GAS5* begin their regulatory functions and modulate the lineage commitment and differentiation embryonic stem cells towards vascular cell types. Cell proliferation, migration, and vessel formation capacity, considered as the defining characteristics of endothelial and smooth muscle cells, are also controlled by various lncRNAs such as *MEG3*, *GAS5*, *MIAT*, *MALAT1* and *SMILR* suggesting that the functionality of lncRNAs is not limited to a particular state of the cell (Table 1). Another feather in the cap of lncRNAs is their potential to drive and manipulate multiple pathophysiological conditions. Be it diabetes mellitus, atherosclerosis, hypertension or various disease-related characters like cellular senescence, apoptosis, autophagy and inflammation, lncRNAs such as *SENCR*, *MEG3*, *LincRNA-p21*, *and TUG1* have been found to be deeply involved as modulators of various underlying pathways. Of notable interest is the extensive diversity in the mode of actions implied by

Table 1
List of lncRNAs involved in vascular biology along with their functions and mechanisms.

lncRNA	Cell type	Function	Mechanism	References
SENCR	SMCs	Promotes contractile phenotype	Induce expression of Myocardin	[13]
	ECs	Endothelial cell lineage commitment	Increases $CD326^{low}CD56^{high}$ progenitors	[32]
MYOSLID	SMCs	Promotes contractile phenotype	Mediates SMAD2 phosphorylation	[14]
GAS5	SMCs	Reduces cell proliferation and migration; Decreases SMC differentiation	Interacts with Annexin A2; Suppression of TGF-β/Smad3 interaction	[18,19]
	ECs	Inhibits hypertension induced vascular remodeling; Promotes atherosclerosis	Inhibits nuclear translocation of β-Catenin; Exosome mediated pathway	[39,44]
TUG1	SMCs	Promotes F-actin polymerization	Interacts with EZH2	[20]
	ECs	Maintains blood-tumor barrier	Sponges miR-144	[55]
SMILR	SMCs	Increases cell proliferation	Positive regulation of proximal gene *HAS2*	[21]
TIE-1 AS	ECs	Decreases vessel formation	Regulation of *Tie-1* transcript levels	[23]
NRON	ECs	Hinders cell migration and tube formation	Unknown	[24]
MIAT	ECs	Promotes microvascular dysfunction	Sponges miR-150-5p	[26]
MEG3	SMCs	Decreases cell proliferation, migration and cell cycle progression	Regulation of PCNA, Cyclin A, Cyclin E and p53 Sponging miR-21	[35,37]
	ECs	Decreases Angiogenesis and proliferation Regulates microvascular dysfunction Cell senescence modulation	Sponging of miR-9 PI3K-Akt Signaling	[27,28,50]
NONHSAT073641	ECs	Improves tube formation	Unknown	[29]
MALAT1	ECs	Regulates proliferation and neovascularization; Induction of pulmonary hypertension	Cell cycle blockage; Regulation by HIF-1α	[31,38]
Dll4-AS	ECs	Decreases cell proliferation, migration and angiogenic capacity	Unknown	[33]
LincRNA-p21	SMCs	Regulates atherosclerosis	Inhibits MDM2-p53 interaction	[40,43]
	ECs	Induces cellular apoptosis	Sponging of miR-130b	[54]
ASncmtRNA-2	ECs	Cell senescence modulation	Precursor for hsa-miR-4485 and hsa-miR-1973	[49]
H19	ECs	Promotes atherosclerosis; Increases angiogenic capacity	Increases MAPK and NF-kB signaling; Sponges miR-29c	[46,57]
TGFB2-OT1	ECs	Regulates autophagy and inflammation	ceRNA for miR-3960,miR-4488 and miR-4459	[51]
LOC100129973	ECs	Suppresses cellular apoptosis	ceRNA for miR-4707-5p and miR-4767	[52]

various lncRNAs to perform their functions. Their modi operandi can include cis/trans acting direct interaction with the promoter region of target genes (*tie-1 AS*), recruitment of transcriptional machinery to the transcription start site of target genes (*NONHSAT073641*), communicating with key pathway regulators such as p53 and EZH2 (*MEG3, TUG1*), modulation of target proteins by direct binding (*GAS5*) or working as a competitive endogenous RNA (ceRNA) to sponge the target miRNAs (*LOC100129973, LincRNA-p21*), further modulating their downstream pathways as summarized in Table 1. Even though these studies have successfully added to our understanding of the relatively new area of lncRNA biology, there is a tremendous untapped potential in lncRNAs as therapies and biomarkers against fatal vascular diseases. The successful story of lncRNA *PCA3* (Prostate cancer antigen 3) to be used as an FDA approved biomarker for the detection of prostate cancer only signifies the high relevance of lncRNAs based biomarker systems [58]. We believe that with the advent of sophisticated sequencing techniques to identify new lncRNAs and efficient technologies to manipulate their expression, researchers will be able to better understand the overall lncRNA picture.

Conflict of interest

TT and SKG have filed and licensed patents for non-coding RNAs. TT is the founder of Cardior Pharmaceuticals GmbH.

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