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Long non-coding RNA in regulation of vascular smooth muscle cells plasticity

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Abstract

Phenotypic plasticity of vascular smooth muscle cells (VSMCs) is a functional property that is essential for vascular remodeling in vessel injury healing. During phenotypic switch, quiescent VSMCs loss contractile capacity but acquire ability to proliferate and migrate to the injured site where they differentiate again to the quiescent state. However, in pathological conditions such as endothelial dysfunction or atherosclerosis, phenotypic changes in arterial VSMCs become deregulated leading to elevated VSMC dedifferentiation, proliferation, excessive extracellular matrix deposits, and intimal thickening. VSMC hyperplasia is a complex mechanism that is coordinated by a network of various regulatory factors. Long non-coding (lnc)RNAs represent an important part of this regulatory network. Some of lncRNAs are involved in VSMC differentiation, apoptosis, and maintenance of quiescence, while other lncRNAs promote VSMC dedifferentiation, proliferation, and motility. In this review, we characterize these lncRNAs and their function in the context of possible involvement in atherosclerosis.

Аннотация

Гладкомышечные клетки кровеносных сосудов характеризуются пластическим фенотипом, который играет важную роль в восстановительных процессах. Эти клетки могут менять свой фенотип, теряя сократительные свойства и приобретая способность пролиферировать и мигрировать к поврежденным участкам ткани, где они снова дифференцируются и приходят в состояние покоя. Однако, при развитии различных патологий, в том числе, атеросклероза, регуляция фенотипической пластичности гладкомышечных клеток сосудов нарушается, что приводит к неконтролируемой и избыточной пролиферации, отложению межклеточного матрикса и, в конечном итоге, утолщению стенки сосуда. В этом сложном процессе принимают участие различные факторы. Длинные некодирующие РНК (длнкРНК) являются одним из таких факторов. Некоторые длнкРНК участвуют в дифференциации гладкомышечных клеток, апоптозе и поддержании фенотипа покоя, в то время как другие способствуют дедифференциации клеток, их пролиферации и миграции. В этом обзоре обсуждаются различные виды длнкРНК, возможно играющие важную роль в развитии атеросклероза.

Keywords: long non-coding RNA; VSMC; phenotype switch; proliferation; hyperplasia; atherosclerosis

1. Introduction

Vascular smooth muscle cells (VSMCs) reside in the arterial wall, in the layer called tunica media. These cells produce a variety of contractile proteins, ion carriers, and intracellular signaling components all involved in the regulation of vascular tone. Under normal conditions, VSMCs exhibit a quiescent phenotype characterized by contractility and a lack of proliferation and motility. However, adult VSMCs can acquire proliferative phenotype through the mechanism of hyperplasia under specific conditions such as vascular injury or hypoxia. Under stress, VSMCs are able to dedifferentiate to non-contractile cells that have a capacity to proliferate and migrate [1]. Dedifferentiated cells are also characterized by higher

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production of extracellular matrix (ECM) proteins and lower expression of contractile proteins, such as smooth muscle myosin heavy chain (SM-MHC), smooth muscle actin-α (SMA-α), sm-22α, calponin, caldesmon, i.e. typical markers of differentiated SMCs [2].

During vascular tissue repair, dedifferentiated VSMCs undergo several rounds of proliferation and participate in vascular remodeling. This process is characterized by neointima formation, medial stiffening, and intimal thickening, a phenomenon observed in vascular pathologies, such as atherosclerosis or pulmonary hypertension [3, 4]. In atherosclerosis, the phenotypic switch of VSMCs is a frequent event that may involve over 80% of plaque VSMCs [5]. Several studies aimed to understand the abundance of VSMC hyperplasia and identify molecular factors that drive both SMC-specific gene expression and differentiation. This work resulted in the discovery of a complex network of regulatory proteins and non-coding RNAs controlling the phenotypic switch of VSMCs [6-8]. However, precise molecular mechanisms that prime phenotypic changes in VSMCs during vascular remodeling remain to be elucidated. Long non-coding RNA (lncRNA) is a very diverse group of non-protein coding RNA molecules that are present in different organisms, from viruses to mammals [9]. However, it was noted that the abundance of lncRNA increases with the organism's complexity, even exceeding that of protein-coding genes [10]. According to current understanding, lncRNAs perform important regulatory and other functions that are only partly known to date. In this review, we will focus on the role of lncRNA in the control of the VSMCs phenotypic switch.

Origin and functions of long non-coding RNAs

Non-protein-coding lncRNAs are transcripts with a length over 200 nucleotides that are distinct from small non-coding RNA, such as microRNAs that are less than 25 nucleotides long. So far, over 167K and 130K lncRNA genes were identified in human and mouse respectively (NONCODE database version 6.0, http://www.noncode.org) [11]. These numbers exceed greatly the number of human protein-coding genes (circa 20K according to the GENCODE database version 19, http://www.gencodegenes.org/stats.html). However, it is unclear whether all these RNA sequences have a biological function because of the lack of categorizing and validating data about lncRNAs. Current criteria for categorization of lncRNAs are based on association with adjacent protein-coding genes (Figure 1).

The origin of lncRNA remains obscure, largely because of the elusive definition of this RNA class and its remarkable variety [9]. However, a recent hyporthesis proposed a possibility of an evolutionary path between enhancer-derived RNAs (eRNAs), which are short, unspliced and cis-acting, and lncRNAs that are much longer, undergo splicing and can be trans-acting [12]. Examples of intermediate forms were found, such as Lockd [13] and Bloodlinc [14] that support this suggestion. More details on eRNA functions are given below.

The localization of lncRNA genes can vary within the genome. Sense lncRNAs overlap with protein-coding genes and usually share the same promoter. Anti-sense lncRNAs are localized on the opposite strands to protein-coding genes. Intronic and intergenic (linc) RNAs reside in introns of protein-coding genes or between protein-coding genes respectively. Enhancer RNAs (eRNAs) span the enhancer regions of protein-coding genes. Circular RNAs (circRNAs) represent covalently enclosed RNAs that are usually originated from splicing of protein-coding genes. However, this classification does not reflect lncRNA function since only a few lncRNAs were functionally characterized so far. In general, lncRNA functions can be divided into four categories: imprinting, activation of enhancer, molecular sponges, and serving as scaffold/guide for epigenetic/transcription factors.

Imprinting

Imprinting can be generally defined as parental-specific gene expression in diploid cells when only one allele is active, and the other allele is epigenetically silenced [15]. The first lncRNA discovered was X-inactive specific transcript (XIST) [16]. The XIST sequences literally cover a whole chromosome X and perform X-inactivation mainly through interaction with polycomb-repressive complex 2 (PRC2) [17]. Other examples of well-studied lncRNAs involved in imprinting are Airn [18], KCNQ1OT1 [19, 20], and H19 [21]. Generally, imprinting-associated lncRNAs inhibit the expression of neighboring genes, acting as cis-regulators. Imprinting is of great importance for embryogenesis (as in the case of murine Xist) [22]. Therefore, imprinting lncRNAs are functionally significant in the developmental processes.

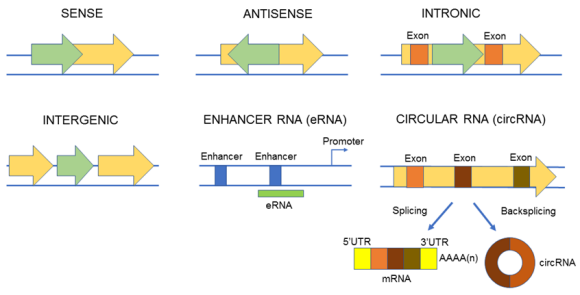

Figure 1 | Nomenclature of non-coding RNAs. Green and orange indicate non-coding RNA and protein-coding genes respectively. UTR, untranslated region. Номенклатура длнкРНК. Зелёным и оранжевым отмечены длнкРНК и кодирующие белок гены, соответственно. UTR – нетранслируемый участок.

Enhancer activation

eRNAs have a compact size that does not exceed 2000 nucleotides. They are transcribed in association with the regulated gene. For eRNAs, several possible mechanisms of action were suggested, including serving as decoys for major transcription factors or their binding sites, facilitating the interaction between the promoter and the enhancer through loop formation, and nucleosome depletion at the promoter region [23]. eRNAs are widely distributed across the genome. For example, in a primary mouse neuronal culture, about 12,000 activity-regulated enhancers were found [24]. Expression levels of these enhancers correlated with expression of adjacent protein-coding genes indicating the presence of enhancer-promoter interaction. Another study showed that 60% of transcribed enhancers are polyadenylated whereas the rest is transcribed without polyadenylation [25]. Similar situation was described for other types of lncRNAs. Polyadenylated eRNAs are unidirectly transcribed to the enhancer and therefore called 1d-eRNAs. By contrast, non-polyadenylated eRNAs (or 2d-eRNAs) are bidirectionally transcribed [26].

The so-called super-enhancer regions of the genome contain enhancer clusters marked by the presence of key tissue-specific transcription factors and mediators. For example, in embryonic stem cells, most genes that control the pluripotent state, have enhancer elements for binding Oct4, Sox2 and Nanog. These transcription factors are responsible for maintenance of the characteristic stem cell properties. After binding to an enhancer, these factors recruit Mediator, a transcriptional coactivator, to induce the expression of target genes [27]. For instance, PR (PRD1-BF1 -RIZ1 homologous) domain-containing 16 (PRDM16), a transcriptional coregulator, recruits MED1, a component of the Mediator complex, to super-enhancers at brown fat-selective genes to initiate a brown fat differentiation program [28].

Molecular sponges

circRNAs can originate from introns or exons (ecircRNAs) of coding genes. Circular intronic RNAs are predominantly present in the nucleus, while those originating from the exons are preferentially cytoplasmic [29]. In human fibroblasts, more than 25000 distinct circRNA species were found, that contained non-colinear exons and increased in numbers after exonuclease degradation of linear RNA [30]. CircRNAs are abundant, stable and evolutionary conserved (especially ecircRNAs), however, their function remains largely unknown. Some ecircRNAs may serve as RNA-binding proteins and contribute to miRNA regulation, and, presumably, to the control of parental gene expression and cell proliferation [31].

The detection of miRNA-binding sites in circRNAs allowed suggesting that circRNAs may act as molecular sponges for miRNAs, regulating their function by sequestration [32]. Circular RNA sponges for miR-7 (ciRS-7, also known as CDR1as) may serve as an example of miRNA-binding circRNA [33]. Linear lncRNAs can also act as sponges for miRNA. A muscle-specific linc-MD1 catches miR-133 and miR-135 to induce a differentiation switch in myoblasts [34, 35]. lncRNAs highly up-regulated in liver cancer (HULC) promote tumorigenesis by sequestering miR -372 [36]. Imprinting lncRNA H19 binds let-7 family of miRNAs, which in turn increases invasiveness of many cancers [37]. Protein-coding transcripts termed competing endogenous RNAs (ceRNAs), which are able to compete with miRNAs, also exist. These transcripts express concordantly with PTEN and possess regulatory properties by suppressing or supporting tumor growth [38].

Scaffolds for epigenetic/transcription factors

Scaffolding function of nuclear lncRNAs was first described in a study focusing on two histone modification complexes and polycomb repressive complex 2 (PRC2) and the LSD1/CoREST/REST complex binding to chromatin [39]. In mouse embryonic cells, the expression of lncRNAs is controlled by key transcription factors including stem cell markers Oct4 and Nanog while lincRNAs transcripts interact with numerous chromatin regulatory proteins to influence shared gene expression programs [40]. Inactivation of dozen of lincRNAs caused either loss of the pluripotent state or induction of alternative differentiation programs. These data indicated the importance of lncRNAs in the processes directing pluripotency and lineage commitment programs [41, 42]. A model was proposed, in which lincRNAs act as cell-type–specific flexible scaffolds to bear protein complexes (i.e. epigenetic factors) to induce specific transcriptional programs [40].

Other functions

Small conserved open-reading frames that encode micropeptides were found in annotated lncRNAs. For example, a putative skeletal muscle-specific lncRNA encodes a functional micropeptide termed myoregulin, which regulates muscle contractility by interaction with the endoplasmic reticulum Ca^{2+}-ATPase (SERCA) and inhibition of Ca^{2+} uptake into the sarcoplasmic reticulum [43]. Later, another micropeptide called DWORF was discovered, encoded by a putative skeletal muscle-specific lncRNA. Compared with myoregulin, DWORF possesses opposite functional properties by activating SERCA and enhancing Ca^{2+} load to the endoplasmic reticulum. These findings suggest that many annotated lncRNAs are 'hidden' mRNAs. Finally, lncRNAs may be involved in protein translocation between the nucleus and the cytosol and in the regulation of the stability of protein-coding genes [44].

Long non-coding RNAs that promote vascular smooth muscle cell proliferation and migration

H19

The gene encoding this imprinted lncRNA resides on chromosome 11p15.5, immediately downstream of the IGF2 gene encoding the insulin-like growth factor II (IGF2) [45]. H19 and IGF2 are imprinted in a mutual way. In the maternal chromosome, H19 is expressed but IGF2 is not. In the paternal chromosome, IGF2 is expressed, while H19 is not [21]. While IGF2 supports cell growth and proliferation, H19 exhibits anti-proliferative activity. The IGF2-H19 locus is involved in the regulation of embryogenesis. Hypomethylation of the IGF2-H19 locus on both chromosomes leads to the abolishment of imprinting accompanied by activation of H19 expression and decrease of IGF2 expression. This phenomenon is essential for the maintenance of quiescence of adult pluripotent stem cells. At the same time, hypermethylation of the IGF2-H19 locus on both chromosomes (i.e. loss of imprinting) results in IGF2 overproduction and is associated with tumorigenesis [46].

Expression of H19 was found at highest levels in the fetal muscle, in aortic smooth muscle, and at almost undetectable levels in adult VSMCs [47]. Up-regulation of this lncRNA was observed in the arterial neointima after acute vascular damage [48] and in atherosclerotic lesions [49]. In cell culture, H19 transcripts were undetectable in proliferating neointimal cells, but were highly abundant in differentiated neointimal cells. The first exon of H19 contains miR-675-encoding gene [50]. H19 regulates the processing of miR-675 and cooperates with this miRNA in physiological effects. For example, in the fetus, H19/miR-675 limits the prenatal placenta growth by inhibiting insulin-like growth factor 1 receptor (Igf1r) gene, a target for miR-675 [50]. However, overexpression of H19/miR-675 was shown to enhance proliferation and invasiveness of breast cancer cells by down-regulating members of ubiquitin ligase E3 family (c-Cbl and Cbl-b) and thereby increasing the stability of pro-oncogenic receptors EGFR and HGFR [51]. In the neointima of balloon-injured artery, both regulatory RNAs are overexpressed and promote neointimal formation and arterial restenosis by inhibition of phosphatase and tensin homolog (PTEN), a suppressor of cell growth and proliferation [52]. It seems that pathologic conditions such as vascular proliferative disorders (like atherosclerosis) and cancer, induce global epigenetic reprogramming accompanied by a loss of imprinting at the IGF2-H19 locus. In such conditions, this locus supports VSMC proliferation. In normal conditions, IGF2-H19 imprinting exists that is associated with suppression of VSMC proliferation.

ANRIL

The antisense noncoding RNA in the INK4 locus (ANRIL; also known as CDKN2BAS) is located on chromosome 9p21.3. This lncRNA shares the promoter with the CDKN2A (p14ARF) gene. The first ANRIL exon overlaps with two exons of the CDKN2B (p15INK4B) gene. Both CDKN2A and CDKN2B encode negative cell cycle regulators that target cyclin-dependent kinases CDK4 and CDK6 respectively. This disrupts binding of CDK4/CDK6 to D-cyclins and leads to the cell cycle arrest [53]. In addition, CDKN2A interacts with the E3 ubiquitin-protein ligase MDM2 that results in MDM2 degradation and derepression of p53 [54]. Expression of ANRIL and CDKN2A is coordinated both in steady-state and pathological conditions [55]. ANRIL silences the expression of CDKN2A by recruiting the polycomb repressive complex-2 (PRC2), a chromatin-modifying complex, to the shared promoter thereby permitting the cell cycle progression and cell proliferation [56].

The results of early studies suggested that ANRIL acts as a scaffold for PRC1 and PRC2 mediating the repression of the CDKN2B/ CDKN2A/CDKN1B locus in a cis manner [56, 57]. However, ANRIL exhibits a more complicated regulatory pattern displaying the ability to regulate gene expression in a trans manner as well. Genome-wide association studies showed a strong association of the ANRIL locus with coronary artery disease (CAD), type 2 diabetes, cancer, and intracranial aneurisms [58]. Association between ANRIL and cardiovascular diseases is complex and mediated by extended haplotypes located in the 58-kb region (so called CAD interval) that lacks any known protein-coding gene [40]. Disease-associated ANRIL variants were shown to influence the expression of this lncRNA. It was found that the association between ANRIL and atherosclerosis can be modulated in trans by Alu elements located in ANRIL and the promoters of its target genes [59].

Another study showed that the C/C genotype of single nucleotide polymorphism (SNP) rs1333049, a CAD risk ANRIL variant, was associated with the lowest expression of this lncRNA in VSMCs, increased VSMC proliferation, and the highest content of VSMCs in atherosclerotic plaques [60]. The risk allele C of rs1333049 can decrease the expression of the long ANRIL transcript but increase that of short transcripts, which in turn leads to CDKN2A/B down-regulation and VSMC proliferation [61]. The significance of this genomic region in the pathogenesis of vascular diseases was confirmed by the experiments, in which the orthologous 70-kb non-coding CAD interval was deleted from murine chromosome 4. That led to increased proliferation of VSMCs, reduction of cardiac expression of Cdkn2a/b [62], and vascular aneurism progression [63]. Loss of the CAD interval also resulted in reduced transforming growth factor (TGF)-β-dependent canonical Smad2 signaling [63]. This is consistent with recent observations that alterations in Tgf-β signaling in Cdkn2b-deficient VSMCs due to down-regulation of the inhibitor Smad7 impairs vessel maturation and VSMC recruitment to neovessels under hypoxic

conditions. However, inhibition of CDKN2B in hypoxic VSMCs leads to up-regulation of Tgf-β itself [64]. These findings suggest that overexpression of ANRIL in VSMCs promotes dedifferentiation, growth, proliferation, and profibrotic activity in a TGF-β-dependent manner.

Up-regulation of ANRIL contributes to atherogenesis. It was demonstrated that knockdown of ANRIL in VSMCs correlated with reduction of cell growth [65]. However, while linear forms of ANRIL appear to promote VSMC proliferation, a recently identified atheroprotective circular ANRIL (circANRIL) isoform exhibits opposite effects by inducing VSMC apoptosis and inhibiting proliferation. Mechanistically, circANRIL binds to pescadillo homologue 1 (PES1), an essential 60S-preribosomal assembly factor, that leads to the abnormalities in exonuclease-mediated pre-rRNA processing and ribosome biogenesis in VSMCs and macrophages. As a result, this circular RNA induces nucleolar stress and p53 activation with subsequent apoptosis [66]. In summary, ANRIL exerts complex effects on VSMC behavior and function. In human aortic SMCs, different ANRIL splice variants may have distinct roles by influencing the expression of genes involved in cell proliferation, apoptosis, vascular remodeling, and inflammation.

SMILR

The intergenic lncRNA (chromosome 8q24.13) has been recently identified in VSMCs stimulated with interleukin (IL)-1α and platelet-derived growth factor (PDGF) [67]. Followed stimulation, levels of this RNA called smooth muscle-induced lncRNA enhances replication (SMILR). SMILR activates VSMC proliferation. Interestingly, SMILR depletion led to down-regulation of hyaluronan synthase 2 (HAS2), an enzyme involved in the extracellular matrix synthesis during neointimal formation [68]. High levels of SMILR were observed in vulnerable atherosclerotic plaques suggesting for a possible proatherogenic role.

HAS2-AS1

Hyaluronan synthase 2 antisense RNA1 (HAS2-AS1) is located on chromosome 8q24.13, in the vicinity to the HAS2 gene, but seems to have a distinct promoter [69]. Expression of this antisense RNA is coordinated with that of HAS2. In renal proximal tubular epithelial cells, expression of both genes can be induced by IL-1β or TGF-β. Furthermore, for HAS2-AS1 and HAS2, the possibility to form a heterodimer was shown in silico [69]. O-GlcNAcylation modulates HAS2 -AS1 promoter up-regulation through mobilization of the NF-κB subunit p65, while HAS2-AS1 itself performs cis-regulation of HAS2 expression by chromatin remodeling about the proximal HAT2 promoter via O-GlcNAcylation and acetylation [70]. Overactivity of HAS2/HAS2-AS1 may contribute to diabetic macrovascular complications by stimulating dedifferentiation and proliferation of VSMCs since diabetic vessels are rich of matrix hyaluronan, a stimulator of HAS2 synthesis.

MIR222HG

The non-coding miR-222 host gene (MIR222HG; also known as Lnc-Ang362) is located on chromosome Xp11.3 and serves as a host transcript for miR-221 and miR-222, two RNAs that stimulate proliferation of VSMCs. In VSMCs, MIR222HG expression can be induced by angiotensin II. MIR222HG knockdown reduces VSMC proliferation thereby suggesting that this lncRNA possesses proproliferative properties [71].

Long non-coding RNAs that promote vascular smooth muscle differentiation or inhibit proliferation

SENCR

The gene encoding this cytoplasmic lncRNA is located on chromosome 11q24.3. SENCR (Smooth muscle and Endothelial cell-enriched migration/differentiation-associated long non-coding RNA) is transcribed antisense at 5'end of the Friend leukemia integration 1 transcription factor (FLI1) and contains two splice variants [72]. This lncRNA is enriched in vascular cells such as VSMCs and the endothelial cells. Despite the overlap with the FLI gene, little or no cis-effect of SENCR to FLI1 or neighboring gene expression was observed [72]. SENCR knock-down was accompanied with significant down-regulation of the expression of myocardin and multiple smooth muscle contractile genes. Accordingly, down-regulation of SENCR also led to the activation of VSMC proliferation and migration mediated through up-regulation of the transcription factor Forkhead box protein O1 (FoxO1) and transient receptor potential cation channel C6 (TRPC6) [73]. These data indicate that this SMC-enriched RNA is involved in the regulation of SMC differentiation program. Overexpression of SENCR could reverse proproliferative effects of high glucose on VSMC suggesting for a potential vasculoprotective function in diabetes. Recently, it was reported that expression of SENCR significantly correlated with left ventricular (LV) mass to LV end-diastolic volume ratio, a marker of cardiac remodeling, in type 2 diabetic patients [74]. This indeed indicates a role of SENCR as an independent predictor of remodeling in diabetes. In summary, SENCR supports VSMC differentiation and is expected to play an anti-atherosclerotic role. Further studies are required to identify the molecular targets and signaling pathways regulated by this lncRNA.

MYOSLID

Recently, a new SMC-specific lncRNA was identified, named MYOcardin-induced Smooth muscle LncRNA, Inducer of Differentiation (MYOSLID) [75]. The MYOSLID

gene is situated on chromosome 2q33.3, upstream the LINC01802 gene that encodes long intergenic non-protein coding RNA 1802. Myocardin drives the transcription of MYOSLID. In VSMCs, this RNA is involved in propagation of the VSMC differentiation and inhibition of proliferation. MYOSLID did not influence expression of any transcription factors. However, knock-down of this RNA in VSMCs abolished formation of actin stress fibers and prevented the translocation of MYOCD-related transcription factor A (MKL1) to the nucleus. In addition, down-regulation of MYOSLID inhibited TGF-β-mediated activation of SMAD2, an intracellular effector that mediates the effects of this growth factor on cell differentiation [75].

LincRNA-p21

LincRNA-p21 (also known as tumor protein p53 pathway corepressor 1, TRP53COR1) is a p53-induced intergenic lncRNA located on chromosome 6p21.2. By binding to MDM2, lincRNA-p21 derepresses p53 and stimulates p53 interaction with p300. P53/p300 complex then binds to the promoters or enhancers of target genes to promote their expression [76]. LincRNA-p21 inhibits VSMC proliferation and induces apoptosis of cultured VSMCs and macrophages. Expression of this RNA is decreased in CAD patients. In in a murine carotid artery injury model, lincRNA-p21 knockdown promoted neointima formation [77]. Together, these data indicate that lincRNA-p21 suppresses neointimal hyperplasia of VSMCs and may therefore have anti-atherosclerotic properties.

HIF1A-AS1

This lncRNA spans the 5' region of the hypoxia-inducible factor-1α-encoding HIF1A gene in the antisense direction (chromosome 14q23.2). It is unknown whether HIF1A-AS1 influences HIF1A expression in cis. In VSMCs, this RNA is induced by Brahma-related gene 1 (BRG1), a transcription factor. Both BRG1 and HIF1A-AS1 are overexpressed in the aortic media of patients with thoracic aortic aneurysms that leads to increased apoptosis and suppressed proliferation of aortic SMCs in a p53-dependent manner and finally to aortic dissection [78]. It was found that inhibition of HIF1A-AS1 in VSMCs resulted in suppression of caspase-3 and caspase-8, both are proapoptotic proteins, and stimulation Bcl2, an anti-apoptotic protein [79]. It is likely that HIF1A-AS1 plays a pathogenic role in aortic aneurism by suppressing proliferation and activating apoptosis of VSMCs.

GAS5

LncRNA Growth arrest specific 5 (GAS5) is located on chromosome 1q25.1 within the cluster of 11 small nucleolar (sno)RNAs. According to current understanding, snoRNAs act as guide non-coding RNAs, which are involved in biogenesis (modifications) of other small nuclear RNAs [80]. GAS5 and the cluster of GAS5 resides between two protein-coding genes, namely DARS2 (encodes aspartyl-tRNA synthetase 2, mitochondrial) and ZBTB37 (encodes zinc finger and BTB domain containing 37, a transcriptional regulator). However, it is unknown whether GAS5 controls the expression of ZBTB37 and DARS2 in a cis manner. The cluster also contains a gene encoding GAS5-AS1 RNA that may potentially be responsible for the control of GAS5 expression. GAS5 exhibits multiple functions. The most prominent role of this RNA is to serve as a tumor suppressor, since GAS5 blocks the growth and spreading of tumor cells in many cancer types [81]. Overexpression of GAS5 is able to suppress the proliferation of non-cancer cells, for example, vein SMCs. It was found that GAS5 overproduction inhibited proliferation and migration, but also reduced apoptosis of human saphenous vein SMCs [82]. However, when expressed at low levels, this RNA contributes to the formation of primary varicose great saphenous veins by stimulating VSMC proliferation and motility. In the vascular wall, GAS5 is expressed in the endothelial cells and VSMCs, and can contribute to controlling functional activity of these cells. In hypertension, vascular expression of GAS5 was shown to be reduced [83]. GAS5 plays a vasculoprotective role by repressing neointimal hyperplasia of VSMCs. Hypertension-induced down-regulation of GAS5 leads to microvascular dysfunction associated with the vessel leakage and retinal neovascularization. In hypertension, GAS5 regulates vascular remodeling by controlling function of endothelial cells and VSMCs through β-catenin-dependent pathway [83].

Other lncRNAs

Recently, at least three lncRNAs (i.e. ADCY5, ARHGEF12, and FGF12) were found to influence the expression of myocardin, a key transcription factor in launching SMC-specific differentiation program [84]. Depletion of these competitive endogenous RNAs resulted in down-regulation of myocardin and phenotypic transformation of VSMCs from contractile cells to undifferentiated cells characterized by advanced proliferation, enhanced ECM production, and mural thrombi formation. These characteristics may cause an abnormal tissue repair and chronical maintenance of intracranial aneurysms. Therefore, these lncRNAs appear to be involved in the regulation of mature SMC contractility.

Long non-coding RNA detection and use

Numerous studies have identified particular lncRNAs as clinically useful biomarkers, mostly, for detection of various cancers [85, 86], but also for other human diseases, such as ischemic stroke [87] and other cardiovascular disorders [88]. Correspondingly, the interest in improved methods of lncRNA detection is growing. To date, the two most commonly used methods for lncRNA detection are

microarrays that were first developed to detect protein-coding RNAs, and RNA sequencing (RNA-seq) [89]. Microarrays are well-characterized and powerful tools for detection of particular lncRNA with previously known sequences. RNA-seq approach requires a more complicated analysis, but has a broader coverage of lncRNA transcriptome than microarrays. Libraries for RNA-seq can be created using oligo dT beads to enrich and detect polyadenylated lncRNAs (together with mRNAs) or via depletion of rRNA to analyze all other lncRNAs, including circRNAs. The methodological difference between the two methods naturally suggests using RNA-seq approach mostly for screening for novel lncRNAs associated with various human diseases, and microarrays – for robust detection of established lncRNA biomarkers.

The discovery of new lncRNAs and better characterization of the known ones increases the number of novel biomarkers and potential therapeutic targets for treatment of cardiovascular diseases, including atherosclerosis. The lncRNAs discussed in this review likely represent only the beginning of the long list of lncRNAs implicated in cardiovascular diseases that remains to be established. Recently, the diagnostic and therapeutic potential of circRNAs for treatment of cardiovascular diseases has been discussed [88]. Silencing disease-associated lncRNAs that promote cell proliferation appears to be an attractive therapeutic possibility, which has already been explored in animal models of some cancers. Inhibition of lncRNAs can be achieved by different modern methods, such as the use of small interfering RNA (siRNAs) or short hairpin RNAs (shRNAs) and knock-down via CRISPR/Cas9 editing. Some of these methods have already been employed at pre-clinical level and are described in a recent review [90]. More studies are needed, however, to translate the accumulating knowledge of regulatory lncRNAs into clinical applications.

5. Concluding Remarks

Phenotypic switch of VSMCs followed by hyperplasia and neointima formation is an essential stage of pro-atherosclerotic vascular remodeling. LncRNAs play an essential role in controlling this process. Some RNAs suppress proliferation/migration of VSMCs thereby preventing phenotype changes, while others up-regulate VSMC proliferation and hence support VSMC dedifferentiation and neointimal formation. Expression of proproliferative VSMCs can be up-regulated in atherosclerotic plaques and therefore inhibition of their expression may have therapeutic interest in order to target aberrant arterial wall remodeling in atherosclerosis. However, at present, using lncRNAs for clinical purposes is not yet considered. Only a few lncRNA are functionally characterized so far. In VSMCs, lncRNAs form a complex regulatory network that controls VSMC function and behavior. This network becomes even more complex since lncRNAs interacts with miRNAs and regulatory proteins. In addition, not all lncRNAs exclusively expressed or enriched in VSMCs. Therefore, targeting widely distributed lncRNAs may increase risk of target-off effects and adverse influences on other cells. Using cell-type specific delivery approaches may reduce off-target effects since quiescent and hyperplastic VSMCs express distinct sets of biomarkers. Circulating lncRNAs can probably be also used as diagnostic and prognostic biomarkers, and this possibility is already being explored in some studies. In conclusion, although lncRNAs have promising therapeutic potential, they have not attained the stage that would provide an option of easy clinical application.

Заключение

Смена фенотипа гладкомышечных клеток кровеносных сосудов, сопровождающаяся гиперплащией и утолщением сосудистой стенки является важным этапом в развитии атеросклеротической бляшки, и длнкРНК играют важную роль в этом процессе. Отдельные виды длнкРНК подавляют пролиферацию и миграцию гладкомышечных клеток, таким образом, препятствуя смере фенотипа, в то время как другие виды наоборот, способствуют пролиферации и миграции и, тем самым, формированию неоинтимы. Пролиферация гладкомышечных клеток наблюдается в атеросклеротических бляшках, и подавление этого процесса может иметь важное терапевтическое значение. Однако, в настоящее время еще рано говорить о терапевтическом применении длнкРНК. Лишь несколько длнкРНК были функционально охарактеризованы к настоящему моменту. В то же время, в гладкомышечных клетках сосудов может существовать развитая регулирующая сеть, включающия различные длнкРНК, которые также могут взаимодействовать с малыми интерферирующими РНК и регуляторными белками. Кроме того, не все описанные длнкРНК экспрессируются преимущественно в гладкомышечных клетках, что создает риск побочных эффектов при применении этих длнкРНК. Решением этой проблемы может стать разработка методов направленной доставки терапевтических молекул в гладкомышечные клетки атеросклеротических бляшек, так как эти клетки имеют некоторые характерные биомаркеры, которые позволяют различать клетки, находящиеся в состоянии покоя от пролиферирующих клеток. длнкРНК, присутствующие в системе кровообращения, могут также служить в качестве диагностических и прогностических факторов, и эта возможность в настоящее время активно исследуется. Таким образом, длнкРНК обладают интересным потенциалом для диагностики и лечения атеросклероза, который, однако, в настоящий момент еще не достаточно исследован.

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