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REVIEW ARTICLE

Noncoding RNAs in vascular smooth muscle cell function and neointimal hyperplasia

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Keywords

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Neointimal hyperplasia (NIH) is a pathological process occurring in the blood vessel wall during atherosclerosis and in-stent restenosis (ISR). Due to the abundance of vascular smooth muscle cells (VSMCs) within neointimal lesions, VSMCs have long been considered as a key cellular target in preventing NIH. Noncoding RNA molecules such as microRNA (miRNAs), long noncoding RNA (lncRNAs) and circular RNAs (circRNAs) expressed in VSMCs offer unique therapeutic targets for tackling VSMC phenotype switching, proliferation, migration and apoptosis processes responsible for promoting NIH. In this review, we provide an extensive overview of VSMC RNA biology, highlighting the most recent discoveries in the field of lncRNAs and circRNAs, with the aim of identifying key molecular players that could be harnessed for future therapeutic interventions, in our quest to halt NIH in vascular disease.

Abbreviations

BANCR, BRAF-activated noncoding RNA; CABG, coronary artery bypass grafting; CAD, coronary arteries disease; CARMEN, (CAR)diac (M)esoderm (E)nhancer-associated (N)oncoding RNA; CENPF, centromere protein F; circActa2, circRNA Acta2; circRNAs, circular RNAs; CVD, cardiovascular diseases; DES, drug-eluting stents; ECM, extracellular matrix; EVI1, ecotropic virus integration site 1 protein homolog; GAS5, growth arrest specific 5; ISR, in-stent restenosis; KLF4, Krüppel-like factor 4; LIPCAR, mitochondrial long noncoding RNA uc022bqs.1; LncRNAs, long noncoding RNAs; LncRNA-SRA, lncRNA-steroid receptor RNA activator; MDM2, mouse double minute 2; MECP2, methyl-CpG binding protein 2; MI, myocardial infarction; miRNAs, microRNAs; mRNA, messenger RNA; mTOR, mammalian target of rapamycin; MYOSLID, MYOcardin-induced Smooth muscle LncRNA, Inducer of Differentiation; NcRNAs, noncoding RNAs; NEAT1, nuclear paraspeckle assembly transcript 1; NIH, Neointimal hyperplasia; PCI, percutaneous coronary intervention; PDGF-BB, platelet-derived growth factor BB; PRC2, polycomb repressive complex 2; PTEN, phosphatase and tensin homolog; SM22α, smooth muscle-22α; SMMHC, smooth muscle myosin heavy chain; SMαA, smooth muscle-α-actin; STEMI, ST-segment elevation myocardial infarction; TGFβ1, transforming growth factor β1; VSMCs, vascular smooth muscle cells; WDR5, WD repeat domain 5.

Introduction

Neointimal hyperplasia (NIH) is a process that describes the rapid proliferation and migration of vascular smooth muscle cells (VSMCs) into the neointima, the inner layer of diseased or injured arteries. NIH often occurs following vascular procedures to prevent or treat heart attacks, such as percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) [1,2]. Although similar to the physiological process of 'intimal hyperplasia', which occurs in newborns during closure of the ductus arteriosus in the heart [3,4], NIH leads to increased deposition of extracellular matrix (ECM) proteins, resulting in 'neointimal thickening' of the vessel wall [5]. Following a CABG procedure, greater arterial pressures across the wall of grafted veins further exacerbate NIH, leading to greater neointimal thickening and therefore greater arterial narrowing [6,7].

During the advanced stages of atherosclerosis, a disease whereby the inner layers of arteries accumulate lipid deposits, immune cells and ECM proteins, the migration and proliferation of VSMCs into these areas, otherwise known as atherosclerotic plaques, are largely considered to have beneficial and protective roles. Clinical evidence from patients with coronary arteries disease (CAD) has revealed an inverse correlation between VSMC content in the outer layers of the plaque, also known as the fibrous cap, and the likelihood of plaque rupture. This adverse vascular event can lead to blockage of the artery feeding the heart muscle, and result in a myocardial infarction (MI), also known as a heart attack [8]. Unfortunately, the protective benefit of VSMC proliferation and migration into the neointima during atherosclerosis is hindered by VSMC senescence and apoptosis (i.e. programmed cell death) [9]. Importantly, NIH is also a key determinant of in-stent restenosis (ISR), a phenomenon of arterial re-narrowing by $\geq 50\%$ of a previously blocked coronary artery. ISR occurs in 20–40% of heart attack patients within 6–12 months of undergoing PCI [10,11], a nonsurgical procedure involving the insertion of a stent to restore blood flow in a blocked coronary artery. The extent of ISR is assessed by angiographic imaging or intravascular ultrasonography [12] and results in a severe reduction of blood flow to downstream cardiac tissues, leading to the onset of cell death in the heart muscle wall, also known as myocardial ischaemia [13] and often presents as progressive recurrent angina [12]. Chronic inflammation in the vessel wall, brought about by endothelial dysfunction and/or sudden vascular injury, provides an abundant source of growth factors [14,15], pro-inflammatory cytokines [16-18] and chemoattractant proteins [19,20] capable of further promoting VSMC proliferation and migration [21], as well as inducing VSMC phenotype switching [22-24]. Moreover, since the discovery of distinct stem/progenitor cell populations resident in the vascular wall [25], capable of contributing to the VSMC pool in vascular disease, more research has been carried out to assess the therapeutic benefit of targeting these alternative cell types during NIH [26-28]. Therapies aimed at modulating VSMC functions such as proliferation, migration and apoptosis during NIH as well as uncovering VSMC-specific molecular pathways responsible for phenotype switching and (de)differentiation will prove vital in preventing ISR in vulnerable patients with heart disease.

Currently, several mechanical and pharmacological techniques are used to prevent ISR in patients who have undergone PCI (Fig. 1). Dual antiplatelet therapies and other anti-coagulant drugs are prescribed to patients after PCI to reduce the risk of blood clot formation within the stents, also known as 'stent thrombosis' [29]. Several drugs have also been trialled for preventing ISR due to their anti-inflammatory properties including corticosteroids [30], statins [31], antioxidants [32], and nitric oxide [33]. However, local delivery of drugs using drug-eluting stents (DES) is considered the most successful innovation for reducing rates of ISR and the need for repeated revascularisation in PCI [34]. Following revascularisation of the blocked coronary artery, insertion of a stent that releases antiproliferative drugs, which prevent rapid cell proliferation, has shown dramatic improvements in clinical outcomes for patients. The antiproliferative drug, sirolimus, has been successful in reducing the incidence of ISR by preventing NIH, in single primary lesions and complex coronary lesions [35,36], whereas other antiproliferative drugs have been less successful, with some studies revealing higher rates of ISR and major cardiac events occurring with actinomycin [37], and increased stent thrombosis with 7-hexanoyltaxol [38]. A recent innovation in stent mechanics involving the use of bioresorbable vascular scaffolds, which gradually become resorbed leaving the vessel free of foreign and thrombogenic material, has been shown to lower the incidence of restenosis or occlusion [39]. The BIOSTEMI trial demonstrated an improvement in target lesion failure rates after 1 year, in patients with acute MI who had received biodegradable polymer sirolimus-eluting stents versus durable polymer everolimus-eluting stents [40]. Unfortunately, rates of restenosis are still high [41-43] with the most recent clinical trial, NORSTENT, showing that repeat

revascularisation is still required for 16.5% of PCI patients who received a DES compared to 19.8% receiving bare-metal stent [34]. Additionally, the widespread antiproliferative effect of these drugs on the vascular wall delays re-endothelisation, which promotes clot formation and neo-atherosclerosis, and ultimately increases the likelihood of another adverse cardiovascular event [44,45]. Therefore, a cell-specific method of targeting NIH is needed to address VSMC-mediated NIH in the vascular wall. As such, noncoding RNA (ncRNA)-based therapy may offer an alternative approach to targeting VSMC and preventing NIH or ISR [46,47].

General introduction to noncoding RNAs

Noncoding RNAs (ncRNAs) are a class of RNA molecules that are transcribed from the genome but do not code for a protein. Similar to protein-coding mRNA, which only constitutes 2–3% of the transcribed genome [48], they can travel into the cytoplasm and interact with other organelles and proteins [49]. Although long considered a 'by-product' of mRNA biosynthesis, ncRNAs can interact with numerous signalling pathways and alter cell function and cell fate [50].

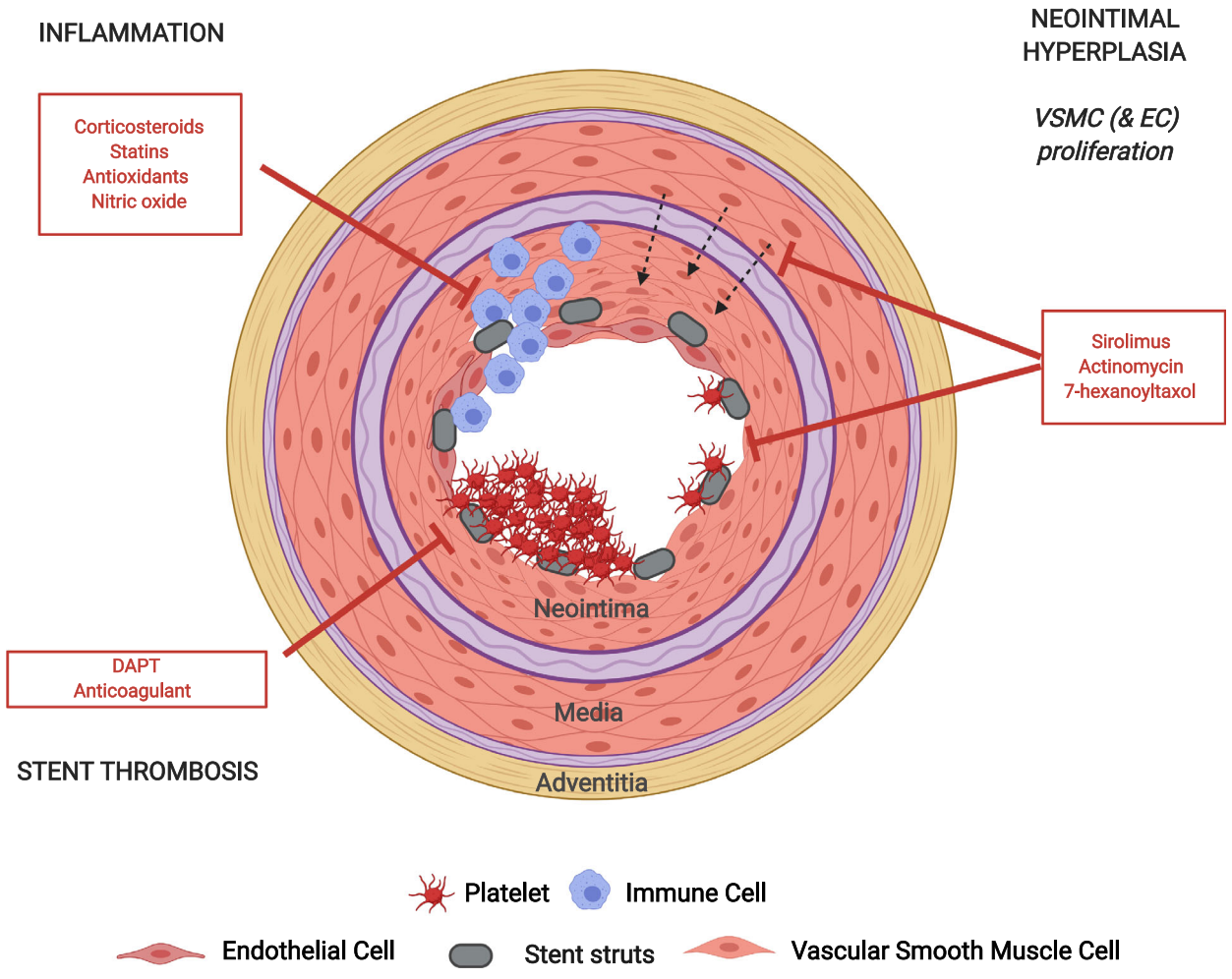

Fig. 1. Current pharmacological methods of ISR prevention. Multiple drugs can be used to target different pathophysiological processes responsible for promoting ISR. Dual antiplatelet therapies (DAPT) and other anti-coagulant drugs prevent platelet aggregation, thereby reducing the risk of stent thrombosis. Drugs with anti-inflammatory properties (corticosteroids, statins, antioxidants and nitric oxide) reduce the influx of immune cells responsible for promoting NIH in the artery wall. Antiproliferative drugs (sirolimus, actinomycin, 7-hexanoyltaxol) inhibit NIH by preventing VSMCs proliferation and re-endothelialisation, increasing the likelihood of stent thrombosis. Black (dashed) arrows indicate cellular proliferation and migration. Red (closed) arrows indicate inhibition of process. ISR, in-stent restenosis; NIH, neointimal hyperplasia; VSMC, vascular smooth muscle cell; EC, endothelial cell. This diagram was created with Biorender.com.

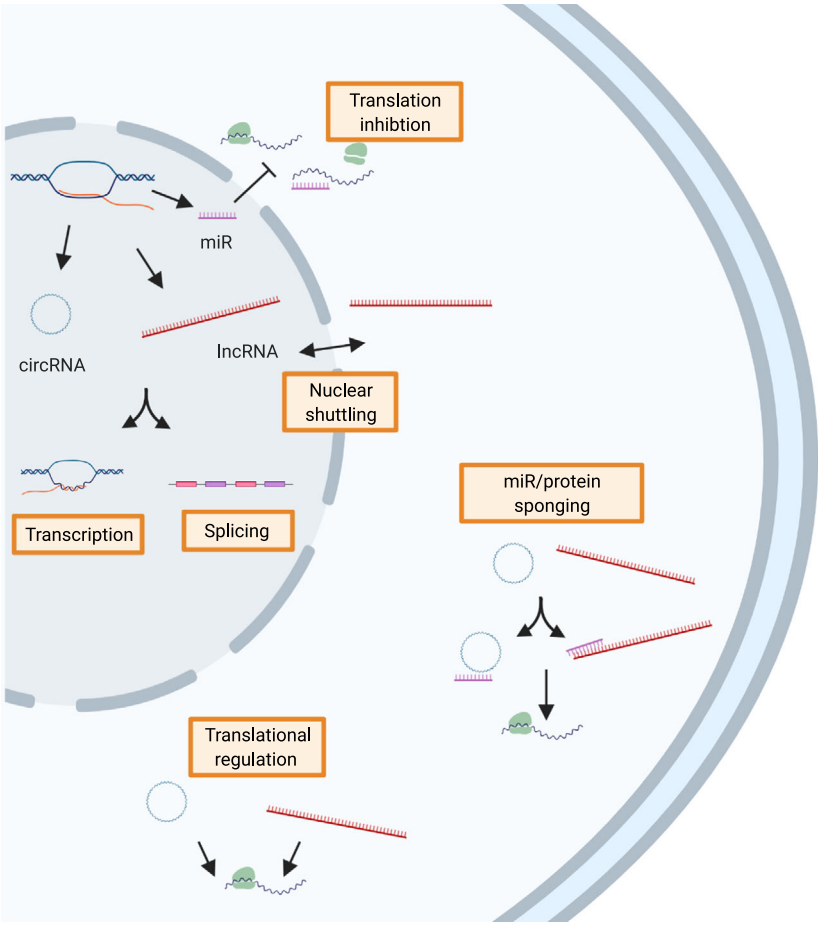

Fig. 2. Noncoding RNAs molecular functions within the cell. All three major NcRNAs (miRs, lncRNAs and circRNAs) are all transcribed at the nucleus and can migrate to different parts of the cell to carry out their molecular functions. MiRs migrate into the cytoplasm to prevent translation of mRNA. LncRNA and circRNA can operate in the nucleus to modulate gene transcription and RNA splicing or migrate into the cytoplasm to sponge miRs or regulate mRNA translational processes. LncRNA can also regulate nuclear shuttling. NcRNA, noncoding RNA; miR, microRNA; lncRNA, long noncoding RNA; circRNA, circular RNA. This diagram was created with Biorender.com.

Several important ncRNA classes have been described in cardiovascular diseases (CVDs), namely microRNAs (miRs) [51,52], long noncoding (lncRNAs) [53] and circular RNAs (circRNAs) with important molecular functions (Fig. 2) [54]. miRs are defined as 20–22 nucleotides long, single strand of RNA, capable of preventing messenger RNA (mRNA) translation by binding to the 3' untranslated region (UTR), and in some cases the 5' UTR, of its target mRNA [55-57]. They are transcribed in the nucleus by RNA polymerase II or III enzymes, and cleaved using a protein complex comprised of an RNAse III endonuclease, called Drosha, and a double-stranded RNA binding protein, called Di George syndrome critical region gene 8. This precursor to miR is then exported to the cytoplasm for further cleavage in an enzyme complex. Following this, the double-stranded miR is loaded onto the RNA-induced silencing complex ready to capture and initiate degradation of the target mRNA [58]. LncRNA molecules are typically defined as ≥ 200 nucleotides in length. They exhibit more specific expression profiles than mRNA and alter expression profiles depending cell-type and disease state [59]. They are transcribed by RNA polymerase II and III and undergo extensive post-transcriptional modifications such as 5′-capping, splicing, polyadenylation and, in some cases, alternative splicing [60]. The field of lncRNA biology has been rapidly expanding with new transcripts identified as capable of regulating epigenetic events [61,62], gene transcription in both *cis* [63] and *trans* [64], protein translation [65], RNA [66], protein 'sponging' [67] and nuclear/cytoplasmic 'shuttling' [68]. Finally, circRNAs are a nonlinear lncRNAs, with a unique circular structure formed through backsplicing of pre-mRNA, which in the absence of a 5'cap and a poly A tail confers resistance to miR-induced deadenylation and decay [69-71].

All three classes of ncRNAs have members involved in cardiovascular development (e.g. miR-145/143 [72], lncRNA *Braveheart* [73], circRNA cZNF292 [74]) as well as members, which could serve as circulating biomarkers for CVDs, such as CAD (e.g. miRNA-765 [75], lncRNA OTTHUMT00000387022 [76] and circular RNA Hsa_circ_0004104 [77]). Several key miRs

have been identified as important players in neointimal formation, including miR-22, which can reduce VSMC proliferation and limit neointima formation in a mouse model of ISR, by promoting degradation of target genes: ecotropic virus integration site 1 protein homolog (EVI1) and methyl-CpG binding protein 2 (MECP2). Reduced expression of miR-22, as well as increased expression of EVI1 and MECP2 in diseased human femoral arteries, confirmed its regulatory role in VSMC proliferation and thus presents a new VSMC-specific target for preventing NIH and therefore neointimal formation [78]. Another miRNA, miR-34a, has been identified as a useful ncRNA target not only by preventing VSMC proliferation and migration in the neointima [79] but also promoting VSMC differentiation from stem cells [80]. Moreover, regulation of miR-34a by platelet-derived growth factor (PDGF-BB) and transforming growth factor β1 (TGFβ1), two growth factors involved in regulating VSMC phenotype switching, was found to regulate miR-34a expression in a p53-dependent manner [79]. Cancer studies have established miR-34a as an important tumour suppressor and governed by the transcription factor and oncogene activator, p53 [81], and previous studies have confirmed a similar role in VSMCs, where elevated levels of miR-34a lead to enhanced apoptosis and senescence [82,83]. With a single miR exhibiting several roles in VSMC phenotype and function, it stands to reason that many more ncRNA molecules exist that may present greater potential for modulating specialised functions, which in turn may be advantageous in preventing neointimal formation.

A number of Reviews have discussed the role of miRs in VSMC phenotype switching, proliferation and migration, in the context of NIH, and these molecules are summarised briefly in Table 1. We also refer the reader to the following Reviews for a more in-depth study [84-90]. This Review will seek to examine the more recent discoveries in lncRNA and circRNA functions in VSMC biology, with particular emphasis on their shared signalling pathways with previously uncovered miRs to map out new gene regulatory mechanisms, which may aid in the future to manipulate VSMC behaviour during neointimal formation.

NcRNAs in VSMC phenotypic switching

Contractile versus synthetic VSMC phenotype

Previously, a binary model of VSMC phenotype switching was established whereby TGFβ stimulation promoted a quiescent 'contractile' VSMC phenotype with upregulated expression of contractile SMC markers [smooth muscle-α-actin (SMαA), smooth muscle-22α (SM22α), smooth muscle myosin heavy chain (SMMHC)], whereas PDGF-BB stimulation triggered a drop in SMC gene expression and an increase in extracellular matrix (ECM) protein secretion, leading to the adoption of a pro-migratory, hyperproliferative 'synthetic' VSMC phenotype, which contributed to neointimal formation in vascular disease [22,91,92]. Using this model, several lncRNAs have been found to interact with promoter regions of SMC genes including growth arrest specific 5 (GAS5), which was shown to prevent TGFβ-induced SMC differentiation of VSMCs by blocking Smad3 activity via RNA Smad-binding elements [93]. The lncRNA, nuclear paraspeckle assembly transcript 1 (NEAT1), was found to prevent serum response factor binding to SMC gene promoters, by sequestering the chromatin 'activator' WD repeat domain 5 (WDR5) under PDGF-BB stimulation. In addition, knockdown of NEAT1 could prevent phenotype switching of VSMCs towards a 'synthetic' state, as well as reduce VSMC proliferation and migration resulting in attenuated NIH after carotid artery ligation in mice [94]. Conversely, lncRNA MRAK048635_P1 was able to prevent proliferation, migration and phenotypic switching, as well as promote apoptosis of VSMCs isolated from spontaneously hypertensive rats [95]. Another 'pro-contractile' ncRNA was found to indirectly increase SMαA protein expression by sponging a miR, miR-548f-5p. Under TGFβ stimulation, circRNA Acta2 (circActa2) is activated, leading to the inhibition of miR-548f-5p-mediated translational repression of SMαA mRNA. Further studies are needed to investigate whether enforced expression of circActa2 could prevent phenotype switching of VSMCs during neointimal formation [96,97]. Finally, the VSMC-specific MYOcardin-induced Smooth muscle LncRNA, Inducer of Differentiation (MYOSLID) was identified as a product of myocardin/serum response factor activation essential for the downstream phosphorylation of Smad2 and actin stress fibre formation following TGFB stimulation [98]. Taken together, a large number of new ncRNA molecules have emerged as crucial modulators of SMC gene and protein expression and thus present new opportunities to promote a quiescent 'contractile' phenotype in vascular disease.

VSMC alternative phenotypes

An increasing number of studies have shown that VSMCs can also adopt alternative phenotypes in response to changes in their environment. For

Table 1. Known miRs required for VSMC phenotype switching, proliferation and migration, and their molecular targets. ND, not determine; '↑' and '↓' indicate up- and downregulation, respectively; '+' and '−' represent 'promoting' and 'inhibiting', respectively.

miRNAs	Expression levels in neointimal hyperplasia	Role in VSMC quiescent vs. synthetic phenotype	Proliferation	Migration	Target	Ref
let-7a	↓	ND	– *(PDGF-BB)*	–	c-Myc, K-ras	[143]
miR-21	↑	Promotes PDGF-induced synthetic phenotype	+	ND	PTEN Bcl2	[143,177-179]
miR-22	↓	Promotes TGFβ-induced contractile phenotype and prevents PDGF-induced synthetic phenotype	–	–	EVI-1, MECP2, HDAC4	[78]
miR-24	↓	Promotes PDGF-induced synthetic phenotype	+ *(PDGF)* – (*under adenoviral miR-24 overexpression in vivo)*	ND	Tribbles-like protein 3 Wnt4/Dvl-1/β-catenin signalling pathway	[143] [180] [181]
miR-26a	↓	Promotes PDGF-induced synthetic phenotype	+ *(PDGF)* – *(with miR-26a agomir)*	+	Smad1, 4 Mitogen-activated protein kinase 6	[182],[183] [184]
miR-29b	ND	Promotes PDGF-induced synthetic phenotype	ND	ND	SIRT1 NF-κB	[185]
miR-31	↑	ND	+	ND	Large tumour suppressor homolog 2	[186]
miR-34a	↓	ND	–	–	Notch1	[79]
miR-124	↓	ND	–	–	IQGAP1	[187]
miR-133	↑	Prevents PDGF-induced synthetic phenotype *in vitro* and *in vivo*	– *(10% FBS & PDGF-BB)*	– *(10% FBS)*	Sp-1 Moesin SRF KLF4	[188]
miR-137	ND	ND	– *(PDGF-BB)*	–	IGFBP-5	[189]
miR143/145	↓	Prevents PDGF-induced synthetic phenotype *in vitro* and *in vivo*	– *(PDGF-BB)*	ND	CamkII-δ, KLF4, Elk-1, serum response factor, myocardin, Nkx2.5	[72,128-130,143,190,191]
miR-146a	↑	ND	+ *(control conditions and PDGF)*	ND	Krüppel-like factor 4	[143,192,193]
miR-195	↓	ND	– *(ox-LDL)*	– *(ox-LDL)*	Cdc42, cyclin D1, fibroblast growth factor	[194]
miR-204	↑	ND	+ *(PDGF-BB, high glucose)*	ND	Calveolin-1	[143,195]
miR-208	ND	ND	+ *(insulin)*	ND	p21	[196]
miR-214	↓	ND	–	–	NCKAP1	[197]
miR-221/222	↑	ND	+ *(PDGF-BB)*	ND	p27, p57	[143,198]
mir-424/322	↓	Promotes contractile phenotype	– *(PDGF-BB)*	– *(PDGF-BB)*	STIM1, calumenin, cyclin D1	[199]
miRNA-503	↑	ND	– *(PDGF-BB)*	– *(PDGF-BB)*	insulin receptor	[199,200]
miR-541	ND	ND	+	ND	Interferon regulatory factor	[201]
miR-599	ND	ND	–	–	TGFb2	[202]
miR-633	↓	Promotes contractile phenotype	– *(PDGF-BB)*	– *(PDGF-BB)*	JunB	[203]
miR-688	ND	Promotes contractile phenotype	– *(PDGF-BB)*	– *(PDGF-BB)*	NOR1/cyclin D	[204]

instance, a 'pro-inflammatory' VSMC phenotype can be generated under TNFα stimulation [99], a 'foam cell-like' VSMC phenotype can be generated following ox-LDL exposure [100,101], and a stem cell-like phenotype can be generated following vascular injury [102,103]. One study showed that an ncRNA transcript of the SIRT1 gene, circ-Sirt1, could inhibit inflammatory phenotype switching of VSMCs under TNF-α stimulation. This 'anti-inflammatory' circRNA was found to inhibit nuclear translocation of NFκB p65 and enhance expression of its host gene by directly binding to miR-132/212, resulting in reduced transcriptional activity of NFκB [104].

A 'pro-inflammatory' ncRNA, Lnc-Ang362, was found to be essential for angiotensin II-mediated proliferation and migration of human pulmonary artery smooth muscle cells. As the host transcript for miR-221 and miR-222, upregulation of Lnc-Ang362 led to increased expression of miR-221 and miR-222, which in turn increased phosphorylation of NFκB proteins, p65 and IκBα [105].

Under ox-LDL exposure, expression of lncRNA LINC00341 was significantly increased, resulting in enhanced VSMC proliferation and migration. Interestingly, cytoplasmic LINC00341 acted as an endogenous sponge for miR-214, thereby preventing translational repression of FOXO4, a transcription factor, that binds to the TFBS 3 binding motif of promoter region for LINC00341, revealing a positive feedback loop [106]. As such, the LINC00341/miR-214/FOXO4 pathway presents an interesting target to prevent VSMC differentiation towards a foam cell-like phenotype. Another lncRNA urothelial carcinoma-associated (UCA1) was upregulated following ox-LDL exposure and enabled VSMC proliferation and migration. This lncRNA acted as an endogenous sponge for miR-26a, which regulates the expression of phosphatase and tensin homolog (PTEN) required for VSMC apoptosis [107].

VSMCs versus stem/progenitor cells

A recent study revealed the ability of a subpopulation of VSMCs to dedifferentiate into $Sca1^+/CD34^+$ vascular progenitor cells, which undergo cellular expansion in response to vascular injury [103]. Majesky et al. (2017) identified Krüppel-like factor 4 (KLF4) as key to maintaining their progenitor phenotype. A key regulator of VSMC phenotype commitment, KLF4, has been studied extensively with regard to VSMC behaviour during neointimal formation [108-112]. Indeed, several knockdown studies have shown that KLF4 regulates phenotype switching and proliferation of VSMCs [110]. One study revealed that, although SMC-specific deletion of KLF4 could delay phenotype switching, this ultimately resulted in enhanced cellular proliferation and accelerated neointimal formation in a murine model of ISR [113]. The lncRNA, POU3F3, was recently identified as a potential regulator of KLF4 by Zhang et al. [114]. They showed that POU3F3 is upregulated in PCI patients with ISR and that overexpression of POU3F3 in VSMCs downregulates expression of SMC genes but increases VSMC proliferation and migration. Interestingly, POU3F3 overexpression increased KLF4 expression, but this was attenuated by miR-449a, revealing a POU3F3/miR-449a/KLF4 regulatory axis, providing a new regulatory route for modulating KLF4 expression in VSMCs for the treatment of ISR.

This discovery of a subpopulation of VSMCs with stem cell-like properties in the neointima, combined with the existence of multiple stem/progenitors cell families contributing to VSMC populations during vascular remodelling [115-117], adds further complexity to our understanding of cellular responses during neointimal formation [118-120]. Studies have identified numerous stem cell subtypes in both the medial and adventitial layers of mammalian arteries such as $Sca1^+$ $CD34^+$ adventitial stem/progenitor cells [27], $Sox17^+$ $Sox10^+$ multipotent vascular stem/progenitor cells [26] and mesenchymal stem cell-like cells [121] capable of differentiating into neointimal VSMCs. As such, molecular signalling pathways governing the differentiation of these vascular stem cells will be of great interest to both the field of stem cell biology and vascular disease. For instance, miR-34a has been shown to play a role in stem cell specialisation during neointima formation [80]. Several ncRNAs have been studied during embryonic stem cell differentiation, including miR-214 that has been found to promote VSMC differentiation by suppressing its target gene, Quaking (QKI) [122]. Moreover, Guttman et al. (2009) identified over 100 lncRNAs with putative functions in four different murine ESC lines involved in regulating pluripotency as well as cell proliferation using chromatin state mapping [123]. Many lncRNA molecules promote cardiovascular lineage commitment such as Braveheart, which ensures commitment of mesoderm towards to the cardiac fate [73]; Fendrr, which is crucial for heart and body wall development [124]; and (CAR)diac (M)esoderm (E)nhancer-associated (N)oncoding RNA (CARMEN), which maintains cardiac identity of cardiomyocytes from cardiac precursor cells [125]. All three lncRNAs function through epigenetic regulation via the polycomb repressive complex 2 (PRC2); however, Braveheart is not expressed in humans. At

present, studies focussing on lncRNA function in stem cells have mainly identified roles for ncRNAs in maintaining stem cell pluripotency and self-renewal rather than differentiation [126]. However, lncRNAs, Terminator, Alien and Punisher, have all been identified as being vital for different stages of angiogenic processes such as blood vessel development and endothelial tubule formation [127].

As more and more studies investigate the role of newer members of the ncRNA family, it is clear that our understanding of VSMC origin and differentiation will improve and eventually provide us with unique molecular switches to manipulate VSMC towards a desired phenotype and therefore behaviour (Table 2 and Fig. 3).

NcRNAs in VSMC proliferation and migration

A key component of neointimal formation is the influx of rapidly dividing VSMCs. As such, an abundance of ncRNAs have been identified as regulators of VSMC proliferation and migration. One of the earliest known players in VSMC biology, the miR-143/miR-145 cluster, was shown to play a key role in modulating VSMC migration and proliferation (see Table 1). Elia et al. (2009) demonstrated greater migratory and proliferative capacity of miR-143/miR-145 knockout VSMCs, which formed part of a wider response of dedifferentiation towards a pro-migratory, hyperproliferative and synthetic phenotype [128]. Despite this however, *in vivo* knockout of both miRs led to impaired migration of VSMCs due to dysregulated cytoskeletal dynamics, resulting in attenuated neointimal formation [129]. In the same year, these two studies were published, and another study found that miR-143/miR-145-deficient mice exhibited greater neointimal lesion formation, with VSMCs appearing to be 'locked in' a synthetic state [130]. More recently, the circRNA, circ-LRP6, was shown to have several miR-145 binding sites, allowing it to 'sponge' miR-145 as seen by colocalisation of these two RNAs in P-bodies using fluorescence in situ hybridisation [131]. Importantly, silencing of circ-LRP6 led to increased miR-145 levels with VSMCs exhibiting reduced proliferation and migration, and increased VSMC differentiation markers, reinforcing an atheroprotective role for miR-145. However, the authors note that this inverse relationship could only be seen under TGFβ treatment conditions, and that under PDGF stimulation, circ-LRP6 expression followed that of miR-145 expression patterns. Moreover, hypoxic conditions were shown to cause significant downregulation of miR-145 expression, with circ-LRP6 expression levels remaining largely unaffected, a pattern that was observed in atherosclerotic vessels isolated from $ApoE^{-/-}$ knockout mice. Nevertheless, viral delivery of circ-LRP6-shRNA led to reduced NIH. The relationship between miRs and circRNAs highlights the complex nature of RNA regulation in VSMCs, which can substantially alter depending on the cellular conditions and disease context, but provides a promising use for circ-LRP6 as a useful molecular switch to prevent ISR following PCI. Unsurprisingly, circ-LRP6 silencing was found to reduce expression of the miR-145 target, KLF4, which, as discussed previously, plays a vital role in modulating vascular stem cell differentiation.

Countless other signalling pathways governing proliferation and migration have been discovered to be under ncRNA regulation. Indeed, the lncRNA, lncRNA-steroid receptor RNA activator (LncRNA-SRA), is upregulated during NIH in mice following femoral artery wire injury and was found to promote VSMC proliferation and migration by triggering phosphorylation of the MEK/ERK/CREB pathway [132]. The lncRNA, BRAF-activated noncoding RNA (BANCR), was found to promote VSMC proliferation and migration through phosphorylation and activation of the JNK pathway. Importantly, BANCR expression was increased in VSMCs under both TNFα stimulation *in vitro* and in human atherosclerotic tissues *ex vivo* [133]. The nuclear lncRNA, Giver, was shown to play an important role in regulating expression of genes associated with cell proliferation and oxidative stress through epigenetic regulation. Das et al. (2018) showed that Giver expression can be induced following angiotensin II treatment of rat VSMCs by promoting transcription of its neighbouring gene, Nr4a3. Using chromatin immunoprecipitation, Giver was shown to enrich RNA polymerase activity and prevent histone H3 trimethylation of lysine 27 at the Nox1 gene promoter, as well as promote transcription of pro-inflammatory genes, interleukin-6, Ccl-2 and TNF-α [134]. The lncRNA, smooth muscle-induced lncRNA enhances replication (SMILR), was found to regulate the late mitotic phase of cell division and could bind directly to centromere protein F (CENPF), a mitotic centromere protein [135], thereby promoting human saphenous vein VSMC proliferation following treatment with PDGF and interleukin 1-α. Reduced expression of SMILR in unstable atherosclerotic plaques and plasma taken from patients undergoing carotid endarterectomies provides a useful insight into the potential of promoting SMILR expression in vascular disease to reduce the risk of adverse coronary events [136].

Table 2. Summary of newly discovered members of lncRNA and circRNA family involved in regulating VSMC phenotype commitment and specialisation, and ncRNA expression levels under different external stimuli and/or CVD pathologies and their molecular targets. '↑' and '↓' indicate up- and downregulation, respectively; '+' and '−' represent 'promoting' and 'inhibiting', respectively.

ncRNA	Stimulus	Expression following stimulus or in CVD	VSMC phenotype	Promotes/ Inhibits (+/−)	Target	Ref
LncRNAs						
Lnc-Ang362	Angiotensin II	(pulmonary arterial hypertension patients)	Inflammatory	+	miR-221/222	[105]
NEAT-1	PDGF-BB	↑ (PDGF-BB)	Synthetic	+	WDR5	[94]
POU3F3	N/A	↑ (in PCI patients with ISR)	Stem cell	+	KLF4 miR-449a	[114]
GAS5	TGFβ	↓ (CAD patients)	Contractile	−	Smad3	[93,205]
MYOSLID	TGFβ	↓ Neointimal lesions of arteriovenous fistula tissue	Contractile	+	Smad2	[98]
Linc00341	ox-LDL	↑ (ox-LDL)	Foam cell	−	miR-214	[106]
UCA1	ox-LDL	↑ (ox-LDL)	Foam cell	+	miR-26a	[107]
CircRNAs						
Circ-Sirt1	TNF-α	↓ (neointima of atherosclerotic tissues)	Inflammatory	−	p65 miR-132/212	[206]
CircActa2	TGFβ	↑ (TGFβ)	Contractile	+	miR-548f-5p	[96,97]

Two other lncRNAs, lnc-RNCR3 and lncRNA-430945, have recently been identified as regulators of VSMC proliferation and migration, with elevated levels of both lncRNAs seen in human atherosclerotic lesions. LncRNA-430945, in particular, was found to act mainly through activation of the RhoA signalling pathway by promoting the expression of receptor tyrosine kinase-like orphan receptor 2 [137]. Knockdown of lncRNA 430945, using small interfering RNA, led to reduced angiotensin II-induced VSMC proliferation and migration. Similarly, knockdown of lnc-RNCR3 saw a significant drop in VSMC proliferation and migration; however, this was found to further aggravate atherosclerosis and promote inflammation in mice [138]. Interestingly, lnc-RNCR3 appeared to promote EC proliferation by acting as a competing endogenous RNA (ceRNA) for miR-185-5p, resulting in elevated levels of KLF2. Whether a similar regulatory network exists in VSMCs remains to be seen.

The lncRNA, lnc-00113, was also found to be highly expressed in the serum of atherosclerosis patients, and silencing of lcn-00113 was found to suppress proliferation, but promote migration of VSMCs and HUVECs. Lnc-00113-mediated proliferation was considered to occur through activation of the PI3K/Akt/mTOR pathway in HUVECs; however, these findings have yet to be confirmed in VSMCs [139]. Finally, the previously mentioned ceRNA, GAS5, was downregulated following PDGF-BB stimulation in VSMCs. Overexpression of GAS5 could prevent PDGF-BB-induced VSMC proliferation and migration by acting as a 'molecular sponge' for miR-21 [140]. Crucially, exosome release of GAS5 from GAS5-overexpressing ECs could reduce VSMC proliferation and migration, and vice versa, highlighting an important role for GAS5 in VSMC-EC crosstalk. Moreover, GAS5 was found to regulate the β-catenin signalling pathway through nuclear localisation of β-catenin in both VSMCs and ECs [141]. Importantly, this study provides an exception to the rule that most lncRNAs are cell specific, therein providing both an opportunity for multicellular approach to targeting NIH, but also a heightened risk of off-target effects with unwanted consequences on EC growth and consequently stability and integrity of a vulnerable endothelium.

A multitude of lncRNAs and circRNAs has been identified in recent years (Table 3 and Fig. 4), with important roles in VSMC proliferation in particular. In some cases, ncRNAs appear to share the same role in cancer cells, and potentially, this overlap will provide useful clues to investigate previously unknown pathways across the two different cell types and pathologies.

NcRNAs in VSMC apoptosis and survival

Most studies attempt to address NIH, by targeting pathways responsible for the initial presence and activity of VSMCs. Nevertheless, several studies have

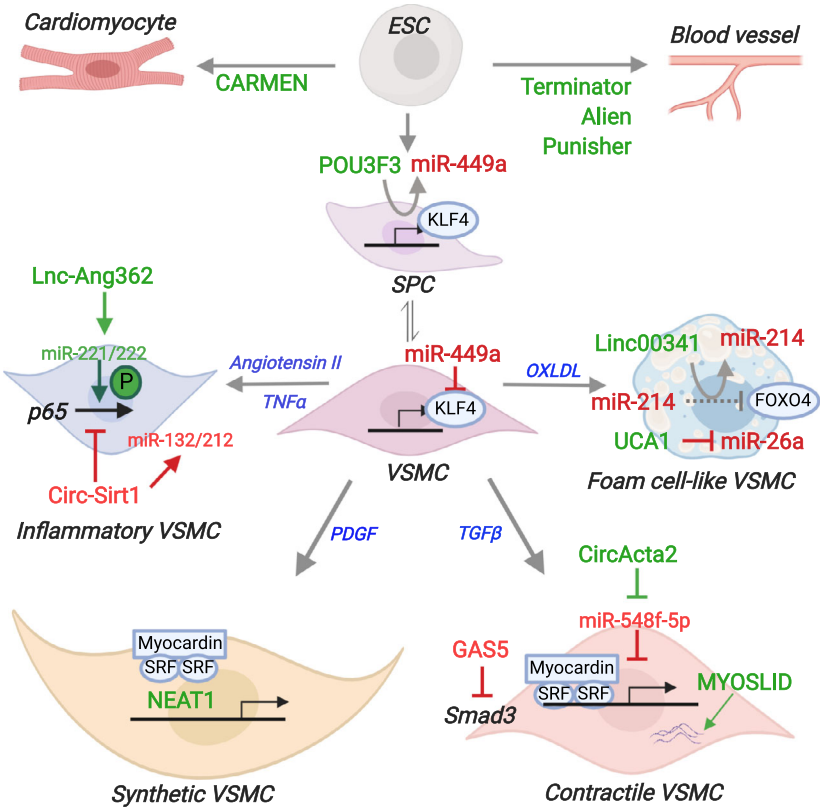

Fig. 3. Diagram representing newly discovered members of lncRNA and circRNA family involved in regulating VSMC phenotype commitment and specialisation under different external stimuli. VSMC phenotype is regulated by different ncRNAs during specialisation towards a 'pro-inflammatory' phenotype (Lnc-Ang362, CirC-Sirt1), 'synthetic' phenotype (NEAT1), 'contractile' phenotype (GAS5, CircActa2, MYOSLID) and 'foam cell-like' phenotype (Linc00341, UCA1). VSMCs can also adopt a SPC phenotype under KLF4 regulation, itself regulated by lncRNA POU3F3. Finally, lncRNA CARMEN is required for cardiomyocyte lineage specification, and Terminator, Alien and Punisher are each required for different stages of cardiovascular development. NcRNAs in green and red highlight ncRNAs that promote and inhibit the relevant molecular pathway, respectively. NcRNA, noncoding RNA; lncRNA, long noncoding RNA; circRNA, circular RNA; ESC, embryonic stem cell; SPC, stem/progenitor cell; KLF4, Krüppel-like factor 4. This diagram was created with Biorender.com.

revealed the merit of targeting ncRNAs involved in subsequent events, which determine the long-term survival of VSMCs in the ever-growing neointima (Table 4 and Fig. 5). Indeed, several miRs have been found to play a key role in regulating VSMC apoptosis including miR-210, miR-21 and miR-26a. Using human carotid artery SMCs, miR-210 expression was shown to prevent VSMC apoptosis in human carotid artery SMCs by directly targeting the tumour suppressor gene and adenomatous polyposis coli [142], thereby providing a novel therapeutic target that could prevent late-stage VSMC apoptosis responsible for fibrous cap rupture. MiR-21 was also found to have a protective role in preventing VSMC apoptosis, promoting cell proliferation and preventing dedifferentiation, by targeting PTEN and B-cell lymphoma 2 (Bcl-2) to induce downregulation and upregulation of its target mRNAs, respectively [143]. Similarly, miR-26a was also shown to target PTEN against H_2O_2-induced apoptosis, thereby conferring protection through activation of the AKT/mammalian target of rapamycin (mTOR) pathway [144].

An important role for PTEN in VSMC function has previously been investigated. One study showed that expression of PTEN increases in apoptotic VSMCs 12 h following balloon injury in rat carotid arteries. Importantly, overexpression of PTEN prevented Akt phosphorylation, resulting in increased VSMC apoptosis [145]. Conversely, PTEN overexpression was found to suppress PDGF-induced VSMC proliferation [146] and angiotensin II-induced VSMC proliferation and migration [147]. Given PTEN's instrumental role in regulating VSMC apoptosis, proliferation and migration, VSMC-specific ncRNAs, capable of manipulating PTEN activity, present a useful means of targeting VSMC behaviour. More recently, the circular RNA and miRNA sponge, circSLC8A1, was found to modulate PTEN activity by sponging miR130b/miR-494 to suppress progression of bladder cancer cells [148].

Studies have detected the expression of miR-130b in murine embryonic stem cell cultures as well as adult tissues [149]. Importantly, miR-494 was found to have a proliferative role in human coronary artery SMCs, with overexpression resulting in reduced proliferation of murine SMCs and attenuated neointimal formation following femoral arterial wire injury [150]. Whether the circSLC8A1/miR-130b/miR-494/PTEN axis exists in VSMCs, and exerts a similar effect as seen in cancer cell lines, has yet to be determined. Nevertheless,

Table 3. Summary of ncRNA family involved in regulating VSMC proliferation and migration, expression levels under different external stimuli and/or CVD pathologies and their molecular targets. ND, not determine; '↑' and '↓' indicate up- and downregulation, respectively; '+' and '−' represent 'promoting' and 'inhibiting', respectively.

ncRNA	Expression following stimulus and/or in CVD	Promotes/Inhibits proliferation (+/−)	Promotes/Inhibits migration (+/−)	Target	Ref
miR-145	↑ (TGFβ) ↓ (PDGF-BB) ↓(hypoxia) ↓ (aneurysm) ↓ (murine atherosclerosis) ↑ (murine hypertension)	−	−	KLF4 Myocardin ELK-1	[72,131]
circ-LRP6	↓ (TGFβ) ↓ (PDGF-BB) No change (hypoxia) No change (aneurysm) No change (murine atherosclerosis) No change (murine hypertension)	+ (TGFβ)	+ (TGFβ)	miR-145	[131]
LncRNA-SRA	↑ (mouse model ISR)	+	+	MEK/ERK/CREB	[132]
BANCR	↑ (TNF-α)	+	+	Jnk	[133]
Giver	↑ (angiotensin II)	+ (Angiotensin II)	ND	NONO	[134]
SMILR	↓ (unstable atherosclerotic plaques)	+ (PDGF, IL1-α)	ND	CENPF	[207,208]
lnc-RNCR3	↑ox-LDL treatment ↑ (human atherosclerotic lesions)	+	+	miR-185-5p (EC)	[138]
lncRNA-430945	↑(human atherosclerotic lesions)	+ (Angiotensin II)	+	receptor tyrosine kinase-like orphan receptor 2 (ROR2	[137]
lnc-00113	↑(human atherosclerosis	+	−	PI3K/Akt/mTOR (EC)	[139]
GAS5	↓(Hypertension)	− (VSMC and EC)	− (VSMC and EC)	β-Catenin nuclear translocation	[141]

research conducted thus far presents a compelling case for the exploration of this pathway in VSMCs, which may prove useful in redefining the rates of VSMC proliferation and apoptosis during neointimal formation.

Several other ncRNAs have also been implicated in the regulation of VSMC apoptosis pathways, most notably through the p53 pathway, an important tumour suppressor pathway responsible for triggering cell cycle arrest and apoptosis. One study showed that Linc-p21, a lncRNA transcribed upstream of a critical cell cycle regulator, *Cdkn1a* [151], acts as a downstream repressor of p53 target genes by binding to hnRNP-K and guiding it to target genes destined for transcriptional repression [152]. Moreover, Linc-p21 was found to form a positive feedback loop, further promoting p53 transcriptional activity by preventing ubiquitin–proteasome degradation by binding to mouse double minute 2 (MDM2), an E3 ubiquitin–protein ligase in human aortic VSMCs. Importantly, Linc-p21 expression was found to be significantly downregulated in atherosclerotic plaques of $ApoE^{-/-}$ mice as well as patients with coronary heart disease.

As mentioned previously, GAS5 plays an important role in preventing TGFβ-induced SMC differentiation [93] and becomes downregulated under PDGF-BB stimulation [140]. It is associated with high blood pressure and has been shown to play a key role in VSMC apoptosis [141]. Knockdown of GAS5 in VSMCs was found to not only protect against H_2O_2-induced apoptosis but also accelerate VSMC proliferation and migration, and promote dedifferentiation towards a synthetic phenotype.

The known lncRNA molecule, ANRIL, transcribed from the CVD risk locus on chromosome 9p21, was found capable of forming a circular RNA molecule, circ-ANRIL. Despite the finding that a high circ-ANRIL to ANRIL ratio was associated with a lower risk of CAD in patients, overexpression of circ-ANRIL in HEK293 cells and human primary SMCs was found to promote cell apoptosis and prevent cell proliferation through p53 activation. circ-ANRIL was also found to bind to RNA binding proteins required for ribosomal assembly complex and RNA splicing [153]. Finally, another ncRNA associated with VSMC

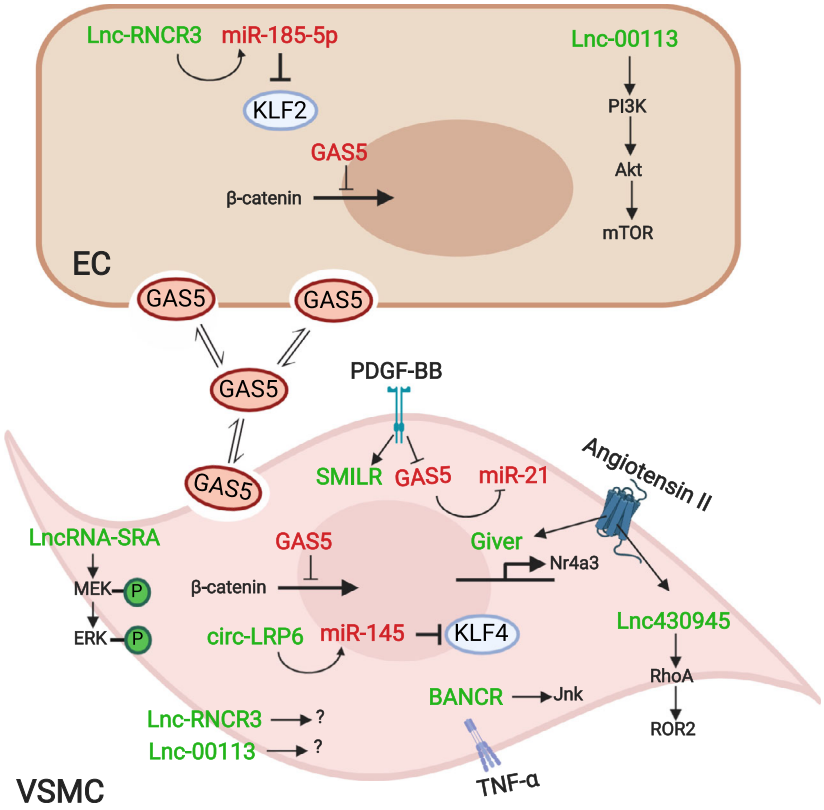

Fig. 4. Noncoding RNAs govern signalling pathways required for VSMC and EC proliferation and/or migration. Different pro-inflammatory stimuli (PDGF-BB, angiotensin II, TNF-α) triggers upregulation of ncRNAs (SMILR, Lnc430945 and Giver, BANCR). LncRNA-SRA promotes activation of the MEK/ERK/CREB pathway. Meanwhile, Lnc-RNCR3 and Lnc-00113 are upregulated in proliferative ECs and VSMCs, with their molecular signalling pathways delineated in ECs only. Finally, GAS5-mediated VSMC-EC cross-talking controls both VSMC and EC proliferation and migration by affecting β-catenin nuclear translocation. NcRNAs in green and red promote and inhibit relevant molecular pathway, respectively. '?' denotes unknown pathway. NcRNA, noncoding RNA; lncRNA, long noncoding RNA; EC, endothelial cell; VSMC, vascular smooth muscle cell; KLF2, Krüppel-like factor. This diagram was created with Biorender.com.

apoptosis has been investigated in the context of thoracic aortic aneurysms. LncRNA, HIF 1α-antisense RNA 1 (HIF1α-AS1), is upregulated in patients with aneurysms and promotes apoptosis by regulating the expression of caspases 3 and 8, and Bcl2 proteins [154].

Often considered two sides of the same coin, VSMC apoptosis and proliferation are frequently regulated by the same ncRNA molecule. As these two vital events can determine the rate of neointimal thickening, ncRNAs targeting both may significantly improve our chances of tackling pathological remodelling of the vessel wall.

Noncoding RNAs as biomarkers for MI and CAD

Numerous ncRNAs have been found to play key role in VSMC biology and vascular pathology. However, in the absence of any concrete methods for targeting these ncRNA in vascular disease, other clinical uses for these ncRNAs have been put forward, including their suitability as CVD biomarkers. Several ncRNAs have been identified as useful predictors of MI, the culminating event of neointimal formation whereby the advanced atherosclerotic plaque has either ruptured or encroached into the blood vessel significantly enough to require revascularisation [155]. MiRs, miR-1, miR-133, miR-208 and miR-499, were shown to be upregulated in the serum of patients following acute MI [156]. Elevated levels of lncRNAs, HIF1α-AS1, member 1 opposite strand/antisense transcript 1 and mitochondrial long noncoding RNA uc022bqs.1 (LIPCAR), were also positively correlated with MI. Moreover, LIPCAR upregulation was found to have the greatest predicative ability for patients with ST-segment elevation myocardial infarction (STEMI) [157] and was found to be associated with left ventricular remodelling and heart failure [158]. On the other hand, circRNA_081881 was significantly downregulated in the plasma samples of acute MI patients, which appeared to target PPARγ expression in macrophages to prevent foam cell formation [159]. Another CVD pathology whereby VSMC function plays an important role is in CAD. Reduced expression of platelet-derived miRs, miR-126 and mir-199, was also associated with CAD [160]. Unfortunately, miR-126 findings were not replicable [161]. The lncRNA, ANRIL and circ-ANRIL precursor, is another known biomarker for CAD in patients with type II diabetes [162]. More pertinently, elevated plasma expression levels of ANRIL are increased in patients with ISR [163], and high levels of circ-ANRIL are

Table 4. Summary of ncRNAs involved in regulating VSMC apoptosis and survival in neointimal hyperplasia, their expression in CVD and molecular targets. '↑' and '↓' indicate up- and downregulation, respectively; '+' and '−' represent 'promoting' and 'inhibiting', respectively.

ncRNA	Expression in CVD	Promotes/Inhibits (+/−)	Target	Ref
miR-21	↑	−	PTEN Bcl2	[143,209]
miR-26a	↓	−	PTEN	[144]
miR-210	↑ (human atherosclerosis) ↓ (advanced atherosclerosis)	−	adenomatous polyposis coli	[210,211]
circ-ANRIL	↓(high CAD burden)	+	P53	[153]
circSLC8A1 (bladder cancer cells)	*N/A*	+	*miR-130b/miR-494*	[148]
Linc-p21	↓ (murine atherosclerosis)	+	hnRNP-K MDM2	[152]
GAS5	↓(murine microvascular dysfunction & arterial hypertension)	+ (H_2O_2)	β-catenin nuclear translocation	[141]
HIF1α-AS1	↑ (TAA patients)	+	Caspase 3 & 8 Bcl2	[154]

associated with less severe CAD due in part to its atheroprotective role [164]. Nine other circRNAs (circ_0089378, circ_0083357, circ_0082824, circ_0068942, circ_0057576, circ_0054537, circ_0051172, circ_0032970 and circ_0006323) were differentially expressed in CAD patients [165], and their mechanism of action appeared to converge on the miR-130a-3p responsible for modulating expression of transient receptor potential cation channel subfamily M member 3, which regulates VSMC contractility and proliferation [166]. However, due to a small samples size, the significance of these circRNAs requires further investigation.

The role of miRs as biomarkers in various CVDs is well established [167]. However, conflicting reports of miRs expression levels in pathology, as seen with miR-210 (Table 4), present an important difficulty in assessing their predictive value [52]. Studies have also been carried out to examine lncRNA biomarker potential; however, due to their low detectability and sporadic expression levels, they may not prove as useful as other ncRNAs [168]. CircRNAs are more abundant, have greater cytoplasmic accessibility and are more stable within the body [169]. As such, circRNAs present the most potential for monitoring and detecting CVD development and pathology, and future studies should attempt to not only delineate their signalling network and cellular function, but also seek to determine any predictive value they may present in the diagnosis and treatment of CVDs.

Future perspectives and conclusion

As evidenced by the latest research in ncRNA biology, numerous avenues are available to modulate VSMC presence and behaviour in the neointima (Fig. 6). With the emergence of newer sequencing technologies for identifying and characterising complex ncRNA molecules and their targets, identifying unique molecular signalling pathways governing VSMCs during neointimal formation have become easier to unravel [170,171]. Despite this, novel treatment strategies targeting these ncRNAs directly are sorely lacking. Moreover, lncRNAs present additional difficulties as future therapeutic targets due to their low evolutionary conservation, resulting in an absence of murine homologues through which to test their therapeutic potential [172].

Fortunately, our understanding of ncRNAs in cancer therapy resistance has shed a useful light on a complex disease, and as such has spurred the development of specialised nanotechnologies and RNA-guided precision medicine [173], which may pave the way for future CVD treatment. However, the evident overlap of ncRNAs, which regulate VSMC proliferation and apoptosis as well as cancer cell proliferation and invasion, presents an additional tumorigenic risk for these molecules in the treatment of vascular disease.

As it stands, the less invasive use of ncRNAs as biomarkers provides a compelling therapeutic tool for CVD, and genetic variation in the coding regions of several ncRNAs has revealed important associations for CVD risk [153,174-176]. As ncRNA signalling networks that govern VSMC phenotype, apoptosis, proliferation and migration become mapped out over time, more techniques will develop to harness the potential of ncRNAs and provide new ways of fine-tuning the vascular microenvironment with the aim of preventing NIH, and its adverse outcomes.

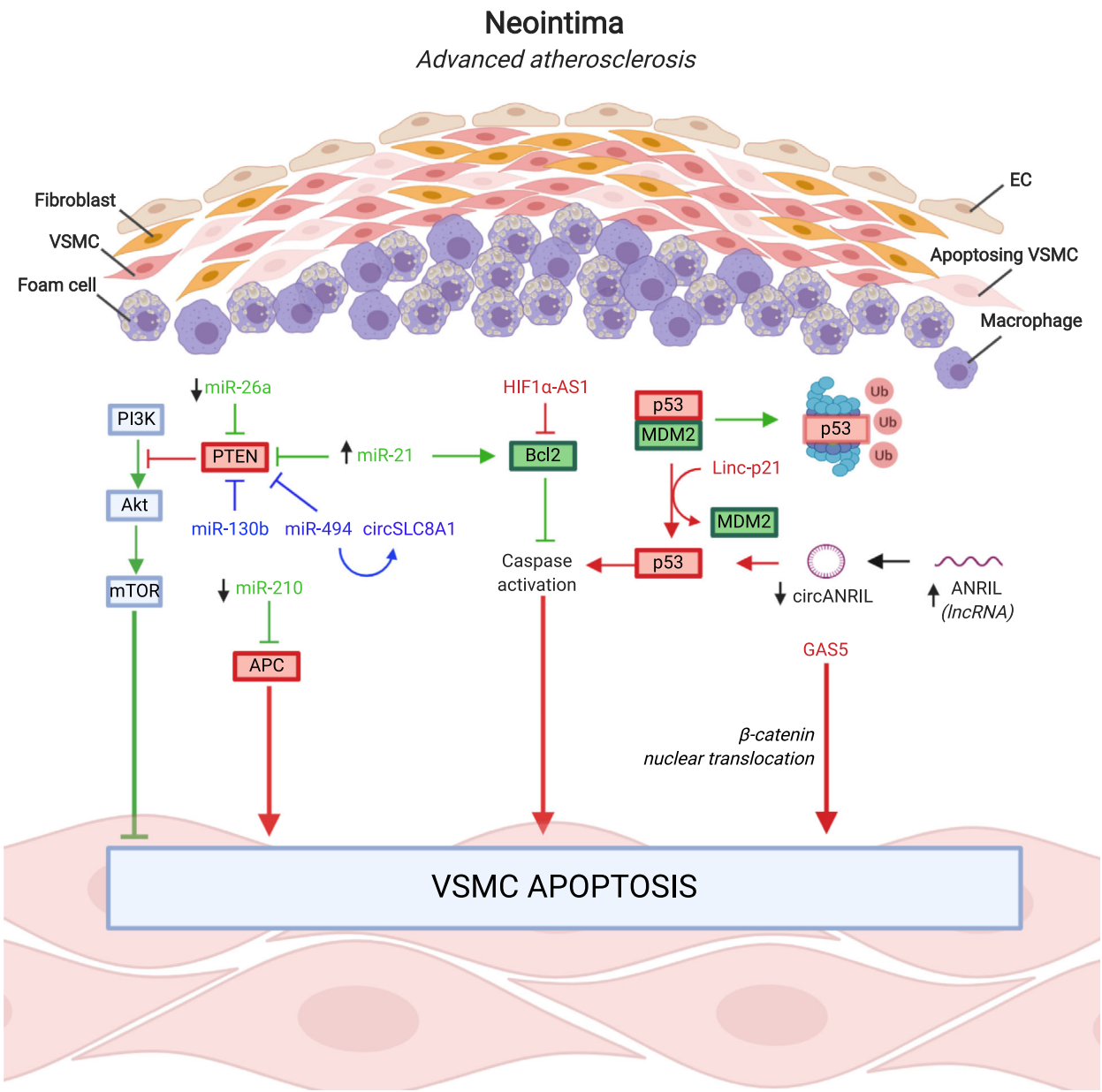

Fig. 5. Noncoding RNA regulators of VSMC apoptosis and survival pathways. Several miRs regulate PTEN expression in VSMCs to target activity of the PI3K/Akt/mTOR pathway (miR-26a, miR-21). Other ncRNAs (blue) have been shown to modulate PTEN activity and apoptosis in bladder cancer cells (miR-130b, miR-494, circSLC8A1). NcRNAs also regulate caspase activation, either through Bcl2-mediated regulation (miR-21, HIF1α-AS1), or through the tumour suppressor, p53 (Linc-p21, circ-ANRIL). Interference of β-catenin nuclear translocation is dependent on GAS5, which promotes VSMC apoptosis or inhibits VSMC viability. Black arrows indicate ncRNA expression levels in the neointima. Green designates anti-apoptotic/pro-proliferative pathways, whereas red designates pro-apoptotic/antiproliferative pathways. NcRNA, noncoding RNA; miRs, microRNAs; PTEN, phosphatase and tensin homolog; EC, endothelial cell; VSMC, vascular smooth muscle cell. This diagram was created with Biorender.com.

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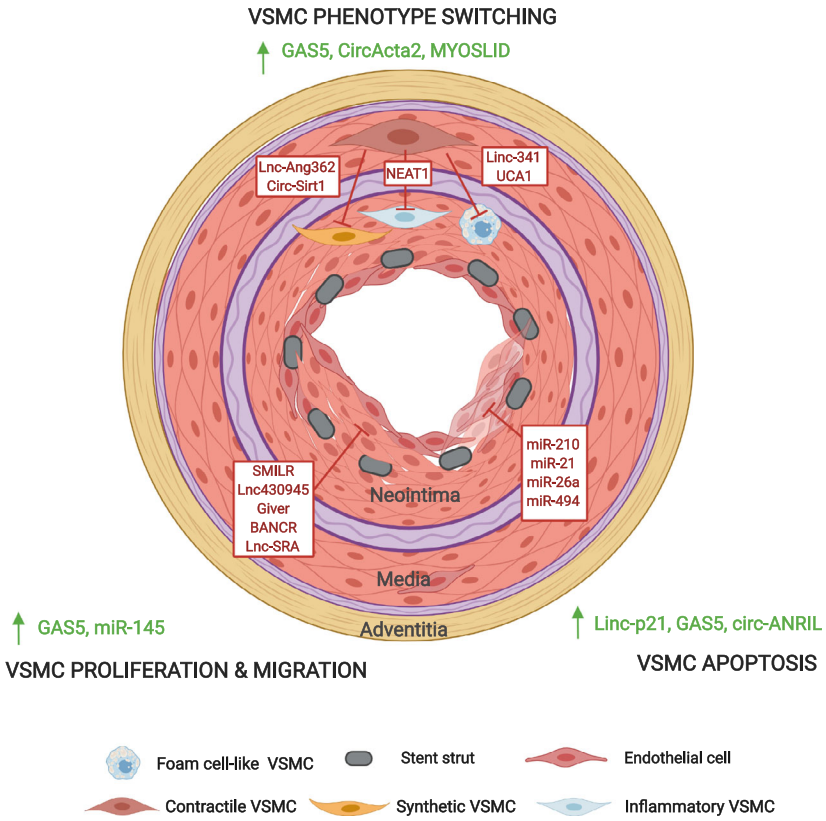

Fig. 6. Future ncRNA-based potential methods for ISR prevention. NIH can be attenuated by targeting different aspects of VSMC biology: phenotype switching, proliferation, migration and apoptosis. Upregulation of GAS5, CircACTA2 and MYOSLID will promote differentiation towards a VSMC contractile phenotype. Whereas downregulation of other ncRNAs will prevent dedifferentiation of the VSMC phenotype towards a synthetic phenotype (Lnc-Ang362 and Circ-Sirt1), an inflammatory phenotype (NEAT1) and a foam cell-like phenotype (Linc-341 and UCA1). Upregulation of GAS5 and miR-145 and/or downregulation of SMILR, Lnc430945, Giver, BANCR and Lnc-SRA will prevent VSMC proliferation and migration. Finally, upregulation of Linc-p21, GAS5 and circ-ANRIL and/or downregulation of miR-210, miR-21, miR-26a and miR-494 will promote VSMC apoptosis and slow NIH. Green arrows denote upregulation of ncRNAs, and red (closed) arrows denote downregulation of pathways required to prevent NIH. ISR, in-stent restenosis; NIH, neointimal hyperplasia; NcRNA, noncoding RNA; VSMC, vascular smooth muscle cell. This diagram was created with Biorender.com.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

EMM involved in the original draft and revision. QX performed the design, supervision and critical revision.

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