Noncoding RNAs in vascular smooth muscle cell function and neointimal hyperplasia

Eithne Margaret Maguire¹ and Qingzhong Xiao¹,²

1 Centre for Clinical Pharmacology, William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, UK

2 Key Laboratory of Cardiovascular Diseases at The Second Affiliated Hospital, Guangzhou Municipal and Guangdong Provincial Key Laboratory of Protein Modification and Degradation, School of Basic Medical Sciences, Guangzhou Medical University, China

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 (Noncoding 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, IncRNA-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.
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 ≥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, non-coding 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.
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 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 IncRNA 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 IncRNAs have been found to interact with promoter regions of SMC genes including growth arrest specific 5 (GASS), which was shown to prevent TGFβ-induced SMC differentiation of VSMCs by blocking Smad3 activity via RNA Smad-binding elements [93]. The IncRNA, 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, IncRNA 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 TGFβ 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                                | [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)        | ND        | Tribbles-like protein 3             | [143]       |
|                 |                                             |                                                | (under adenoviral miR-24 overexpression in vivo) |           | Wnt4/Dvl-1/β-catenin signalling pathway | [181]       |
| miR-26a         | ↓                                           | Promotes PDGF-induced synthetic phenotype     | (PDGF)        | +         | Smad1, 4                            | [182],[183] |
|                 |                                             |                                                | (with miR-26a agomir) |           | Mitogen-activated protein kinase 6  | [164]       |
| miR-29b         | ND                                          | Promotes PDGF-induced synthetic phenotype     | ND            | ND        | SIRT1                               | [185]       |
| miR-31          | ↑                                           | ND                                            | +             | ND        | Large tumour                        | [186]       |
| miR-34a         | ↓                                           | ND                                            | −             | −         | Notch1                              | [79]        |
| miR-124         | ↓                                           | ND                                            | −             | −         | IGFAP1                              | [187]       |
| miR-133         | ↑                                           | Prevents PDGF-induced synthetic phenotype in vitro and in vivo | (10% FBS & PDGF-BB) | − (10% FBS) | Sp-1                                | [188]       |
| miR-137         | ND                                          | Prevents PDGF-induced synthetic phenotype in vitro and in vivo | (PDGF-BB)     | −         | IGFBP-5                             | [189]       |
| miR143/145      | ↓                                           | ND                                            | (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) | +         | Kruppel-like factor 4              | [143,192,193]|
| miR-195         | ↓                                           | ND                                            | (ox-LDL)      | (ox-LDL) | Cdc42, cyclin D1                    | [194]       |
| miR-204         | ↑                                           | ND                                            | (PDGF-BB, high glucose) | ND        | fibroblast growth factor 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)     | −         | STIM1, calumenin, cyclin D1         | [199]       |
| miRNA-503       | ↑                                           | ND                                            | (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)     | −         | JunB                                | [203]       |
| miR-688         | ND                                          | Promotes contractile phenotype                | (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 IkBz [105].

Under ox-LDL exposure, expression of lncRNA LINCO0341 was significantly increased, resulting in enhanced VSMC proliferation and migration. Interestingly, cytoplasmic LINCO0341 acted as an endogenous sponge for miR-221 and miR-222, upregulation of LINCO0341 led to increased expression of miR-221 and miR-222, which in turn increased phosphorylation of NFκB proteins, p65 and IkBz [105].

VSMCs versus stem/progenitor cells

A recent study revealed the ability of a subpopulation of VSMCs to dedifferentiate into Scal+/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].
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 proliferation and migration

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

---

**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- | Angiotensin II (pulmonary arterial hypertension patients) | Inflammatory | + | miR-221/222 | [105] |
| Ang362 | II | | | | | |
| NEAT-1 | PDGF-BB | ↑ (PDGF-BB) | Synthetic | + | WDR5 | [94] |
| POU3F3 | N/A | ↑ (in PCI patients with ISR) | Stem cell | + | KLF4 | [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 | [206] |
| CircActa2 | TGFβ | ↑ (TGFβ) | Contractile | + | miR-548f-5p | [96,97] |

---

The FEBS Journal 287 (2020) 5260–5283 © 2020 The Authors. The FEBS Journal published by John Wiley & Sons Ltd on behalf of Federation of European Biochemical Societies
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 H₂O₂-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)                                   | −                                    | −                               | KLF4                    | [72,131] |
|               | ↓ (PDGF-BB)                                | −                                    | −                               | Myocardin               |      |
|               | ↓ (hypoxia)                                | −                                    | −                               | ELK-1                   |      |
|               | ↓ (aneurysm)                               | −                                    | −                               |                         |      |
|               | ◂ (murine atherosclerosis)                 | −                                    | −                               |                         |      |
|               | ◂ (murine hypertension)                    | −                                    | −                               |                         |      |
| circ-LRP6     | ◂ (TGFβ)                                   | + (TGFβ)                            | + (TGFβ)                        | miR-145                 | [131] |
|               | ◂ (PDGF-BB)                                | No change (hypoxia)                  | No change (aneurysm)            |                         |      |
|               | No change (murine atherosclerosis)         | No change (murine hypertension)      | No change (murine hypertension) |                         |      |
| LncRNA-SRA    | ↑ (mouse model ISR)                       | +                                    | +                               | MEK/ERK/CREB            | [132] |
| BANCR         | ↑ (TNF-α)                                  | +                                    | +                               | Jnk                     | [133] |
| Giver         | ↑ (angiostatin II)                         | + (Angiostatin II)                   | ND                              | NO/NO                   | [134] |
| SMILR         | ◂ (unstable atherosclerotic plaques)       | + (PDGF, IL1-α)                     | ND                              | CENPF                   | [207,208] |
| Lnc-RNCR3     | ◂ ox-LDL treatment                         | +                                    | +                               | miR-185-5p (EC)         | [136] |
| LncRNA-430945 | ◂ (human atherosclerotic lesions)          | + (Angiostatin II)                   | ND                              |                         |      |
| Lnc-00113     | ≤ (human atherosclerosis)                 | +                                    | −                               | PI3K/Akt/mTOR (EC)      | [139] |
| GAS5          | ◂ (Hypertension)                           | −                                    | −                               | β-Catenin nuclear translocation | [141] |

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 H2O2-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...
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 predictive 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 PPAR 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...
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 miR 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.

Acknowledgements

This work was supported by the British Heart Foundation (PG/15/11/31279, PG/15/86/31723 and PG/16/1/31892). This work forms part of the research portfolio for the National Institute for Health Research Biomedical Research Centre at Barts.
**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.

**References**

1 Purcell C, Tennant M & McGeachie J (1997) Neointimal hyperplasia in vascular grafts and its implications for autologous arterial grafting. *Ann R Coll Surg Engl* 79, 164–168.

2 Tennant M, Dilley RJ, McGeachie JK & Prendergast FJ (1990) Histogenesis of arterial intimal hyperplasia and atherosclerosis. *Austral New Zealand J Surg* 60, 79–85.

3 Clyman RI, Chan CY, Mauray F, Chen YQ, Cox W, Seidner SR, Lord EM, Weiss H, Waleh N, Evans SM & *et al.* (1999) Permanent anatomic closure of the ductus arteriosus in newborn baboons: the roles of postnatal constriction, hypoxia, and gestation. *Pediatr Res* 45, 19–29.

4 Waleh N, Seidner S, McCurnin D, Giavedoni L, Hodara V, Goelz S, Liu BM, Roman C & Clyman RI (2011) Anatomic closure of the premature patent ductus arteriosus: The role of CD14+/CD163+ mononuclear cells and VEGF in neointimal mound formation. *Pediatr Res* 70, 332–338.

5 Kijani S, Vazquez AM, Levin M, Boren J & Fogelstrand P (2017) Intimal hyperplasia induced by...
vascular intervention causes lipoprotein retention and accelerated atherosclerosis. *Physiol Rep* 8, e13334.
6 Dobrin PB, Littooy FN & Endean ED (1989) Mechanical factors predisposing to intimal hyperplasia and medial thickening in autogenous vein grafts. *Surgery* 105, 393–400.
7 Desai M, Mirzay-Razzaz J, von Delft D, Sarkar S, Hamilton G & Seifalian AM (2010) Inhibition of neointimal formation and hyperplasia in vein grafts by external stent/sheath. *Vasc Med (London, England)* 15, 287–297.
8 Bauriedel G, Hutter R, Welsch U, Bach R, Sievert H & Luderitz B (1999) Role of smooth muscle cell death in advanced coronary primary lesions: implications for plaque instability. *Cardiovasc Res* 41, 480–488.
9 Lacolley P, Regnault V, Nicoletti A, Li Z & Michel JB (2012) The vascular smooth muscle cell in arterial pathology: a cell that can take on multiple roles. *Cardiovasc Res* 95, 194–204.
10 Leimgruber PP, Roubin GS, Hollman J, Cotsonis GA, Dobrin PB, Littooy FN & Endean ED (1989) Importance of monocyte chemoattractant protein-1 pathway in neointimal hyperplasia after periarterial injury in mice and monkeys. *Circ Res* 90, 1167–1172.
11 Kozinski M, Krzewina-Kowalska A, Kubica J, Zbikowska-Gotz M, Dynek G, Piasecki R, Sukiennik A, Grzesk G, Bogdan M, Chojnicki M et al. (2005) Percutaneous coronary intervention triggers a systemic inflammatory response in patients treated for in-stent restenosis – comparison with stable and unstable angina. *Inflamm Res* 54, 187–193.
12 Rectenwald JE, Moldawer LL, Huber TS, Seeger JM & Ozaki CK (2000) Direct evidence for cytokine involvement in neointimal hyperplasia. *Circulation* 102, 1697–1702.
13 Levine GN, Chodos AP & Loscalzo J (1995) Restenosis after balloon angioplasty in patients with single-vessel disease. *Circulation* 73, 710–717.
14 Nili N, Cheema AN, Giordano FJ, Barolet AW, Francis SE, Hunter S, Holt CM, Gadsdon PA, Rogers Goetze S, Kintscher U, Kaneshiro K, Meehan WP, Collins A, Fleck E, Hsueh WA & Law RE (2001) TNFalpha induces expression of transcription factors c-fos, Egr-1, and Ets-1 in vascular lesions through extracellular signal-regulated kinases 1/2. *Atherosclerosis* 159, 93–101.
NcRNAs in VSMC function and neointimal hyperplasia

E. M. Maguire and Q. Xiao

5276

The FEBS Journal 287 (2020) 5260–5283 © 2020 The Authors. The FEBS Journal published by John Wiley & Sons Ltd on behalf of Federation of European Biochemical Societies

grafts in ApoE-deficient mice. J Clin Investig 113, 1258–1265.
28 Kramar R, Goettsc H, Wonghoonsin J, Iwata H, Schneider RK, Kuppe C, Kaelser N, Chang-Panessa M, Machado FG, Gratwohl S et al. (2016) Adventitial MSC-like cells are progenitors of vascular smooth muscle cells and drive vascular calcification in chronic kidney disease. Cell Stem Cell 19, 628–642.
29 Bode C & Huber K (2008) Antiplatelet therapy in percutaneous coronary intervention. European Heart J Suppl. 10, A13–A20.
30 Ribichini F, Tomai F, Pesarini G, Zivelonghi C, Rognoni A, De Luca G, Bocuzzi G, Presbitero P, Ferrero V, Ghini AS et al. (2013) Long-term clinical follow-up of the multicentre, randomized study to test immunosuppressive therapy with oral prednisone for the prevention of restenosis after percutaneous coronary interventions: Cortisone plus BMS or DES versus BMS alone to Eliminate Restenosis (CEREAD). Eur Heart J 34, 1740–1748.
31 Walter DH, Fichtlscherer S, Britten MB, Rosin P, Auch-Schwelk W, Schächinger V & Zeiher AM (2001) Statin therapy, inflammation and recurrent coronary events in patients following coronary stent implantation. J Am Coll Cardiol 38, 2006–2012.
32 Liu J, Li M, Lu H, Qiao W, Xi D, Luo T, Xiong H & Guo Z (2015) Effects of probucol on restenosis after percutaneous coronary intervention: a systematic review and meta-analysis. PLOS ONE 10, e0124021.
33 Bahnsen ESM, Kassam HA, Moyer TJ, Bianco W, Morgan CE, Vercammen JM, Jiang Q, Flynn ME, Stupp SI & Kibbe MR (2016) Targeted nitric oxide delivery by supramolecular nanofibers for the prevention of restenosis after arterial injury. Antioxid Redox Signal 24, 401–418.
34 Bonaa KH, Mannsverk J, Wiseth R, Aaberge L, Myreng Y, Nygard O, Nilsen DW, Klow NE, Uchto M, Trovik T et al. (2016) Drug-eluting or bare-metal stents for coronary artery disease. New Engl J Med 375, 1242–1252.
35 Morice M-C, Serruys PW, Sousa JE, Fajadet J, Ban Hayashi E, Perin M, Colombo A, Schulz G, Barragan P, Guagliumi G et al. (2002) A randomized comparison of a sirolimus-eluting stent with a standard stent for coronary revascularization. N Engl J Med 346, 1773–1780.
36 Kelbaek H, Thuesen L, Helqvist S, Kløvgaard L, Jorgensen E, Aljabbari S, Saunamäki K, Krusell LR, Jensen GV, Botker HE et al. (2006) The Stenting Coronary Arteries in Non-stress/benestent Disease (SCANDSTENT) trial. J Am Coll Cardiol 47, 449–455.
37 Serruys PW, Orniston JA, Sianos G, Sousa JE, Grube E, den Heijer P, de Feyter P, Buszman P, Schömig A, Marco J et al. (2004) Actinomycin-eluting stent for coronary revascularization: a randomized feasibility and safety study: the ACTION trial. J Am Coll Cardiol 44, 1363–1367.
38 Grube E, Lansky A, Hauptmann KE, Di Mario C, Di Sciascio G, Colombo A, Silber S, Stumpf J, Reifart N, Fajadet J et al. (2004) High-dose 7-hexanoyltaxol-eluting stent with polymer sleeves for coronary revascularization: one-year results from the SCORE randomized trial. J Am Coll Cardiol 44, 1368–1372.
39 Garg S & Serruys PW (2010) Coronary stents: looking forward. J Am Coll Cardiol 56, S43–78.
40 Iglesias JF, Muller O, Heg D, Roffi M, Kurz DJ, Moaroof I, Weilemann D, Kaiser C, Tapponnier M, Stortecky S et al. (2019) Biodegradable polymer sirolimus-eluting stents versus durable polymer everolimus-eluting stents in patients with ST-segment elevation myocardial infarction (BIOSTEMI): a single-blind, prospective, randomised superiority trial. Lancet (London, England) 394, 1243–1253.
41 Katsaros KM, Kastl SP, Zorn G, Maurer G, Wojta J, Huber K, Christ G & Speidl WS (2010) Increased restenosis rate after implantation of drug-eluting stents in patients with elevated serum activity of matrix metalloproteinase-2 and -9. JACC Cardiovasc Interv 3, 90–97.
42 Byrne RA, Joner M & Kastrati A (2015) Stent thrombosis and restenosis: what have we learned and where are we going? The Andreas Gruntzig Lecture ESC 2014. Eur Heart J 36, 3320–3331.
43 Bagyura Z, Kiss L, Berta B, Szilágyi Á, Hirschberg K, Széplaki G, Lux Á, Szélid Z, Soós P & Merkely B (2017) High rate of in-stent restenosis after coronary intervention in carriers of the mutant mannose-binding lectin allele. BMC Cardiovasc Disord 17, 4.
44 Habib A & Finn AV (2016) Anti-proliferative drugs for restenosis prevention. Int Cardiol Clin 5, 321–329.
45 Lüscher TF, Steffel J, Eberli FR, Joner M, Nakazawa G, Tanner FC & Virmani R (2007) Drug-eluting stent and coronary thrombosis. Circulation 115, 1051–1058.
46 Leeper NJ & Maegdefessel L (2017) Non-coding RNAs: key regulators of smooth muscle cell fate in vascular disease. Cardiovasc Res 114, 611–621.
47 Das A, Samidurai A & Salloum FN (2018) Deciphering non-coding RNAs in cardiovascular health and disease. Front Cardiovasc Med 5, 73.
48 International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. Nature 431, 931–945.
49 da Silva DCP, Carneiro FD, de Almeida KC & Fernandes-Santos C (2018) Role of miRNAs on the pathophysiology of cardiovascular diseases. Arq Bras Cardiol 111, 738–746.
50 Fasolo F, Di Gregoli K, Maegdefessel L & Johnson JL (2019) Non-coding RNAs in cardiovascular cell
bially and atherosclerosis. *Cardiovasc Res* **115**, 1732–1756.

51 Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* **116**, 281–297.

52 Zhou S-S, Jin J-P, Wang J-Q, Zhang Z-G, Freedman JH, Zheng Y & Cai L (2018) miRNAs in cardiovascular diseases: potential biomarkers, therapeutic targets and challenges. *Acta Pharmacol Sin* **39**, 1073–1084.

53 Sallam T, Sandhu J & Tontonoz P (2018) Long noncoding RNA discovery in cardiovascular disease: decoding form to function. *Circ Res* **122**, 155–166.

54 Altesha MA, Ni T, Khan A, Liu K & Zheng X (2019) Circular RNA in cardiovascular disease. *J Cell Physiol* **234**, 5588–5600.

55 Wightman B, Ha I & Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. *Cell* **75**, 855–862.

56 Ha M & Kim VN (2014) Regulation of microRNA biogenesis. *Nat Rev Mol Cell Biol* **15**, 509–524.

57 Broughton JP, Lovci MT, Huang JL, Yeo GW & Pasquinelli AE (2016) Pairing beyond the seed directs HDAC3.

58 Macfarlane LA & Murphy PR (2010) MicroRNA: Biogenesis, function and role in cancer. *Curr Genom* **11**, 537–561.

59 Quinn JJ & Chang HY (2016) Unique features of long non-coding RNA biogenesis and function. *Nat Rev Genet* **17**, 47–62.

60 Dhanoo KJ, Sethi RS, Verma R, Arora JS & Mukhopadhyay CS (2018) Long non-coding RNA: its evolutionary relics and biological implications in mammals: a review. *J Animal Sci Technol* **60**, 25.

61 McHugh CA, Chen CK, Chow A, Surka CF, Tran C, McDonel P, Pandya-Jones A, Blanco M, Burghard C, Moradian A et al. (2015) The Xist IncRNA interacts directly with SHARP to silence transcription through HDAC3. *Nature* **521**, 232–236.

62 Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R & Willard HF (1991) A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. *Nature* **349**, 38–44.

63 Engelitz JM, Haines JE, Perez EM, Munson G, Chen J, Kane M, McDonel PE, Guttmann M & Lander ES (2016) Local regulation of gene expression by IncRNA promoters, transcription and splicing. *Nature* **539**, 452–455.

64 Atianand MK, Hu W, Satpathy AT, Shen Y, Ricci EP, Alvarez-Dominguez JR, Bhatta A, Schattgen SA, McGowan JD, Blii J et al. (2016) A long noncoding RNA IncRNA-EPS acts as a transcriptional brake to restrain inflammation. *Cell* **165**, 1672–1685.

65 Carrieri C, Cinamiti L, Biagioli M, Beugnet A, Zucchelli S, Fedele S, Pesce E, Ferrer I, Collavin L, Santoro C et al. (2012) Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. *Nature* **491**, 454–457.

66 Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK & Kjems J (2013) Natural RNA circles function as efficient microRNA sponges. *Nature* **495**, 384–388.

67 Tichon A, Gil N, Lubelsky Y, Havkin Solomon T, Lemze D, Itzkovitz S, Stern-Ginossar N & Ulitsky I (2016) A conserved abundant cytoplasmic long noncoding RNA modulates repression by Pumilio proteins in human cells. *Nat Commun* **7**, 12209.

68 Yap K, Mukhina S, Zhang G, Tan JSC, Ong HS & Makeyev EV (2018) A short tandem repeat-enriched RNA assembles a nuclear compartment to control alternative splicing and promote cell survival. *Mol Cell* **72**, 525–540.e13.

69 Eulalio A, Huntzinger E, Nishihara T, Rehwinkel J, Fauser M & Izaurralde E (2009) Deadenylation is a widespread effect of miRNA regulation. *RNA* **15**, 21–32.

70 Djuranovic S, Nahvi A & Green R (2012) miRNA-mediated gene silencing by translational repression followed by mRNA deadenylation and decay. *Science (New York, NY)* **336**, 237–240.

71 Li Y, Zheng Q, Bao C, Li S, Guo W, Zhao J, Chen D, Gu J, He X & Huang S (2015) Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. *Cell Res* **25**, 981–984.

72 Cordes KR, Sheehy NT, White MP, Berry EC, Morton SU, Muth AN, Lee TH, Miano JM, Ivey KN & Srivastava D (2009) miR-145 and miR-143 regulate smooth muscle cell fate and plasticity. *Nature* **460**, 705–710.

73 Klattenhoff CA, Scheuermann JC, Surface LE, Bradley RK, Fields PA, Steinhauser ML, Ding H, Butty VL, Torrey L, Haas S et al. (2013) Brakeheart, a long noncoding RNA required for cardiovascular lineage commitment. *Cell* **152**, 570–583.

74 Boeckel JN, Jae N, Heumuller AW, Chen W, Boon RA, Stellos K, Zeiher AM, John D, Uchida S & Dimmeler S (2015) Identification and characterization of hypoxia-regulated endothelial circular RNA. *Circ Res* **117**, 884–890.

75 Sayed AS, Xia K, Li F, Deng X, Salma U, Li T, Deng H, Yang D, Haoyang Z, Yang T & et al. (2015) The diagnostic value of circulating microRNAs for middle-aged (40–60-year-old) coronary artery disease patients. *Clinics (Sao Paulo, Brazil)* **70**, 257–263.

76 Cai Y, Yang Y, Chen X, Wu G, Zhang X, Liu Y, Yu J, Wang X, Fu J, Li C et al. (2016) Circulating 'IncRNA OTTHUMT0000387022' from monocytes as a novel biomarker for coronary artery disease. *Cardiovasc Res* **112**, 714–724.
NcRNAs in VSMC function and neointimal hyperplasia

E. M. Maguire and Q. Xiao

77 Wang L, Shen C, Wang Y, Zou T, Zhu H, Lu X, Li L, Yang B, Chen J, Chen S et al. (2019) Identification of circular RNA Hsa_circ_0001879 and Hsa_circ_0004104 as novel biomarkers for coronary artery disease. Atherosclerosis 286, 88–96.

78 Yang F, Chen Q, He S, Yang M, Maguire EM, An W, Afzal TA, Luong LA, Zhang L & Xiao Q (2018) miR-22 is a novel mediator of vascular smooth muscle cell phenotypic modulation and neointima formation. Circulation 137, 1824–1841.

79 Chen Q, Yang F, Guo M, Wen G, Zhang C, Luong LA, Zhu J, Xiao Q & Zhang L (2015) miRNA-34a reduces neointima formation through inhibiting smooth muscle cell proliferation and migration. J Mol Cell Cardiol 89, 75–86.

80 Yu X, Zhang L, Wen G, Zhao H, Luong LA, Chen Q, Huang Y, Zhu J, Ye S, Xu Q et al. (2015) Upregulated sirtuin 1 by miRNA-34a is required for smooth muscle cell differentiation from pluripotent stem cells. Cell Death Differ 22, 1170–1180.

81 Chang T-C, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, Feldmann G, Yamakuchi M, Ferlito M, Lowenstein CJ et al. (2007) Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Mol Cell 26, 745–752.

82 Badi I, Burba I, Ruggeri C, Zeni F, Bertolotti M, Scopece A, Pompilio G & Raucci A (2014) MicroRNA-34a induces vascular smooth muscle cells senescence by SIRT1 downregulation and promotes the expression of age-associated pro-inflammatory secretory factors. J Gerontol Series A 70, 1304–1311.

83 Wang H, Wang F, Wang X, Wu X, Xu F, Wang K, Xiao M & Jin X (2019) Friend or foe: a cancer suppressor microRNA-34 potentially plays an adverse role in vascular diseases by regulating cell apoptosis and extracellular matrix degradation. Med Sci Monit 25, 1952–1959.

84 Kang H & Hata A (2012) MicroRNA regulation of smooth muscle gene expression and phenotype. Curr Opin Hematol 19, 224–231.

85 Ballantyne MD, McDonald RA & Baker AH (2016) lncRNA/MicroRNA interactions in the vasculature. Clin Pharmacol Ther 99, 494–501.

86 Johnson JL (2019) Elucidating the contributory role of microRNA to cardiovascular diseases (a review). Vascular Pharmacol 114, 31–48.

87 Fiedler J, Baker AH, Dimmeler S, Heymans S, Mayr M & Thum T (2018) Non-coding RNAs in vascular disease – from basic science to clinical applications: scientific update from the Working Group of Myocardial Function of the European Society of Cardiology. Cardiovasc Res 114, 1281–1286.

88 Xie C, Zhang J & Chen YE (2011) MicroRNA and vascular smooth muscle cells. Vitam Horm 87, 321–339.

89 Maegdefessel L, Rayner Katey J & Leeper Nicholas J (2015) MicroRNA regulation of vascular smooth muscle function and phenotype. Arterioscler Thromb Vasc Biol 35, 2–6.

90 Davis-Dusenbery BN, Wu C & Hata A (2011) Micromanaging vascular smooth muscle cell differentiation and phenotypic modulation. Arterioscler Thromb Vasc Biol 31, 2370–2377.

91 Alexander MR & Owens GK (2012) Epigenetic control of smooth muscle cell differentiation and phenotypic switching in vascular development and disease. Annu Rev Physiol 74, 13–40.

92 Thyberg J (1998) Phenotypic modulation of smooth muscle cells during formation of neointimal thickenings following vascular injury. Histol Histopathol 13, 871–891.

93 Tang R, Zhang G, Wang YC, Mei X & Chen SY (2017) The long non-coding RNA GAS5 regulates transforming growth factor beta (TGFB-beta)-induced smooth muscle cell differentiation via RNA Smad-binding elements. J Biol Chem 292, 14270–14278.

94 Ahmed ASI, Dong K, Liu J, Wen T, Yu L, Xu F, Kang X, Osman I, Hu G, Bunting KM et al. (2018) Long noncoding RNA NEAT1 (nuclear paraspeckle assembly transcript 1) is critical for phenotypic switching of vascular smooth muscle cells. Proc Natl Acad Sci USA 115, E8660–E8667.

95 Fang G, Qi J, Huang L & Zhao X. (2019) LncRNA MRAK048635_P1 is critical for vascular smooth muscle cell function and phenotypic switching in essential hypertension. Biosci Rep 39, BSR20182229.

96 Weiser-Evans MCM (2017) Smooth muscle differentiation control comes full circle: the circular noncoding RNA, circActa 2. Functions as a miRNA sponge to fine-tune alpha-SMA expression. Circ Res 121, 591–593.

97 Sun Y, Yang Z, Zheng B, Zhang X-H, Zhang M-L, Zhao X-S, Zhao H-Y, Suzuki T & Wen J-K (2017) A novel regulatory mechanism of smooth muscle α-actin expression by NRG-1/circACTA2/miR-548f-5p axis. Circ Res 121, 628–635.

98 Zhao J, Zhang W, Lin M, Wu W, Jiang P, Tou E, Xue M, Richards A, Jourd’heuil D, Asif A et al. (2016) MYOSLID Is a novel serum response factor-dependent long noncoding RNA that amplifies the transforming growth factor beta (TGF-beta)-induced alpha-SMA expression. Mol Cell 26, 745–752.

99 Alexander MR & Owens GK (2012) Epigenetic control of smooth muscle cell differentiation and phenotypic switching in vascular development and disease. Annu Rev Physiol 74, 13–40.

100 YinYW, Liao SQ, Zhang MJ, Liu Y, Li BH, Zhou Y, Chen L, Gao CY, Li JC & Zhang LL (2014) TLR4-mediated inflammation promotes foam cell formation.
of vascular smooth muscle cell by upregulating ACAT1 expression. Cell Death Dis 5, e1574–e1574.

101 Maguire EM, Pearce SWA & Xiao Q (2019) Foam cell formation: A new target for fighting atherosclerosis and cardiovascular disease. Vascul Pharmacol 112, 54–71.

102 Shankman LS, Gomez D, Cherepanova OA, Salmon M, Alencar GF, Haskins RM, Sliwatkowska P, Newman AA, Greene ES, Straub AC et al. (2015) KLF4-dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis. Nat Med 21, 628–637.

103 Majesky Mark W, Horita H, Ostriker A, Lu S, Regan Jenna N, Bagchi A, Dong Xiu R, Poczobutt J, Nemenoff Raphael A & Weiser-Evans Mary CM (2017) Differentiated smooth muscle cells generate a subpopulation of resident vascular progenitor cells in the adventitia regulated by Klf4. Circ Res 120, 296–311.

104 Kong P, Yu Y, Wang L, Dou Y-Q, Zhang X-H, Cui Y, Wang H-Y, Yong Y-T, Liu Y-B, Hu H-J et al. (2019) circ-Sirt1 controls NF-kB activation via sequence-specific interaction and enhancement of SIRT1 expression by binding to miR-132/212 in vascular smooth muscle cells. Nucleic Acids Res 47, 3580–3593.

105 Wang H, Qin R & Cheng Y (2019) LncRNA-Ang362 promotes pulmonary arterial hypertension by regulating miR-221 and miR-222. Shock (Augusta, Ga). https://doi.org/10.1097/SHK.0000000000001410

106 Liu X, Ma B-D, Liu S, Liu J & Ma B-X (2019) Long noncoding RNA LINC00341 promotes the vascular smooth muscle cells proliferation and migration via miR-214/FOXO4 feedback loop. Am J Transl Res 11, 1835–1842.

107 Tian S, Yuan Y, Li Z, Gao M, Lu Y & Gao H (2018) LncRNA UCA1 sponges miR-26a to regulate the migration and proliferation of vascular smooth muscle cells. Gene 673, 159–166.

108 Cheng W-L, She Z-G, Qin J-J, Guo J-H, Gong F-H, Zhang P, Fang C, Tian S, Zhu X-Y, Gong J et al. (2017) Interferon regulatory factor 4 induces neointima formation by engaging Krüppel-like factor 4 signaling. Circulation 136, 1412–1433.

109 Zheng B, Han M, Bernier M, Zhang X-H, Meng F, Miao S-B, He M, Zhao X-M & Wen J-K (2009) Krüppel-like factor 4 inhibits proliferation by platelet-derived growth factor receptor beta-mediated, not by retinoic acid receptor alpha-mediated, phosphatidylinositol 3-kinase and ERK signaling in vascular smooth muscle cells. J Biol Chem 284, 22773–22785.

110 Zheng B, Han M & Wen J-K (2010) Role of Krüppel-like factor 4 in profibrotic switching and proliferation of vascular smooth muscle cells. IUBMB Life 62, 132–139.

111 Kee HJ, Kwon JS, Shin S, Ahn Y, Jeong MH & Kook H (2011) Trichostatin A prevents neointimal hyperplasia via activation of Kruppel like factor 4. Vascul Pharmacol 55, 127–34.

112 Wang C, Han M, Zhao XM & Wen JK (2008) Krüppel-like factor 4 is required for the expression of vascular smooth muscle cell differentiation marker genes induced by all-trans retinoic acid. J Biochem 144, 313–21.

113 Yoshida T, Kaestner KH & Owens GK (2008) Conditional deletion of Kruppel-like factor 4 delays downregulation of smooth muscle cell differentiation markers but accelerates neointimal formation following vascular injury. Circ Res 102, 1548–57.

114 Zhang J, Gao F, Ni T, Lu W, Lin N, Zhang C, Sun Z, Guo H & Chi J (2019) Linc-POU3F3 is overexpressed in in-stent restenosis patients and induces VSMC phenotypic transformation via POU3F3/miR-449a/KLF4 signaling pathway. Am J Transl Res 11, 4481–4490.

115 Yu B, Chen Q, Le Bras A, Zhang L & Xu Q (2017) Vascular stem/progenitor cell migration and differentiation in atherosclerosis. Antioxid Redox Signal 29, 219–235.

116 Zhang L, Issa Bhaloo S, Chen T, Zhou B & Xu Q (2018) Role of resident stem cells in vessel formation and arteriosclerosis. Circ Res 122, 1608–1624.

117 Wang D, Li HK, Dai T, Wang A & Li S (2018) Adult stem cells in vascular remodeling. Theranostics 8, 815–829.

118 Nguyen Anh T, Gomez D, Bell Robert D, Campbell Julie H, Clowes Alexander W, Gabbiani G, Giachelli Cecilia M, Parmacek Michael S, Raines Elaine W, Rusch Nancy J et al. (2013) Smooth muscle cell plasticity. Circ Res 112, 17–22.

119 Yang J, Zhang L, Yu C, Yang XF & Wang H (2014) Monocyte and macrophage differentiation: circulation inflammatory monocyte as biomarker for inflammatory diseases. Biomark Res 2, 1.

120 Herring BP, Hoggatt AM, Burlak C & Offermanns S (2014) Previously differentiated medial vascular smooth muscle cells contribute to neointima formation following vascular injury. Vasc Cell 6, 21.

121 Tigges U, Komatsu M & Stallcup WB (2013) Adventitial pericyte progenitor/mesenchymal stem cells participate in the restenotic response to arterial injury. J Vase Res 50, 134–144.

122 Wu Y, Li Z, Yang M, Dai B, Hu F, Yang F, Zhu J, Chen T & Zhang L (2017) MicroRNA-214 regulates smooth muscle cell differentiation from stem cells by targeting RNA-binding protein QKI. Oncotarget 8, 19866–19878.

123 Guttmann M, Amit I, Garber M, French C, Lin MF, Feldser D, Huarte M, Zuk O, Carey BW, Cassidy JP
et al. (2009) Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 458, 223.

124 Grote P, Wittler L, Hendrix D, Koch F, Wahrisch S, Beisaw A, Macura K, Blass G, Kellis M, Werber M et al. (2013) The tissue-specific IncRNA Fendrr is an essential regulator of heart and body wall development in the mouse. Dev Cell 24, 206–214.

125 Ounzain S, Micheletti R, Arnarn C, Plaisance I, Cecchi D, Schroen B, Reverter F, Alexanian M, Gonzales C, Ng SY et al. (2015) CARMEN, a human super enhancer-associated long noncoding RNA controlling cardiac specification, differentiation and homeostasis. J Mol Cell Cardiol 89, 98–112.

126 Sheik Mohamed J, Gaughwin PM, Lim B, Robson P & Lipovich L (2010) Conserved long noncoding RNAs transcriptionally regulated by Oct4 and Nanog modulate pluripotency in mouse embryonic stem cells. RNA (New York, NY) 16, 324–337.

127 Kurian L, Aguirre A, Sancho-Martinez I, Benner C, Hishita T, Nguyen TB, Reddy P, Nivet E, Krause MN, Nelles DA et al. (2015) Identification of novel long noncoding RNAs underlying vertebrate cardiovascular development. Circulation 131, 1278–1290.

128 Elia L, Quintavalle M, Zhang J, Contu R, Cossu L, Latronico MVG, Peterson KL, Indolfi C, Catalucci D, Chen J et al. (2009) The knockout of miR-143 and -145 alters smooth muscle cell maintenance and vascular homeostasis in mice: correlates with human disease. Cell Death Differ 16, 1590–1598.

129 Xin M, Small EM, Sutherland LB, Qi X, McAnally J, Plato CF, Richardson JA, Bassel-Duby R & Olson EN (2009) MicroRNAs miR-143 and miR-145 modulate cytoskeletal dynamics and responsiveness of smooth muscle cells to injury. Genes Dev 23, 2166–2178.

130 Boettiger T, Beetz N, Kostin S, Schneider J, Kruger M, Hein L & Braun T (2009) Acquisition of the contractile phenotype by murine arterial smooth muscle cells depends on the Mir143/145 gene cluster. J Clin Investig 119, 2634–2647.

131 Hall IF, Climent M, Quintavalle M, Farina FM, Schorn T, Zani S, Carulpo P, Kunderfranco P, Civilini E, Condorelli G et al. (2019) Circ_Rrp6, a circular RNA enriched in vascular smooth muscle cells, acts as a sponge regulating miRNA-145 function. Circ Res 124, 498–510.

132 Zhang C-J, Liu C, Wang Y-X, Zhu N, Hu Z-Y, Liao D-F & Qin L (2019) Long non-coding RNA-SRA promotes neointimal hyperplasia and vascular smooth muscle cells proliferation via MEK-ERK-CREB pathway. Vasc Pharmacol 116, 16–23.

133 Li H, Liu X, Zhang L & Li X (2017) LncRNA BANCR facilitates vascular smooth muscle cell proliferation and migration through JNK pathway. Oncotarget 8, 114568–114575.

134 Das S, Zhang E, Senapati P, Amaram V, Reddy MA, Stapleton K, Leung A, Lanting L, Wang M, Chen Z et al. (2018) A novel angiotensin II-induced long noncoding RNA giver regulates oxidative stress, inflammation, and proliferation in vascular smooth muscle cells. Circ Res 123, 1298–1312.

135 Mahmoud Amira D, Ballantyne Margaret D, Miscianinov V, Pinel K, Hung J, Scanlon Jessica P, Iyinikkel J, Kaczynski J, Tavares Adriana S, Bradshaw Angela C et al. (2019) The human-specific and smooth muscle cell-enriched LncRNA SMILR promotes proliferation by regulating mitotic CENPF mRNA and drives cell-cycle progression which can be targeted to limit vascular remodeling. Circ Res 125, 535–551.

136 Ballantyne MD, Pinel K, Dukin R, Vesey AT, Diver L, Mackenzie R, Garcia R, Welsh P, Sattar N, Hamilton G et al. (2016) Smooth muscle enriched long noncoding RNA (SMILR) regulates cell proliferation. Circulation 133, 2050–2065.

137 Cui C, Wang X, Shang XM, Li L, Ma Y, Zhao GY, Song YX, Geng XB, Zhao BQ, Tian MR et al. (2019) LncRNA 430945 promotes the proliferation and migration of vascular smooth muscle cells via the ROR2/RhoA signaling pathway in atherosclerosis. Mol Med Rep 19, 4663–4672.

138 Shan K, Jiang Q, Wang XQ, Wang YN, Yang H, Yao MD, Liu C, Li XM, Yao J, Liu B et al. (2016) Role of long non-coding RNA-RNCR3 in atherosclerosis-related vascular dysfunction. Cell Death Dis 7, e2248.

139 Yao X, Yan C, Zhang L, Li Y & Wan Q (2018) LncRNA ENST00113 promotes proliferation, survival, and migration by activating PI3K/Akt/mTOR signaling pathway in atherosclerosis. Medicine 97, e0473.

140 Liu K, Liu C & Zhang Z (2019) LncRNA GAS5 acts as a ceRNA for miR-21 in suppressing PDGF-bb-induced proliferation and migration in vascular smooth muscle cells. J Cell Biochem 120, 15233–15240.

141 Wang Y-N-Z, Shan K, Yao M-D, Yao J, Wang J-J, Li X, Liu B, Zhang Y-Y, Ji Y, Jiang Q & et al. (2016) Long noncoding RNA-GAS5. Hypertension 68, 736–748.

142 Benchabane H & Ahmed Y (2009) The adenomatous polyposis coli tumor suppressor and Wnt signaling in the regulation of apoptosis. Adv Exp Med Biol 656, 75–84.

143 Ji R, Cheng Y, Yue J, Yang J, Liu X, Chen H, Dean DB & Zhang C (2007) MicroRNA expression signature and antisense-mediated depletion reveal an essential role of MicroRNA in vascular neointimal lesion formation. Circ Res 100, 1579–1588.
144 Peng J, He X, Zhang L & Liu P (2018) MicroRNA26a protects vascular smooth muscle cells against H2O2-induced injury through activation of the PTEN/AKT/mTOR pathway. \textit{Int J Mol Med} \textbf{42}, 1367–1378.

145 Sedding DG, Widmer-Teske R, Mueller A, Stieger P, Daniel JM, Gunduz D, Pullamsetti S, Nef H, Moellmann H, Troidl C \textit{et al.} (2013) Role of the phosphatase PTEN in early vascular remodeling. \textit{PLoS ONE} \textbf{8}, e55445.

146 Hu C, Liu S, Sun Y, Shi G & Li Y (2014) Effect of recombinant hPTEN gene expression on PDGF-induced VSMC proliferation. \textit{Cell Biochem Biophys} \textbf{70}, 1185–1190.

147 Dong X, Yu LG, Sun R, Cheng YN, Cao H, Yang KM, Dong YN, Wu Y & Guo XL (2013) Inhibition of PTEN expression and activity by angiotensin II induces proliferation and migration of vascular smooth muscle cells. \textit{J Cell Biochem} \textbf{114}, 174–182.

148 Lu Q, Liu T, Feng H, Yang R, Zhao X, Chen W, Jiang B, Qin H, Guo X, Liu M \textit{et al.} (2019) Circular RNA circSLC8A1 acts as a sponge of miR-130b/miR-494 in suppressing bladder cancer progression via regulating PTEN. \textit{Mol Cancer} \textbf{18}, 111.

149 Houhabiyy HB, Murray MF & Sharp PA (2003) Embryonic stem cell-specific MicroRNAs. \textit{Dev Cell} \textbf{5}, 351–358.

150 Knoepp K, Dutzmann J, Donde K, Korte L, Daniel JM, Bauersachs JM & Sedding D (2018) MicroRNA-494 – A crucial player in smooth muscle cell proliferation and vascular remodeling. \textit{Europ Heart J}, \textbf{39}, doi: 10.1093/eurheartj/ehy563.P3201

151 Huarte M, Guttmann M, Feldser D, Garber M, Koziol MJ, Kenzelmann-Broz D, Khalil AM, Zuk O, Amit I, Rabani M \textit{et al.} (2010) A large intergenic noncoding RNA induced by p53 mediates global gene repression in the p53 response. \textit{Cell} \textbf{142}, 409–419.

152 Wu G, Cai J, Han Y, Chen J, Huang Z-P, Chen C, Cai Y, Huang H, Yang Y, Liu Y \textit{et al.} (2014) LincRNA-p21 regulates neointima formation, vascular smooth muscle cell proliferation, apoptosis, and atherosclerosis by enhancing p53 activity. \textit{Circulation} \textbf{130}, 1452–1465.

153 Holdt LM, Stahringer A, Sass K, Pichler G, Kulak NA, Wilbert W, Kohlmaier A, Herbst A, Northoff BH, Nicolau A \textit{et al.} (2016) Circular non-coding RNA ANRIL modulates ribosomal RNA maturation and atherosclerosis in humans. \textit{Nat Commun} \textbf{7}, 12429.

154 Zhao Y, Feng G, Wang Y, Yue Y & Zhao W (2014) Regulation of apoptosis by long non-coding RNA HIF1A-AS1 in VSMCs: implications for TAA pathogenesis. \textit{Int J Clin Exp Pathol} \textbf{7}, 7643–7652.

155 Takaoka M, Uemura S, Kawata H, Imagawa K-I, Takeda Y, Nakatani K, Naya N, Horii M, Yamano S, Miyamoto Y \textit{et al.} (2006) Inflammatory response to acute myocardial infarction augments neointimal hyperplasia after vascular injury in a remote artery. \textit{Arterioscler Thromb Vasc Biol} \textbf{26}, 2083–2089.

156 Wang G-K, Zhu J-Q, Zhang J-T, Li Q, Li Y, He J, Qin Y-W & Jing Q (2010) Circulating microRNA: a novel potential biomarker for early diagnosis of acute myocardial infarction in humans. \textit{Eur Heart J} \textbf{31}, 659–666.

157 Li M, Wang Y-F, Yang X-C, Xu L, Li W-M, Xia K, Zhang D-P, Wu R-N & Gan T (2018) Circulating long noncoding RNA LIPCAR acts as a novel biomarker in patients with ST-segment elevation myocardial infarction. \textit{Med Sci Monit} \textbf{24}, 5064–5070.

158 Kumarawamy R, Bauters C, Volkman I, Maury F, Fetisch J, Holzmann A, Lemesle G, de Groot P, Pinet F & Thum T (2014) Circulating long noncoding RNA, LIPCAR, predicts survival in patients with heart failure. \textit{Circ Res} \textbf{114}, 1569–1575.

159 Deng Y-Y, Zhang W, She J, Zhang L, Chen T, Zhou J & Yuan Z (2016) GW27-e1167 circular RNA related to PPARy function as ceRNA of microRNA in human acute myocardial infarction. \textit{J Am Coll Cardiol} \textbf{68}, C51–C52.

160 Fichtlscherer S, De Rosa S, Fox H, Schwietz T, Fischer A, Liebetrau C, Weber M, Hamm CW, Roxe T, Muller-Ardogan M \textit{et al.} (2010) Circulating microRNAs in patients with coronary artery disease. \textit{Circ Res} \textbf{107}, 677–684.

161 Sun X, Zhang M, Sanagawa A, Mori C, Ito S, Iwaki S, Satoh H & Fujii S (2012) Circulating microRNA-126 in patients with coronary artery disease: correlation with LDL cholesterol. \textit{Thromb J} \textbf{10}, 16.

162 Rahimi E, Ahmadi A, Boroumand MA, Mohammad Soltani B & Behmanesh M (2018) Association of ANRIL expression with coronary artery disease in type 2 diabetic patients. \textit{Cell J} \textbf{20}, 41–45.

163 Wang F, Su X, Liu C, Wu M & Li B (2017) Prognostic value of plasma long noncoding RNA ANRIL for in-stent restenosis. \textit{Med Sci Monit} \textbf{23}, 4733–4739.

164 Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A, Kwiatkowski DP, McCarthy MI, Ouwehand WH, Samani NJ \textit{et al.} (2007) Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. \textit{Nature} \textbf{447}, 661–678.

165 Pan RY, Liu P, Zhou HT, Sun WX, Song J, Shu J, Cui GJ, Yang ZJ & Jia EZ (2017) Circular RNAs promote TRPM3 expression by inhibiting hsa-miR-130a-3p in coronary artery disease patients. \textit{Oncotarget} \textbf{8}, 60280–60290.

166 Naylor J, Li J, Milligan CJ, Zeng F, Sukumar P, Hou B, Sedo A, Yuldasheva N, Majeed Y, Beri D \textit{et al.} (2010) Pregnenolone sulphate- and cholesterol-regulated TRPM3 channels coupled to vascular...
smooth muscle secretion and contraction. Circ Res 106, 1507–1515.
167 Fichtlscherer S, Zeiher AM & Dimmeler S (2011) Circulating microRNAs: biomarkers or mediators of cardiovascular diseases? Arterioscler Thromb Vasc Biol 31, 2383–2390.
168 Schlosser K, Hanson J, Villeneuve PJ, Dimitroulakos J, McIntyre L, Pilote L & Stewart DJ (2016) Assessment of circulating LncRNAs under physiologic and pathologic conditions in humans reveals potential limitations as biomarkers. Sci Rep 6, 36596.
169 Wang W, Wang Y, Piao H, Li B, Huang M, Zhu Z, Li D, Wang T, Xu R & Liu K (2019) Circular RNAs as potential biomarkers and therapeutics for cardiovascular disease. PeerJ 7, e6831.
170 Gandhi S, Ruehle F & Stoll M (2019) Evolutionary patterns of non-coding RNA in cardiovascular biology. Noncoding RNA 5, 15.
171 Das A, Samidurai A & Salloum FN. (2018) Deciphering non-coding RNAs in cardiovascular health and disease. Front Cardiovasc Med 5, 73.
172 Diederichs S (2014) The four dimensions of noncoding RNA conservation. Trends Genet 30, 121–123.
173 Wang W-T, Han C, Sun Y-M, Chen T-Q & Chen Y-Q (2019) Noncoding RNAs in cancer therapy resistance and targeted drug development. J Hematol Oncol 12, 55.
174 Vausort M, Salgado-Somoza A, Zhang L, Leszek P, Scholz M, Teren A, Burkhardt R, Thiery J, Wagner DR & DeVALUE Y (2016) Myocardial infarction-associated circular RNA predicting left ventricular dysfunction. J Am Coll Cardiol 68, 1247–1248.
175 Zhang J, Xu Y, Xu S, Liu Y, Yu L, Li Z, Xue X & Wang H (2018) Plasma circular RNAs, Hsa_circRNA_025016. Predict postoperative atrial fibrillation after isolated off-pump coronary artery bypass grafting. J Am Heart Assoc Cardiov Cerebrovasc Dis 7, e006642.
176 Zhang Z, Gao W, Long QQ, Zhang J, Li YF, Liu DC, Yan JJ, Zhang JG & Wang LS (2017) Increased plasma levels of lncRNA H19 and LIPCAR are associated with increased risk of coronary artery disease in a Chinese population. Sci Rep. 7, 7491.
177 Wang X-W, Zhang C, Lee K-C, He X-J, Lu Z-Q, Huang C & Wu Q-C (2017) Adenovirus-mediated gene transfer of microRNA-21 sponge inhibits neointimal hyperplasia in rat vein grafts. Int J Biol Sci. 13, 1309-1319.
178 Alshanwani AR, Riches-Suman K, O’Regan DJ, Wood IC, Turner NA & Porter KE (2018) MicroRNA-21 drives the switch to a synthetic phenotype in human saphenous vein smooth muscle cells. IUBMB Life 70, 649–657.
179 Kilari S, Cai C, Zhao C, Sharma A, Chernogubova E, Simeon M, Wu C-C, Song H-L, Maegdefessel L & Misra S (2019) The role of microRNA-21 in venous neointimal hyperplasia: implications for targeting miR-21 for VNH treatment. Mol Ther 27, 1681–1693.
180 Chan MC, Hilyard AC, Wu C, Davis BN, Hill NS, Lal A, Lieberman J, Lagna G & Hata A (2010) Molecular basis for antagonism between PDGF and the TGFbeta family of signalling pathways by control of miR-24 expression. EMBO J 29, 559–573.
181 Yang J, Fan Z, Yang J, Ding J, Yang C & Chen L (2016) MicroRNA-24 attenuates neointimal hyperplasia in the diabetic rat carotid artery injury model by inhibiting Wnt4 signaling pathway. Int J Mol Sci 17, 765.
182 Yang X, Dong M, Wen H, Liu X, Zhang M, Ma L, Zhang C, Luan X, Lu H & Zhang Y (2017) MiR-26a contributes to the PDGF-BB-induced phenotypic switch of vascular smooth muscle cells by suppressing Smad1. Oncotarget 8, 75844–75853.
183 Leeper NJ, Raisedana A, Kojima Y, Chun HJ, Azuma J, Maegdefessel L, Kandu RK, Quertermous T, Tsao PS & Spin JM (2011) MicroRNA-26a is a novel regulator of vascular smooth muscle cell function. J Cell Physiol 226, 1035–1043.
184 Tan J, Yang L, Liu C & Yan Z (2017) MicroRNA-26a targets MAPK6 to inhibit smooth muscle cell proliferation and vein graft neointimal hyperplasia. Sci Rep 7, 46602.
185 Sun Q-R, Zhang X & Fang K (2018) Phenotype of Vascular Smooth Muscle Cells (VSMCs) is regulated by miR-29b by targeting sirtuin 1. Med Sci Monit 24, 6599–6607.
186 Liu X, Cheng Y, Chen X, Yang J, Xu L & Zhang C (2011) MicroRNA-31 regulated by the extracellular regulated kinase is involved in vascular smooth muscle cell growth via large tumor suppressor homolog 2. J Biol Chem 286, 42371–42380.
187 Zhang L, Chen Q, An W, Yang F, Maguire EM, Chen D, Zhang C, Wen G, Yang M, Dai B et al. (2017) Novel pathological role of hnRNP1 (Heterogeneous nuclear ribonucleoprotein A1) in vascular smooth muscle cell function and neointima hyperplasia. Arterioscler Thromb Vasc Biol 37, 2182–2194.
188 Torella D, Iaconetti C, Catalucci D, Ellinson GM, Leone A, Waring CD, Bochicchio A, Vicinanza C, Aquila I, Curcio A et al. (2011) MicroRNA-133 controls vascular smooth muscle cell phenotypic switch in vitro and vascular remodeling in vivo. Circ Res 109, 880–893.
189 Pan J, Li K, Huang W & Zhang X (2017) MiR-137 inhibited cell proliferation and migration of vascular smooth muscle cells via targeting IGFBP-5 and modulating the mTOR/STAT3 signaling. PLoS One 12, e0186245.
190 Cheng Y, Liu X, Yang J, Lin Y, Xu DZ, Lu Q, Deitch EA, Huo Y, Delphin ES & Zhang C (2009)
MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. *Circ Res* **105**, 158–166.

191 Zhang Y-N, Xie B-D, Sun L, Chen W, Jiang S-L, Liu W, Bian F, Tian H & Li R-K (2016) Phenotypic switching of vascular smooth muscle cells in the 'normal region' of aorta from atherosclerosis patients is regulated by miR-145. *J Cell Mol Med* **20**, 1049–1061.

192 Sun SG, Zheng B, Han M, Fang XM, Li HX, Miao SB, Su M, Han Y, Shi HJ & Wen JK (2011) miR-146a and Kruppel-like factor 4 form a feedback loop to participate in vascular smooth muscle cell proliferation. *EMBO Rep* **12**, 56–62.

193 Cao BJ, Wang XW, Zhu L, Zou RJ & Lu ZQ (2019) MicroRNA-146a sponge therapy suppresses neointimal formation in rat vein grafts. *IUBMB Life* **71**, 125–133.

194 Wang Y-S, Wang H-YJ, Liao Y-C, Tsai P-C, Chen K-C, Cheng H-Y, Lin R-T & Joo S-HH (2012) MicroRNA-195 regulates vascular smooth muscle cell phenotype and prevents neointimal formation. *Cardiovasc Res* **95**, 517–526.

195 Torella D, Iaconetti C, Tarallo R, Marino F, Giurato G, Veneziano C, Aquila I, Scalise M, Mancuso T, Cianflone E et al. (2018) miRNA regulation of the hyperproliferative phenotype of vascular smooth muscle cells in diabetes. *Diabetes* **67**, 2554–2566.

196 Zhang Y, Wang Y, Wang X, Zhang Y, Eisner GM, Asico LD, Jose PA & Zeng C (2011) Insulin promotes vascular smooth muscle cell proliferation via microRNA-208-mediated downregulation of p21. *J Hypertens* **29**, 1560–1568.

197 Afzal TA, Luong LA, Chen D, Zhang C, Yang F, Chen Q, An W, Wilkes E, Yashiro K, Cutillas PR et al. (2016) NCK associated protein 1 modulated by miRNA-214 determines vascular smooth muscle cell migration, proliferation, and neointima hyperplasia. *J Am Heart Assoc* e004629.

198 Liu X, Cheng Y, Zhang S, Lin Y, Yang J & Zhang C (2009) A necessary role of miR-221 and miR-222 in vascular smooth muscle cell proliferation and neointimal hyperplasia. *Circ Res* **104**, 476–487.

199 Merlet E, Atassi F, Motiani RK, Mougenot N, Jacquet A, Nadaud S, Capiod T, Trehik M, Lompré A-M & Marchand A (2013) miR-424/322 regulates vascular smooth muscle cell phenotype and neointimal formation in the rat. *Cardiovasc Res* **98**, 458–468.

200 Bi R, Ding F, He Y, Jiang L, Jiang Z, Mei J & Liu H (2016) miR-503 inhibits platelet-derived growth factor-induced human aortic vascular smooth muscle cell proliferation and migration through targeting the insulin receptor. *BioMed Pharmacother* **84**, 1711–1716.

201 Yang F, Xu Z, Duan S & Luo M (2016) MicroRNA-541 promotes the proliferation of vascular smooth muscle cells by targeting IRF7. *Am J Transl Res* **8**, 506–515.

202 Xie B, Zhang C, Kang K & Jiang S (2015) miR-599 inhibits vascular smooth muscle cells proliferation and migration by targeting TGFB2. *PLoS One* **10**, e0141512.

203 Li P, Zhu N, Bi W, Wang N, Chen M, You X, Zhao X, Solomides Charalambos C, Qin Y & Sun J (2013) MicroRNA-663 regulates human vascular smooth muscle cell phenotypic switch and vascular neointimal formation. *Circ Res* **113**, 1117–1127.

204 Li P, Liu Y, Bi W, Wang G, You X, Zhao X, Summer R, Qin Y & Sun J (2013) MicroRNA-638 is highly expressed in human vascular smooth muscle cells and inhibits PDGF-BB-induced cell proliferation and migration through targeting orphan nuclear receptor NOR1. *Cardiovasc Res* **99**, 185–193.

205 Yin Q, Wu A & Liu M (2017) Plasma long non-coding RNA (IncRNA) GAS5 is a new biomarker for coronary artery disease. *Med Sci Monit* **23**, 6042–6048.

206 Kong P, Yu Y, Wang L, Dou YQ, Zhang XH, Cui Y, Wang HY, Yong YT, Liu YB, Hu JH et al. (2019) circ-Sirt1 controls NF-kappaB activation via sequence-specific interaction and enhancement of SIRT1 expression by binding to miR-132/212 in vascular smooth muscle cells. *Nucleic Acids Res* **47**, 3580–3593.

207 Ballantyne MD, Pinel K, Dakin R, Vesey AT, Diver L, Mackenzie R, Garcia R, Welsh P, Sattar N, Hamilton G et al. (2016) Smooth Muscle Enriched Long Noncoding RNA (SMILR) regulates cell proliferation. *Circulation* **133**, 2050–2065.

208 Mahmoud AD, Ballantyne MD, Miscâinov V, Pinel K, Hung J, Scanlon JP, Iyinikkel J, Kaczynski J, Tavares AS, Bradshaw AC et al. (2019) The human-specific and smooth muscle cell-enriched LncRNA SMILR promotes proliferation by regulating mitotic CENPF mRNA and drives cell-cycle progression which can be targeted to limit vascular remodeling. *Circ Res* **125**, 535–551.

209 Cheng Y & Zhang C (2010) MicroRNA-21 in cardiovascular disease. *Cardiovasc Transl Res* **3**, 251–255.

210 Eken SM, Jin H, Chernogubova E, Li Y, Simon N, Sun C, Korzunowicz G, Busch A, Backlund A, Osterholm C et al. (2017) MicroRNA-210 enhances fibrous cap stability in advanced atherosclerotic lesions. *Circ Res* **120**, 633–644.

211 Raithoharju E, Lyytikainen LP, Levula M, Oksala S, Memmander A, Tarkka M, Klopp N, Illig T, Kahonen M, Karhunen PJ et al. (2011) miR-21, miR-210, miR-34a, and miR-146a/b are up-regulated in human atherosclerotic plaques in the Tampere Vascular Study. *Atherosclerosis* **219**, 211–217.