

Non-coding RNA in endothelial-to-mesenchymal transition

Melanie S. Hulshoff^{1,2,3†}, Gonzalo del Monte-Nieto ^{4†}, Jason Kovacic⁵, and Guido Krenning ^{1*}

¹Laboratory for Cardiovascular Regenerative Medicine, Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Hanzeplein 1 (EA11), 9713GZ, Groningen, The Netherlands; ²Department of Cardiology and Pneumology, University Medical Center of Göttingen, Georg-August University, Göttingen, Germany; ³German Centre for Cardiovascular Research (DZHK), Partner Site, Göttingen, Germany; ⁴Australian Regenerative Medicine Institute, Monash University, Clayton 3800, VIC, Australia; and ⁵Dept. Cardiology, Zena and Michael A. Wiener Cardiovascular Institute, Icahn School of Medicine at Mount Sinai, New York, NY, USA

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Abstract

Endothelial-to-mesenchymal transition (EndMT) is the process wherein endothelial cells lose their typical endothelial cell markers and functions and adopt a mesenchymal-like phenotype. EndMT is required for development of the cardiac valves, the pulmonary and dorsal aorta, and arterial maturation, but activation of the EndMT programme during adulthood is believed to contribute to several pathologies including organ fibrosis, cardiovascular disease, and cancer. Non-coding RNAs, including microRNAs, long non-coding RNAs, and circular RNAs, modulate EndMT during development and disease. Here, we review the mechanisms by which non-coding RNAs facilitate or inhibit EndMT during development and disease and provide a perspective on the therapeutic application of non-coding RNAs to treat fibroproliferative cardiovascular disease.

Keywords

Non-coding RNA • Endothelial-mesenchymal transition (EndMT) • Cardiac development • Cardiovascular disease • Plasticity

1. EndMT during development and pathology

Endothelial cells form the inner layer of every single vessel in the body acting as a barrier between the blood or lymph and the rest of the tissues. Endothelial cells are highly plastic cells, that are able to differentiate into arterial, venous, lymphatic, and endocardial cell fates during development, but also into haematopoietic lineages,^{1,2} or mesenchymal lineages through a process coined endothelial-to-mesenchymal transition (EndMT). During heart development, EndMT is essential for the formation of the valve mesenchyme.^{3,4} These valvulogenic regions are located in the atrioventricular canal (AVC) and the outflow tract (OFT) of the developing heart and undergo differential specification programmes compared to the cardiac chambers.^{5,6} Besides the involvement of EndMT during valve development, EndMT is involved in embryonic pulmonary artery development⁷ and in the formation of the smooth muscle component of the dorsal aorta (Figure 1).⁸ Furthermore, just recently, a partial EndMT process has been suggested pivotal to physiological angiogenic sprouting (Figure 1).⁹ This new concept may open new interpretations of vessel development, but also for heart

development, where recent studies suggested a process similar to sprouting angiogenesis in the endocardium during ventricular trabeculation.¹⁰

EndMT is a specific form of epithelial-to-mesenchymal transition (EMT) and the term was coined to refer specifically to the EMT affecting the endothelium, a squamous type of epithelium. The regulation of EndMT during normal developmental conditions shares many aspects with the general process of EMT; however, the molecular defects promoting pathologic EndMT are less understood. EndMT is characterized by loss of endothelial markers such as VE-Cadherin and CD31, and upregulation of the EndMT transcription factors SNAIL, SLUG, TWIST, ZEB1, and ZEB2 as well as mesenchymal markers such as α -SMA (ACTA2) and S100A4. EndMT-derived mesenchymal cells acquire a highly migratory and invasive potential which is accompanied by morphological changes from a cobble-stone endothelial morphology into a spindle-shaped myofibroblast-like morphology. In recent years, the reactivation of EndMT during adult life has gained increasing attention in the cardiovascular field due to its implication in numerous adult pathologies including pulmonary hypertension,¹¹ atherosclerosis,^{12,13} brain vascular malformations,¹⁴ tissue fibrosis,^{15–17} and cancer progression.^{18,19}

* Corresponding author. Tel: +31 50 361 5181; fax: +31 50 361 9911, E-mail: g.krenning@umcg.nl

† The first two authors contributed equally to the study.

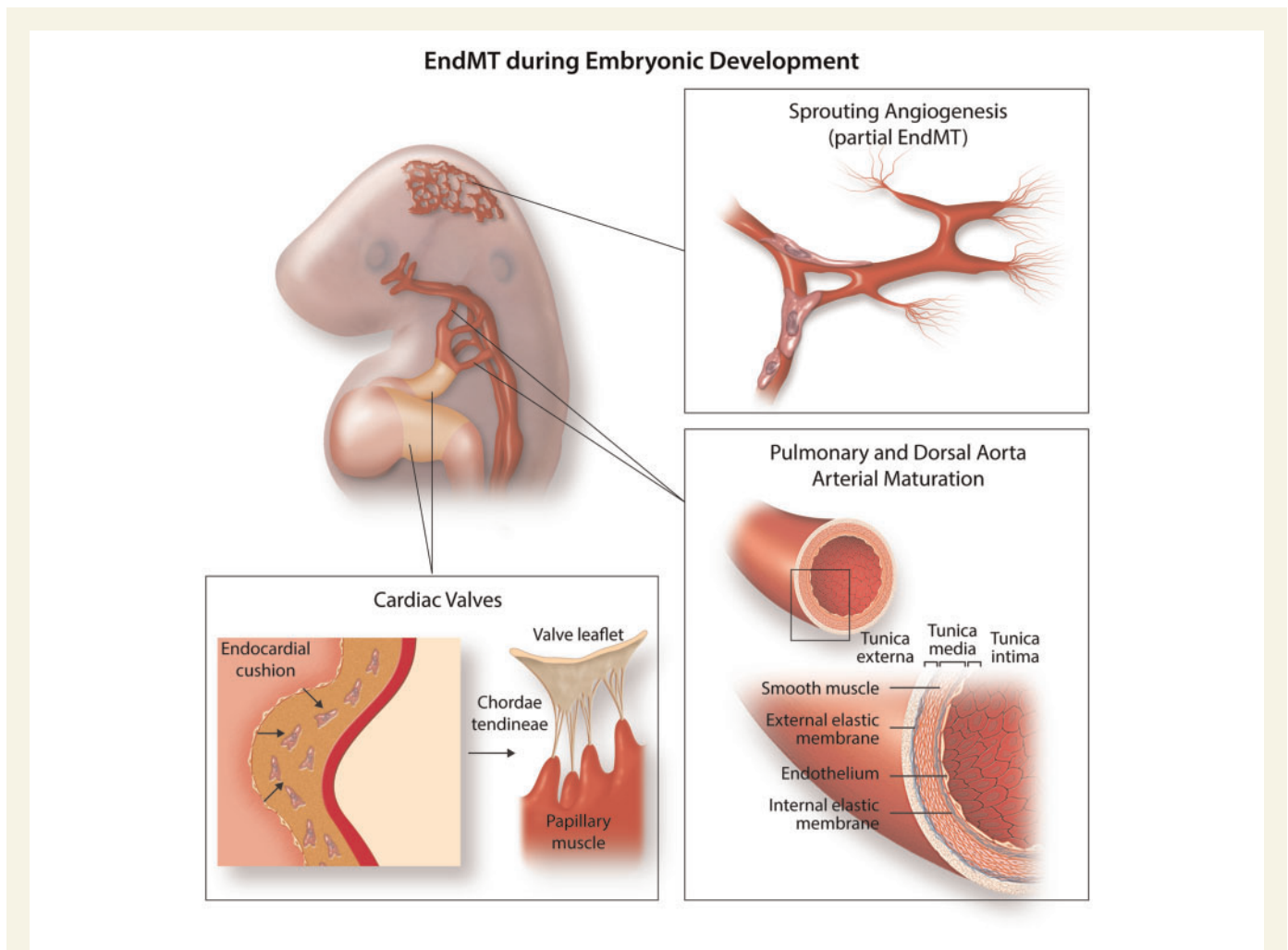


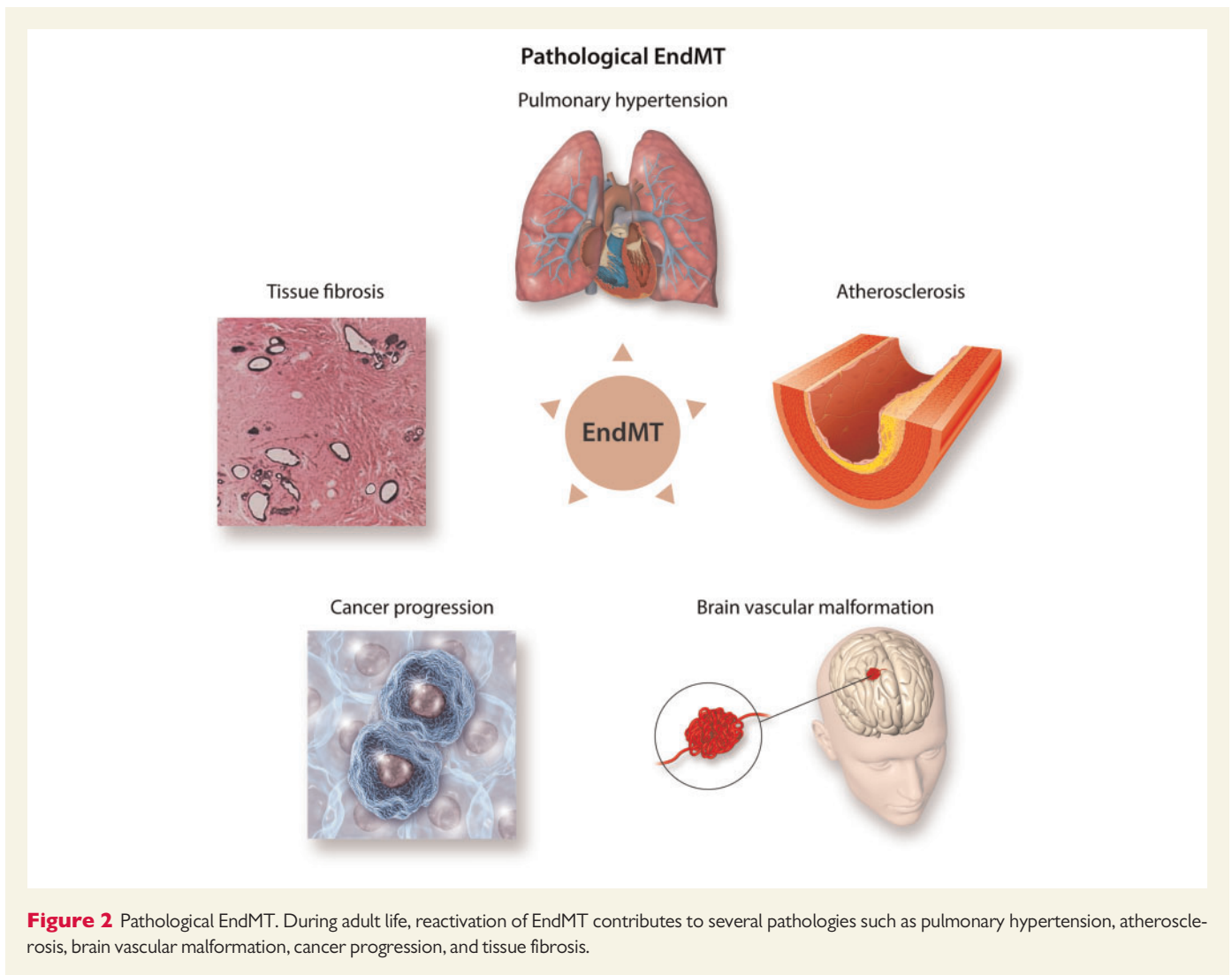
Figure 1 EndMT in development. During embryonic development, EndMT contributes to the cardiac valves, pulmonary and dorsal aorta arterial maturation, and sprouting angiogenesis (partial EndMT).

(Figure 2). Under pathological conditions, several environmental factors induce EndMT, including high glucose, hypoxia, oxidative stress, pro-inflammatory cytokines, and disturbed shear stress (the frictional force of the blood flow on the endothelial cells).^{20–24} These environmental factors trigger the activation of signalling pathways which induce EndMT such as the canonical Transforming Growth Factor Beta (TGF- β) pathway or the non-canonical pathways such as Notch and Wnt.¹³ However, fibroblast growth factor (FGF) and mitogen-activated protein kinase (MAPK) modulate EndMT as inhibitory pathways.²⁵ These signalling pathways are described in more detail below. The environmental factors and, thereby-induced signalling pathways, can also lead to the expression of non-coding RNAs, functional RNA molecules which are not translated into proteins. Non-coding RNAs include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs). These non-coding RNAs influence EndMT pathways during development and pathology.²⁶ In this review, we summarize the current knowledge on the mechanisms by which non-coding RNAs regulate the EndMT process and outline their potential as therapeutic molecules to alleviate fibroproliferative diseases. Readers interested in further detail about the overall EndMT process, or its involvement during development or adult cardiovascular diseases, are referred to other recent articles covering these aspects in depth.^{13,27–29}

2. Canonical EndMT pathways

2.1 The TGF- β signalling pathway

When considering the molecular mechanisms controlling EndMT, the most simplistic overview is a finely regulated network integrating TGF- β , bone morphogenetic proteins (BMPs), and the Notch signalling pathway (Figure 3).³⁰ There are three ligands in the TGF- β family involved in EndMT; TGF- β 1, -2, and -3.³¹ TGF- β ligands signal through tetrameric receptors formed by two type II TGF- β receptors (TGF β R2) and two type I TGF- β receptors (ALK5 and ALK2). Once activated, they induce an intracellular signalling pathway mediated by SMAD2 and SMAD3, which then interact with SMAD4, and the entire SMAD complex translocates to the nucleus and binds DNA-associated proteins to promote the expression of mesenchymal genes. In endothelial cells, TGF- β ligands also induce the activation of the type I TGF- β receptor ALK1 which antagonizes EndMT by signalling through SMAD1, 5, and 8, allowing certain crosstalk between the TGF- β and BMP pathways. TGF- β signalling can also occur in a SMAD-independent manner by activating intracellular signalling pathways such as MAP kinase (MAPK), Rho-like GTPase, ERK1/2, or phosphatidylinositol-3-kinase (PI3K)/AKT pathways,^{32,33} all of which are inducers of EndMT.



2.2 Transcription factors regulating EndMT

TGF- β ligands have been described as master regulators of EndMT by promoting the expression of the transcription factors SNAIL, SLUG, TWIST, ZEB1, and ZEB2.³⁴ Although all of them repress the transcription of VE-Cadherin, they perform overlapping but non-redundant roles during EndMT. The transcription factors SNAIL and SLUG are pivotal to EndMT induction, whereas TWIST, ZEB1, and ZEB2 maintain the migratory phenotype.³⁵ Furthermore, these transcription factors cross-regulate each other and their own expression, highlighting the fine molecular regulation of EndMT.^{36,39} Other transcription factors involved in the EndMT process include GATA4 and the ETS factors ERG and FLI1. Endothelial specific deletion of GATA4 culminates in reduced EndMT whereas the suppression of ERG or FLI1 expression enhances EndMT via activation of the TGF- β pathway, in particular SMAD2/3 transcription factors.^{40,41}

2.3 The BMP signalling pathway

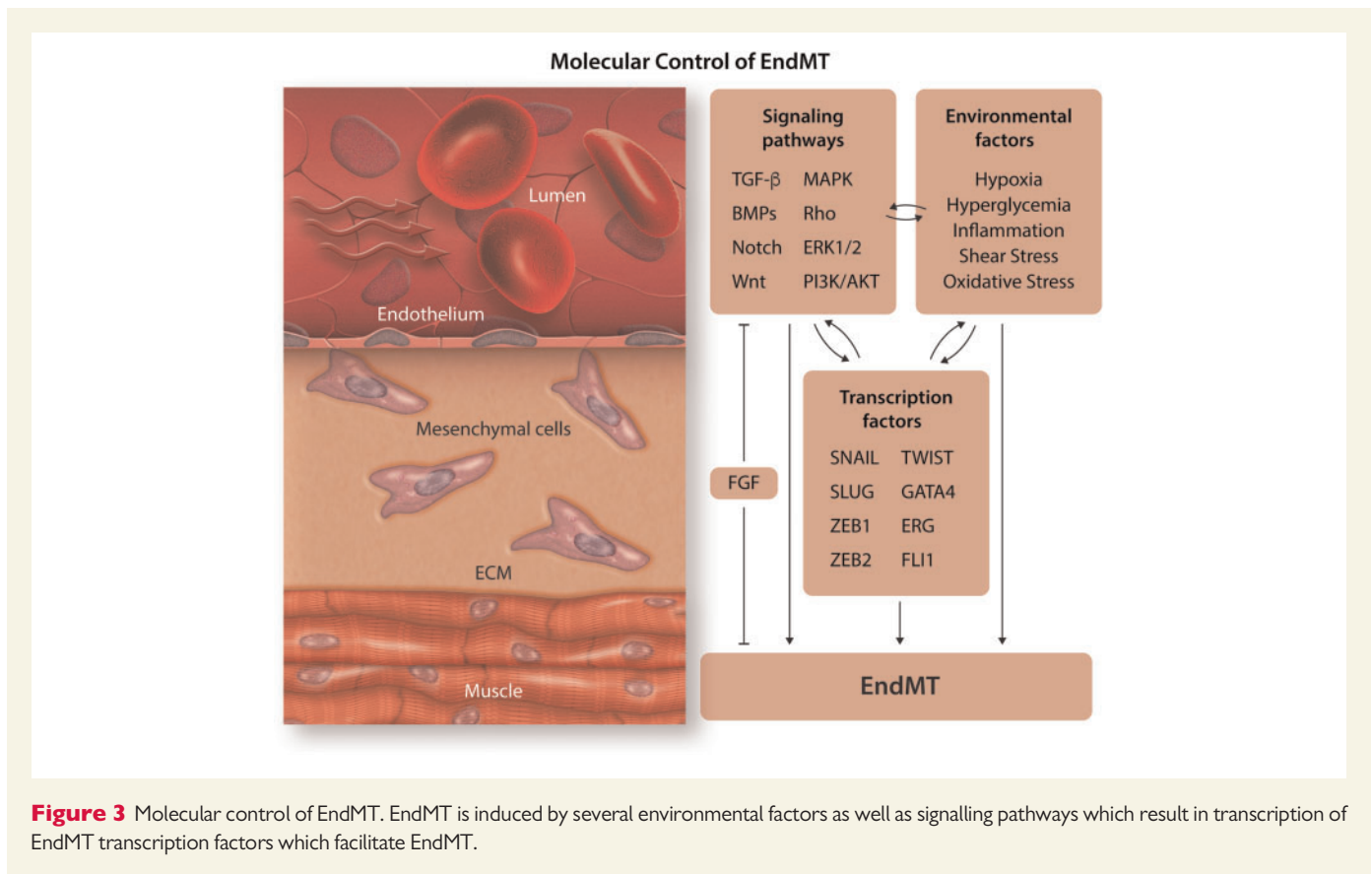
BMPs signal through four possible type I receptors (ALK1, ALK2, ALK3, and ALK6) and three type II receptors (BMPRII, ActRII, or ActRIIB). During valve development, BMP2, 4, 5, 6, and 7 ligands are expressed in the valvulogenic regions. Receptor complex activation promotes intracellular signalling mediated by the SMAD1, 5, and 8, that together with SMAD4 promote the nuclear translocation of the SMAD complex and the activation of BMP-responsive genes. During development, BMPs play

critical roles in the patterning the AVC/OFT myocardium to provide a pro-EndMT environment and promote the transition of endothelial cells into invasive mesenchymal cells. In particular, BMP2 and BMP4 are the two ligands described to be responsible for EndMT in the AVC and OFT.^{5,42,43} It has to be noted that in contrast to its inducing role on EndMT during development, BMPs (in particular BMP7) have been described to inhibit pathological EndMT during adulthood.^{17,44}

3. Non-canonical EndMT pathways

3.1 The notch signalling pathway

Notch is a cell-to-cell signalling pathway formed by four different receptors (Notch1–4). Notch is a key signal in the induction of EndMT during heart valve development. Notch promotes EndMT by inducing the expression of SNAIL and SLUG.^{45,46} Notch1 and 2 mutant embryos show heart valve defects that arise due to defective EndMT.⁴⁵ The Notch pathway acts synergistically with the TGF- β 2 and BMP2 pathways in the control of EndMT.^{46,47} TGF- β 2 and BMP2 are able to induce SNAIL expression and to a weaker extent SLUG expression, whereas Notch activates SLUG and synergistically induces SNAIL in concert with TGF- β 2 and BMP2.^{46,47} Furthermore, Notch controls the BMP2 expression pattern in the AVC myocardium.⁴⁷



3.2 The Wnt signalling pathway

Canonical Wnt signalling involves signal transduction through stabilization of β -catenin. Stabilized β -catenin translocates to the nucleus where it interacts with DNA-associated transcription factors to induce target gene expression. EndMT is strongly inhibited in endothelial-specific β -catenin mutants.^{48,49} Wnt/ β -catenin acts in the AVC endocardium downstream from TGF- β to promote the acquisition of a mesenchymal phenotype during EndMT.⁵⁰ Canonical Wnt signalling induces EndMT by the induction of SNAIL and SLUG expression.^{51,52} In contrast, Wnt signalling through Wnt7a inhibits EndMT,⁵³ highlighting the fine regulation of the EndMT process by the Wnt pathway.

3.3 Inflammatory EndMT

In general, the pathologies in which EndMT is recognized are associated with inflammatory activation in response to tissue damage. During the inflammatory response, pro-inflammatory cytokines including interleukin (IL)-1 β and tumour necrosis factor alpha (TNF- α) activate the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) involved in the activation of other pro-inflammatory cytokines including IL-1, IL-6, TNF- α , or interferon gamma (IFN γ), hence creating a positive feedback regulatory loop. Activation of NF- κ B promotes the induction of TGF- β 1 and TGF- β 2 and promotes EndMT.⁵⁴ But NF- κ B can also induce EndMT in a TGF- β -independent manner by directly activating SNAIL expression.^{55,56}

4. Inhibitory EndMT pathways

4.1 The FGF signalling pathway

The FGF signalling pathway is formed by 22 different ligands that mediate their biological activity by binding to cell surface FGF receptors

(FGFR1–4).⁵⁷ Once activated, FGF receptors recruit similar intracellular signal transduction factors to other tyrosine kinase receptors including PI3K and MKK3/6, and activate similar intracellular signalling pathways including AKT-ERK1/2, Ras, or MAPK (Raf, MEK). The FGF pathway is an important regulator of endothelial TGF- β signalling mainly through FGFR1. Loss of FGFR1 signalling activates the EndMT programme in a TGF- β -dependent manner culminating in intimal hyperplasia and stenosis.⁵⁸

5. Other factors regulating EndMT

Low oscillatory shear stress induces the expression of SNAIL, TWIST, and GATA4 in the endothelium at atheroprone sites,^{59,60} activates TGF- β signalling and decreases FGFR1 expression.¹² Moreover, during hypoxia, HIF-1 α accumulates in the nucleus⁶¹ where it activates hypoxia-induced genes including SNAIL, TWIST, and ALK5.^{62,63} Oxidative stress also promotes EndMT and has additive effects to TGF- β .⁶⁴ Finally, the exposure to long-term high glucose levels, such as in diabetic patients, also promotes EndMT through PI3K/Akt/NF- κ B pathways leading to cardiac fibrosis in diabetic patients.⁶⁵ Altogether, this shows the diversity of factors and signalling pathways that modulate EndMT (Figure 3).

6. Non-coding RNAs

miRNAs are small fragments of RNA (typically 20–25 nucleotides). miRNAs can bind to complementary sequences within mRNA targets and then degrade the mRNA via cleavage or destabilization or inhibit the translation of mRNAs into protein.⁶⁶ This represses gene and protein

expression of direct targets of miRNAs but could also indirectly result in reactivation of gene and protein expression (because their repressors are downregulated by the miRNA). Increasing evidence describes a non-canonical role for miRNAs in transcriptional regulation as well, but the underlying mechanisms remain largely unknown. Non-coding RNAs are classified as lncRNAs when they contain more than 200 nucleotides. Their mechanism of action varies not only between different lncRNAs but also one particular lncRNA can act using different mechanisms. They can act as recruiters, tethers, and scaffolds for other regulatory factors involved in epigenetic modifications, or regulate gene transcription by acting as decoys, coregulators, or polymerase-II inhibitors. They are also involved in the organization of the different components of the transcription and splicing machinery, as well as the organization of subnuclear structures. lncRNAs are also involved in post-transcriptional regulation, controlling processes such as mRNA processing, stability, or translation, acting as sponges for miRNAs blocking their effects, or even can be the source of other small RNAs (for a review see reference 67). lncRNA can be also classified according to their structural conformation. On this regard, in this review we will separate the circRNAs from the rest of the lncRNA described. circRNAs are another subclass of non-coding RNAs generally formed by backsplicing that, similarly to other lncRNAs, function as miRNA sponges, RNA-binding protein sequestering factors, as well as regulators of gene expression by controlling mRNA transcription.^{68,69} Furthermore, circRNAs can also control gene transcription by interactions with phosphorylated Pol II or by competition with the pre-mRNA splicing machinery.^{70,71} circRNAs are highly stable molecules that form a covalently closed continuous loop making them resistant to RNA exonuclease digestion.^{72,73} The mechanisms of action of non-coding RNAs are summarized in Figure 4. Similar to lncRNAs, circRNAs can control EndMT by acting as sponges for miRNAs or by regulating the transcription of genes which can result in EndMT promotion or inhibition. In summary, miRNAs are important modulators as well which can affect gene and protein expression and thus modulate EndMT. lncRNAs and circRNAs, together with miRNAs, are therefore promising targets for therapeutic applications which aim to inhibit EndMT.

7. MicroRNAs inducing/facilitating EndMT

7.1 TGF- β 2-induced EndMT is associated with differential expression of several miRNAs

TGF- β 2-induced EndMT is associated with increased expression of miR-21, miR-27b, miR-30b, miR-125b, miR-195, Let-7c, Let-7g and decreased expression of miR-122a, miR-127, miR-196, and miR-375.^{74,75} This suggests a role for these miRNAs in modulating EndMT. Indeed, miR-21 and miR-27b facilitate EndMT.^{76,77} Inhibition of miR-21 reverts the TGF- β 2-induced repression of VE-Cadherin and induction of S100A4.⁷⁷ Consistently, kallistatin, an endogenous plasma protein, can reverse the TGF- β 1-induced upregulation of miR-21 and thereby inhibit EndMT.⁷⁶ Kallistatin can also reverse the expression of the EndMT transcription factor SNAIL, Akt phosphorylation, and activation of NF- κ B, emphasizing that the inhibiting effect of kallistatin on EndMT is not only due to the inhibition of miR-21 expression.⁷⁶ It has to be noted that inhibition of miR-21 did not affect ionizing radiation-induced EndMT.⁷⁸ Another miRNA that is a positive regulator of EndMT is miR-27b. Inhibition of miR-27b suppresses EndMT by preclusion of ACTA2 and TAGLN

(SM22 α) expression, through an unidentified mechanism.⁷⁵ The expression of another miRNA, miR-31, is not altered upon TGF- β 2 treatment,⁷⁹ yet inhibition of miR-31 suppresses both ACTA2 and TAGLN expression suggesting a role for miR-31 in facilitating EndMT.⁷⁹ However, overexpression of miR-31 has limited effects on TGF- β 2-induced EndMT suggesting that miR-31 does not induce EndMT directly but rather controls the magnitude of EndMT.⁷⁹ Let-7c and Let-7g, two other miRNAs which are upregulated upon TGF- β 2 treatment, are part of the Let-7 family which represses EndMT and will be discussed later in this review. The role of the other differentially expressed miRNAs in the regulation of EndMT remain unknown to date.

7.2 The role of miR-130 in modulating EndMT/EMT

miR-130b is upregulated in high proliferative and angiogenic-prone colorectal cancer, suggesting a role of miR-130b in proliferation, angiogenesis, EndMT, and/or EMT.⁸⁰ Indeed, miR-130b overexpression facilitates tumour growth accompanied by enhanced proliferation, angiogenesis, and EMT.⁸⁰ Overexpression of miR-130b results in decreased expression of E-Cadherin whereas EndMT transcription factors SNAIL and ZEB1 are increased.⁸⁰ miR-130a expression is increased in pulmonary hypertension as well as in TGF- β 1-treated endothelial cells, suggestive of a role for miR-130a in modulating both EndMT and EMT.⁸¹ Indeed, miR-130a enhances the TGF- β 1-induced expression of ACTA2 and decreases CD31 expression.⁸¹ Interestingly, NF- κ B can induce miR-130a expression and vice versa, illustrating the interplay between NF- κ B and miRNAs.⁸¹

7.3 miR-374b and miR-449a modulate EndMT during atherosclerosis

Both miR-374b and miR-449a associate with EndMT in the context of atherosclerosis.^{82,83} Indeed, miR-449a expression culminates in reduced E-Cadherin expression and an increase in the expression of α -SMA and SMAD3.⁸² Importantly, antagonizing miR-449a expression inhibits the development of atherosclerosis in diabetic mice.⁸² Similarly to miR-449a, the expression of miR-374b is elevated in atheroprone regions *in vivo*, as well as TGF- β 1-treated endothelial cells.⁸³ Overexpression of miR-374b results in decreased expression of the endothelial markers VE-Cadherin and eNOS whereas the expression of mesenchymal markers TAGLN and Calponin increases.⁸³ Interestingly, combined overexpression of miR-374b and MAPK7 (a known antagonist of EndMT) precludes the EndMT induction by miR-374b.⁸³ Glucose treatment induces the expression of miR-328,⁸⁴ and induces EndMT evidenced by an increase in the expression of mesenchymal markers Collagen-1 and Collagen-3 accompanied by activation of MEK1/2 and MAPK1/2.⁸⁴ These data highlight the interplay between miRNAs and MAPK signalling, and identify MAPK signalling as a crucial regulator of EndMT.

7.4 The role of miR-9 and miR-342-5p in modulating EndMT and angiogenesis

Contrasting effects of miR-9 and miR-342-5p highlight differential effects of miRNAs on certain biologic processes. miR-9 induces EndMT with decreased VE-Cadherin expression and a corresponding increase in N-Cadherin.⁸⁵ miR-9 also represses NF- κ B expression and inflammation whereas it promotes tube formation.⁸⁵ On the other hand, miR-342-5p also induces EndMT, but in contrast to miR-9, this inhibits lumen formation and angiogenic sprouting.⁸⁶ These data show that although there is redundancy in miRNAs that induce EndMT, there are also distinct

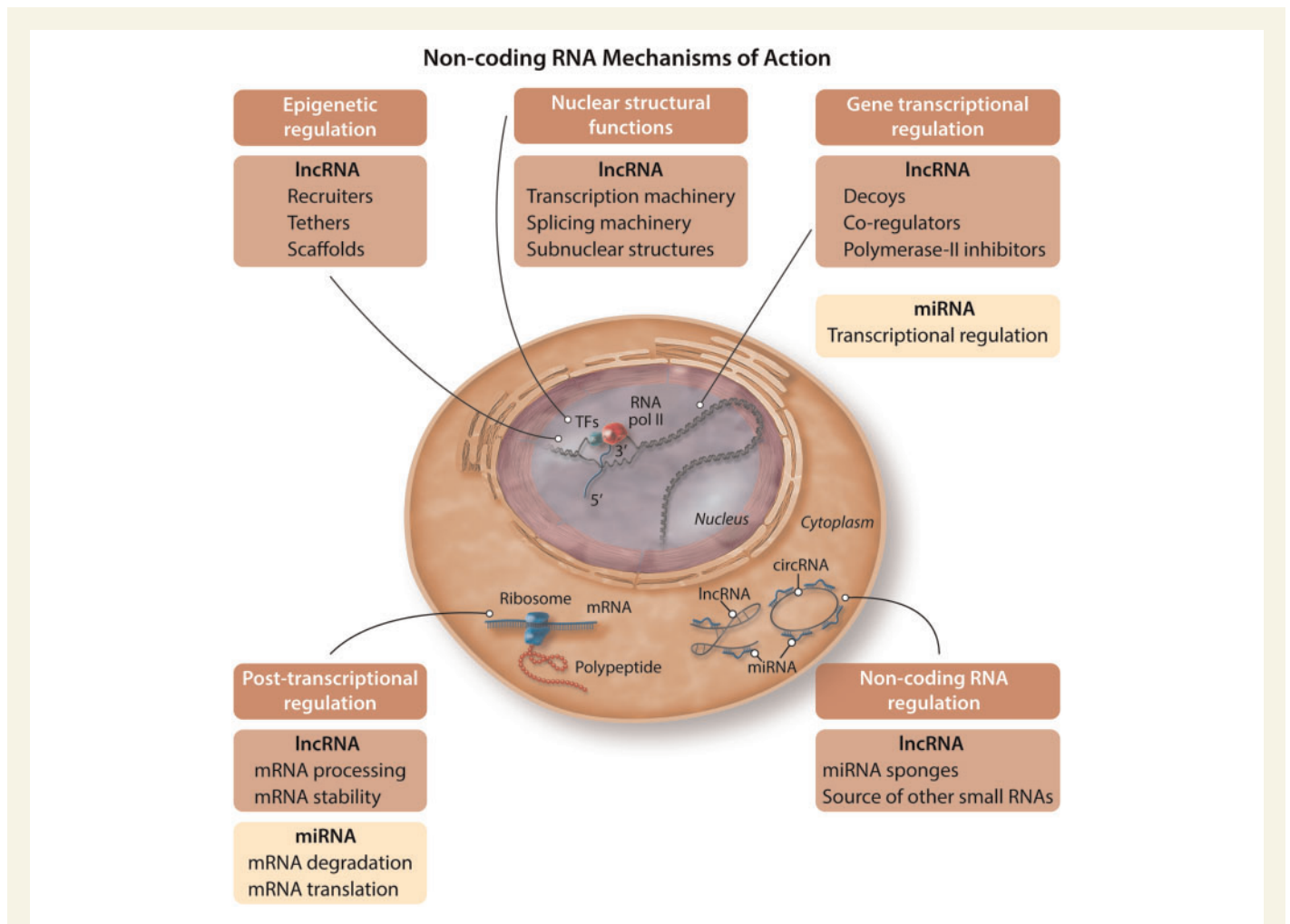


Figure 4 Non-coding RNA mechanisms of action. Examples of how non-coding RNAs interfere with biological processes in cells (e.g. transcriptional regulation, post-transcriptional regulation, and sponging of miRNAs).

outcomes.^{85,86} Indirectly, this also suggests that pro-angiogenic conditions may or may not be pro-EndMT, and vice versa.

8. miRNAs inhibiting EndMT

8.1 miR-15a, miR-23b, and miR-199a as inhibitors of EMT/EndMT during development

While the abovementioned miRNAs function to promote EndMT, others exhibit an inhibitory effect. For example, the levels of miR-15a, miR-23b, and miR-199a are elevated during AVC development in the embryonic chick heart, suggesting a role for these miRNAs in modulating both EMT and EndMT.⁸⁷ Indeed, miR-15a, miR-23b, or miR-199a inhibit EMT/EndMT in AVC explants.⁸⁷ In line with this, miR-23 also inhibits TGF- β -induced EMT/EndMT by inhibiting the TGF- β -induced expression ACTA2 and SNAIL.⁸⁸ Overexpression of miR-199a-5p in human umbilical vein endothelial cells (HUVECs) as well as irradiated HUVECs undergoing EndMT induced the expression of α -SMA and Collagen-1 in co-cultured human foetal lung fibroblasts.⁸⁹ This suggests that miR-199a-5p as well as EndMT in itself are important for myofibroblast activation of neighbouring fibroblasts.⁸⁹

8.2 The role of miR-483 in modulating EndMT

miR-483 reduces oscillatory flow-induced EndMT by decreasing the expression of SNAIL, SLUG, TWIST, and TAGLN,⁹⁰ which coincides with a reduction in inflammatory activation of IL-6, ICAM-1, and VCAM-1.⁹⁰ Concurrently, endothelial cells expressing miR-483 decrease the expression of mesenchymal markers induced by serum of Kawasaki patients, while increasing the expression of endothelial markers.⁹¹ Interestingly, statin (atorvastatin) treatment in combination with serum of Kawasaki patients induces the expression of miR-483 and similarly represses EndMT.⁹¹ This highlights the potential of pharmacological agents to interfere with the EndMT programme through non-coding RNAs. Moreover, it is tempting to speculate that repression of EndMT is among the so-called 'pleiotropic beneficial effects' attributed to statins.

8.3 The role of miR-148 in inhibiting EndMT

Fibrodysplasia ossificans progressiva is a congenital disorder associated with skeletal malformations and heterotopic calcification in which EndMT is involved. While gain of function mutations in ALK2 are associated with this disease, the activin A receptor type I (ACVR1) gene is also

implicated. Notably, both are associated with the TGF- β pathway signalling. ACVR1 is a target of miR-148a, and the constitutive activation of ACVR1 is known to induce EndMT. Indeed, expression of miR-148a represses BMP signalling in endothelial cells,⁹² suggesting that miR-148a can modulate EndMT. To our knowledge, this hypothesis has not been directly examined. In contrast, the expression of miR-148b does not decrease ACVR1 expression, implying that miR-148a and miR-148b have different gene targets.⁹³ Overexpression of miR-148b increases migration, proliferation and angiogenesis in HUVECs.⁹³ On the other hand, inhibition of miR-148b induces EndMT both *in vitro* and in a mouse model of skin wound healing.⁹³ In line with this, TNF- α and IL-1 β treatment in HUVECs decreased miR-148b levels and induced EndMT, which is precluded by the overexpression of miR-148b.⁹³

8.4 Let-7, miR-424, and miR-503 inhibit EndMT

The inflammation-induced loss of FGF signalling decreases the expression levels of Let-7, culminating in the activation of TGF- β signalling and thus EndMT.⁹⁴ Let-7b inhibits EndMT in a murine transplant arteriopathy model.⁹⁴ Moreover, the major plasma metabolite HT-3O sulfate, with antioxidant and anti-inflammatory properties, protects against IL-1 β -induced EndMT by restoring Let-7 expression.⁹⁵ IL-13 treatment induces EndMT accompanied by decreased levels of miR-424 and miR-503, suggesting a role for these miRNAs in modulating EndMT.⁹⁶ Indeed, inhibition of miR-424 increases the expression of the mesenchymal markers α -SMA and N-Cadherin.⁹⁶ Furthermore, miR-424 or miR-503 inhibit the migration of endothelial cells.⁹⁶

8.5 miR-18a-5p and miR-532 inhibit EndMT during cardiac fibrosis and myocardial infarction

miR-18a-5p inhibits glucose-induced EndMT by decreasing the expression of the mesenchymal markers S100A4, Vimentin, and Fibronectin and increasing the expression of CD31.⁹⁷ miR-18a-5p attenuates both cardiac fibrosis and EndMT in diabetic mice.⁹⁷ Since miR-18a-5p targets Notch2, this might explain the underlying mechanism of how miR-18a-5p inhibits EndMT.⁹⁷ Knockdown of another miRNA, miR-532, in a mouse model of myocardial infarction elevates the abundance of Collagen-1/CD31 and α -SMA/CD31 double-positive cells, indicative of active EndMT.⁹⁸ Indeed, knockdown of miR-532 enhanced TGF- β 2-induced EndMT by increasing the expression of Collagen-3, SNAIL, and ACTA2 while decreasing the expression of CD31 and vWF.⁹⁸

8.6 The role of miR-218, miR-221, miR-302c, and miR-494 in inhibiting EndMT

miR-302c inhibits EndMT *in vitro*.⁹⁹ Interestingly, co-implantation of a human hepatocellular carcinoma cell line and endothelial cells with loss or gain of 302c drastically differed hepatocellular carcinoma growth in mice, implying that miR-302c in endothelial cells may suppress endothelial cell-mediated tumour growth.⁹⁹ Reprogramming by the RhoA-Rock-canonical BMP signalling pathways is associated with increased expression of miR-302b and miR-302c.¹⁰⁰ This demonstrates the essential role of miR-302b/c in modulating endothelial cell behaviour. Another miRNA, miR-218, decreases CTGF expression, thereby increasing the expression of E-Cadherin while reducing Vimentin and Fibronectin expression in a human colon cancer cell line (HCT116 cells).¹⁰¹ When HUVECs were treated with conditioned medium from miR-218 overexpressing HCT116 cells, this suppressed angiogenesis.¹⁰¹ These data

suggest that miR-218 not only inhibits EndMT/EMT but also angiogenesis.¹⁰¹ miR-221 also suppresses angiogenesis by downregulating ZEB2 expression in HUVECs.¹⁰² Treatment of HUVECs with conditioned medium from miR-494 overexpressing decidual-derived mesenchymal stem cells also impairs capillary formation.¹⁰³ However, the role of miR-221 and miR-494 in regulating EndMT needs further elucidation.

8.7 miR-192, miR-194, miR-497, miR-29, and Let-7 as inhibitors of EndMT in kidney disease

miR-192 or miR-194 increase E-cadherin and decrease the expression of N-Cadherin and ZEB2,¹⁰⁴ suggesting that a decrease in miR-192 or miR-194 during autosomal dominant polycystic kidney disease contributes to EMT/EndMT.¹⁰⁴ Another miRNA which might be involved in inhibiting EndMT is miR-497.¹⁰⁵ Melatonin inhibits TGF- β 2-induced EndMT by attenuating the TGF- β 2-induced reduction in miR-497 expression, thereby suppressing the expression of ROCK1 and ROCK2.¹⁰⁵ Melatonin also reverses the loss of miR-497 and increase in EndMT in glomeruli of diabetic rats, showing the overall importance of miR-497 in the inhibition of EndMT in the context of kidney disease.¹⁰⁵ Linagliptin, a new dipeptidyl peptidase-4 (DPP-4) inhibitor that is used to treat diabetes, is another pharmacological agent that inhibits EndMT and ameliorates kidney fibrosis in diabetic mice.^{106,107} Importantly, linagliptin modulates miR-29 expression, and the miR-29 family suppresses EndMT, suggesting that linagliptin inhibits EndMT by restoring miR-29 expression levels in chronic kidney disease.¹⁰⁶ Diabetic mice also have decreased expression of N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP), an endogenous anti-fibrotic peptide, which is associated with insufficient levels of anti-fibrotic miRNAs in the kidney such as the miR-29 and Let-7 families.¹⁰⁸ Importantly, administration of AcSDKP to diabetic mice, and also to TGF- β 2-treated endothelial cells, decreases the expression of TGF- β 1 and SMAD3 phosphorylation.^{108,109} AcSDKP restores the expression of both the miR-29 and Let-7 miRNA families,¹⁰⁸ while Linagliptin reverses the decrease in expression of the Let-7 family via restoration of the FGF signalling.¹⁰⁹ This demonstrates that anti-fibrotic interventions induce a similar anti-fibrotic miRNA profile both *in vivo* and *in vitro*.^{106,108} Interestingly, overexpression of Let-7 in combination with TGF- β 2 treatment induces the expression of the miR-29 family, and vice versa.¹⁰⁸ This suggests a crosstalk between the miR-29 and Let-7 miRNA families in facilitating anti-fibrotic and EndMT inhibitory effects.¹⁰⁸

9. miRNAs which have different effects on EndMT in development and pathology

9.1 The role of the miR-200 family in modulating EndMT

miR-200a inhibits TGF- β -induced EndMT.¹¹⁰ Similarly, another miR-200 family member, miR-200b, prevents both glucose- and TGF- β 1-induced EndMT.¹¹¹ Endothelial cell-specific overexpression of miR-200b in diabetic mice prevents glucose-induced EndMT in the heart as well as in retinal tissues.^{111,112} In addition, overexpression of miR-200b associates with angiogenesis suppression.¹¹³ Downregulation of the miR-200 family results in the upregulation of the EndMT transcription factors SNAIL and ZEB1.¹¹⁴ This underlines the role of the miR-200 family in inhibiting EndMT. Individuals with bicuspid aortic valves (and therefore a higher

risk of developing aortic aneurysm) have lower expression of miR-200c suggesting potential activation of both EMT and EndMT.¹¹⁴ Individuals with bicuspid aortic valves are also associated with a non-coding variant, called rs6601627, which is suggested to interact with GATA4.¹¹⁵ Importantly, CRISPR/Cas9-mediated disruption of GATA4 impairs TGF- β 2 and BMP2-induced EndMT in endothelial cells derived from human induced pluripotent stem cells.¹¹⁵ This demonstrates that non-coding RNAs are important in regulating both EMT and EndMT in aortic valves. In contrast to the above, during development, SNAIL-induced repression of the miR-200 family promotes the generation of Flk1-positive endothelial cells, suggesting that during development the miR-200 family has an opposite role and supports the maintenance of the endothelial character.¹¹⁶ Indeed, lower levels of the miR-200 family in human embryonic stem cells is also associated with differentiation into vascular endothelial cells.¹¹⁷ Furthermore, miR-200a did not affect EMT/EndMT during development of the AVC in the developing chicken heart.⁸⁷ This suggests that the miR-200 family has different roles during development and pathological conditions in different species.

9.2 The role of miR-126 in regulating EndMT

Combined knockdown of ERG and FLI1 induces EndMT which is accompanied by low miR-126 expression levels, suggesting a role for miR-126 in modulating EndMT.¹¹⁸ Indeed, miR-126 limits the expression of ACTA2, TAGLN, Collagen-1, and SLUG and partially counteracts the reduction in VE-Cadherin and CD31 expression.¹¹⁸ Interestingly, treatment of HUVECs with conditioned medium from tumours also resulted in decreased expression of both ERG and FLI1, suggesting that the decrease of these transcription factors by soluble mediators from the tumour microenvironment can promote EndMT and therewith tumour progression.¹¹⁸ In line with this, miR-126 suppresses the expression of the mesenchymal genes ACTA2, TAGLN, and myocardin while maintaining the expression of progenitor markers.¹¹⁹ Also, miR-126 reverses the TGF- β 1-induced activation of FoxO3 and SMAD4 and decrease in PI3K and Akt, suggesting novel pathways involved in the modulation of EndMT.¹¹⁹ In contrast to the above data, knockdown of miR-126a-5p reverses the hypoxia-induced decrease in CD31 and increase in α -SMA.¹²⁰ Altogether, while the majority of studies suggest that miR-126 inhibits EndMT, it appears that in specific contexts miR-126 may exert pro-EndMT effects.

9.3 The role of miR-155 and miR-20a in modulating EndMT

Inhibition of miR-155 reverses the TGF- β -induced expression of SNAIL, SLUG, TWIST, and Vimentin, suggesting that miR-155 inhibits EndMT.¹²¹ In contrast, inhibition of miR-155 does not affect TGF- β 3-induced EndMT in mouse embryonic endothelial cells.¹²² Moreover, over-expression of miR-155 inhibits TGF- β 3-induced EndMT, suggesting that induction of miR-155 expression represses EndMT.¹²² This demonstrates that the mechanism by which miR-155 regulates EndMT is different during developmental and pathological contexts, which might be explained by the maturity of the cells or the difference in TGF- β isoform. miR-20a also has differential effect on EndMT in development and adult pathology. During the development of the OFT in the mouse embryos, deletion of BMP4/7 inhibits EMT/EndMT which could be rescued by the expression of miR-17/20a, indicative of an EndMT inductor role for this miRNA.¹²³ In contrast, FGF2-induced expression of miR-20a limits EndMT by reducing the expression of TGF- β receptor 1/2 and SARA

(which recruits SMAD2 and SMAD3 to the TGF- β receptor complex) in adult endothelial cells.¹²⁴

In all, a remarkable number of miRNAs are associated to the EndMT regulatory programme. These are summarized in *Figure 5* and *Table 1*.

10. lncRNAs promoting EndMT

Among all the lncRNAs identified, only a few lncRNAs have been implicated in the regulation of EndMT hitherto. Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is a lncRNA extensively associated with cancer metastasis and recently described as an EndMT inducer. MALAT1 competitively binds to miR-145, a known miRNA that inhibits TGF- β 1-induced EndMT by directly targeting TGF- β 2 and SMAD3. Acting as a miRNA sponge, MALAT1 blocks the inhibitory activity of the miR-145 and promotes EndMT.¹²⁵ Another lncRNA identified as an EndMT promoter is GATA6-AS. The long non-coding antisense transcript of GATA6 (GATA6-AS) interacts with the epigenetic regulator LOXL2 to regulate endothelial gene expression via changes in H3K4me3 methylation, including genes encoding for periostin and cyclooxygenase-2. GATA6-AS expression is induced in endothelial cells by hypoxia. Inhibition of GATA6-AS blocks TGF- β 2-induced EndMT *in vitro* and promotes blood vessel formation in mice.¹²⁶ A third lncRNA that promotes EndMT is PVT1. PVT1 associates with tumour cell proliferation, invasion, and metastasis in different cancer models. In prostate cancer, PVT1 promotes cancer invasion and metastasis in part by the induction of EndMT. PVT1 promotes EndMT by acting as a sponge for miRNA-186-5p and positively regulating TWIST1.¹²⁷

11. lncRNAs inhibiting EndMT

H19 prevents glucose-induced TGF- β 1 expression and therefore, EndMT in diabetic retinopathy models. H19 controls TGF- β 1 mRNA and protein levels through a SMAD-independent MAPK-ERK1/2 pathway, and this regulation is also independent of the actions of miR-200b, a known miRNA (see above) that interacts with H19.¹²⁸ Besides the lncRNAs mentioned above, lncRNA n339260, although not directly related to EndMT, controls the expression of VE-Cadherin during the process of vascular mimicry in a human hepatocellular carcinoma.¹²⁹ Maintenance or reduction of VE-Cadherin in endothelial cells is a critical step in EndMT and therefore, future studies are needed to understand the potential role of lncRNA n339260 in EndMT. Furthermore, this study provided a number of candidate miRNAs that may be regulated by lncRNA n339260 including miR-31-3p, miR-30e-5p, miR-519c-5p, miR-520c-5p, miR-29b-1-5p, and miR-92a-1-5p. These will also require further testing to determine their potential role in the EndMT process. A summary of the currently known lncRNAs that modulate EndMT is provided in *Figure 6* and *Table 2*. We expect this list to grow significantly in the coming years.

12. circRNAs promoting EndMT

circRNAs that are known to promote EndMT include CircHECW2 and CircRNA-MYLK. CircHECW2 promotes EndMT by acting as a sponge for miR-30d and culminating in the increased expression of ATG5, which promotes the activation of the Notch1 pathway to induce pathologic EndMT.¹³⁰ In the case of CircRNA-MYLK, EndMT is promoted by directly binding and inhibiting miR-29a.¹³¹ This promotes EndMT by

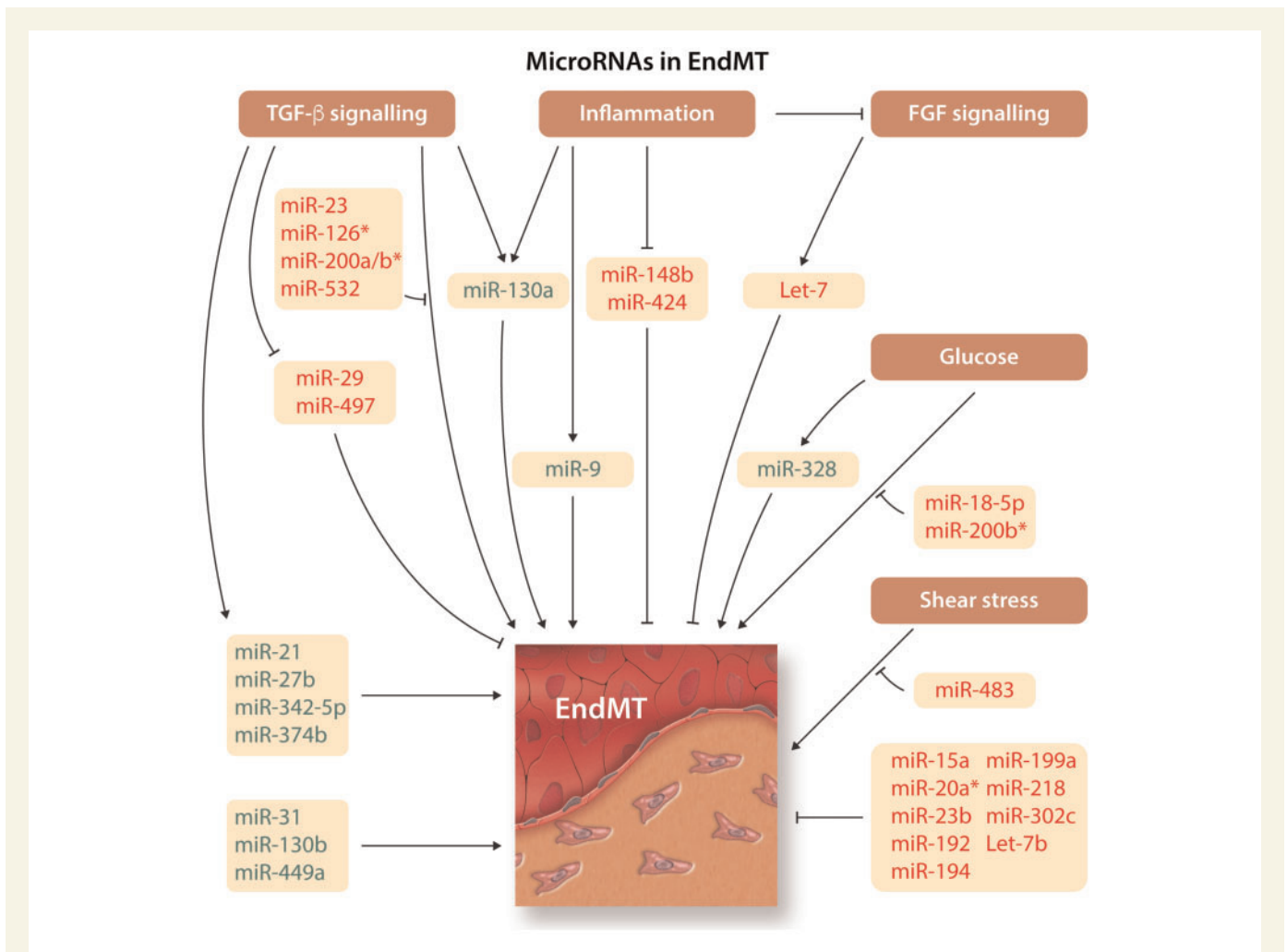


Figure 5 MicroRNA in EndMT Overview of microRNAs (miRNAs) that induce or inhibit EndMT. miRNA inducers and inhibitors of EndMT are depicted in green and red, respectively. *A different role during pathology and development.

activating the TGF- β , NF- κ B, and β -catenin pathways.^{132,133} Besides acting as miRNA sponges, circRNAs can control EndMT by directly regulating the expression of genes. In this regard, circHECTD1 regulates the expression of its host gene E6-AP C-terminal domain E3 ubiquitin protein ligase 1 (HECTD1), which is implicated in the maintenance of endothelial fate and controlled by Wnt signalling through APC-Axin interactions.¹³⁴ Increased expression of circHECTD1 induces EndMT and its derivative fibroblast-like cells contribute to pulmonary fibrosis.¹³⁵

13. circRNAs inhibiting EndMT

Among the circRNAs, the only currently identified EndMT inhibitor is circRNA DLGAP4. DLGAP4 functions as an endogenous miR-143 sponge to allow for the expression of HECTD1, as mentioned above. circRNA DLGAP4 inhibits EndMT and promotes maintenance of endothelial integrity and in the case of cerebral ischaemia it maintains the integrity of the blood–brain barrier.¹³⁶ In addition, circRNAs chr5:90817794|90827570, chr8:71336875|71337745, and chr6:22033342|22038870, were found to be increased in a circRNA screening performed on an EndMT assay,¹³⁷

although their specific functions remain unknown. circRNAs that are currently known to modulate EndMT are summarized in *Figure 6* and *Table 3*.

The identification of the functional relevance of lncRNAs and circRNAs in EndMT is a fast growing field of research. It is expected that our understanding of the regulatory roles of lncRNAs and circRNAs in EndMT will increase accordingly in the coming years and so our understanding of physiologic and pathologic EndMT.

14. Future perspectives: targeting non-coding RNAs to modulate EndMT

As this review has highlighted, non-coding RNAs are key players in the control and modulation of EndMT (*Figure 5* and *6*). However, our overall knowledge of the role of non-coding RNAs in EndMT is in its infancy. Despite this, taking the data reviewed in this article as a whole, we are already able to draw several important conclusions about this field. Firstly, it is immediately

Table 1 MicroRNAs in EndMT

miRNAs	Biological context	Targets	Experimental model
Inducing EndMT			
miR-9	Development/Pathology— Lymphangiogenesis/Inflammation	NF-κB1	<i>In vitro</i> —Rat mesenteric lymphatic endothelial cells ⁸⁵
miR-17	Development—OFT formation	Vegfa	<i>In vivo</i> —Mouse embryos ¹²³ <i>Ex vivo</i> —OFT explant culture
miR-21	Pathology—Fibrosis	?	<i>In vitro</i> —HUVECs ^{76,77} <i>In vivo</i> —Mouse model of cardiac pressure overload
miR-27b	Molecular characterization	Elk1, Nrp2, PlxnA2, PlxnD1	<i>In vitro</i> —Mouse pancreatic microvascular endothelial cells ⁷⁵
miR-31	Pathology—Inflammation	VAV3	<i>In vitro</i> —Mouse pancreatic microvascular endothelial cells ⁷⁹
miR-130a	Pathology—Pulmonary hypertension	BMPR2	<i>In vitro</i> —Lung microvascular endothelial cells ⁸¹ <i>In vivo</i> —pulmonary arterial hypertension mouse model
miR-130b	Pathology—Colorectal cancer	PPARγ	<i>In vitro</i> —Human colorectal adenocarcinoma cell lines ⁸⁰ <i>In vivo</i> —Mouse xenografts and human colorectal cancer samples
miR-143	Pathology—Ischaemic stroke	HECTD1	<i>In vivo</i> —Mouse brain ¹³⁶
miR-328	Pathology—Diabetes	?	<i>In vitro</i> —HUVECs ⁸⁴
miR-342-5p	Development—Angiogenesis	Endoglin	<i>In vitro</i> —HUVECs ⁸⁶ <i>In vivo</i> —Mouse retinal vasculature <i>Ex vivo</i> —Mouse aortic rings
miR-374b	Pathology—Neointimal hyperplasia	RAC1, MAP3K3, MAP3K7, MAPK7, MEF2D, KLF4	<i>In vitro</i> —HUVECs ⁸³ <i>In vivo</i> —Porcine abdominal trifurcations and human coronary arteries
miR-449a	Pathology—Atherosclerosis	AdipoR2	<i>In vitro</i> —HUVECs ⁸² <i>In vivo</i> —Diabetic mice and human carotid atherosclerotic plaques
Inhibiting EndMT			
miR-15a	Development—AVC formation	?	<i>Ex vivo</i> —Chicken AVC explants ⁸⁷
miR-18a-5p	Pathology—Diabetes	Notch2	<i>In vitro</i> —Human aortic valvular endothelial cells ⁹⁷ <i>In vivo</i> —Diabetic mice
miR-23b	Development—AVC formation Development—Cardiac valve formation	?	<i>Ex vivo</i> —Chicken AVC explants ⁸⁷ <i>In vitro</i> —Mouse embryonic endothelial cells ⁸⁸ <i>In vivo</i> —Zebrafish
miR-29a	Pathology—Bladder carcinoma	VEGFA	<i>In vitro</i> —Human bladder carcinoma cells ¹³¹ <i>In vivo</i> —Mouse xenografts
miR-29 family	Pathology—Diabetes-related kidney fibrosis	DPP-4	<i>In vitro</i> —Human dermal microvascular endothelial cells ^{106,108} <i>In vivo</i> —Fibrotic diabetic kidney disease mouse model
miR-30d	Pathology—Neuroinflammatory disorders	ATG5	<i>In vitro</i> —Human brain microvascular endothelial cells ¹³⁰ <i>In vivo</i> —Mouse brain
miR-145	Pathology—Neointimal hyperplasia	TGFBR2, SMAD3	<i>In vitro</i> —Endothelial progenitor cells ¹²⁵
miR-148b	Physiology—Skin wound healing	TGFBR2, SMAD2	<i>In vitro</i> —HUVECs ⁹³ <i>In vivo</i> —Mouse model of skin wound healing
miR-186-5p	Pathology—Prostate cancer	Twist1	<i>In vitro</i> —Prostate cancer cells ¹²⁷
miR-192/194	Pathology—Kidney disease	ZEB2, CDH2	<i>In vitro</i> —Renal epithelial cells ¹⁰⁴ <i>In vivo</i> —Autosomal dominant polycystic kidney disease mouse model and human samples
miR-199a	Development—AVC formation Pathology—Radiation-induced pulmonary fibrosis	?	<i>Ex vivo</i> —Chicken AVC explants ⁸⁷ <i>In vitro</i> —HUVECs ⁸⁹
miR-218	Pathology—Colorectal cancer	CTGF	<i>In vitro</i> —HUVECs and human colon cancer cell line ¹⁰¹ <i>In vivo</i> —Human colorectal cancer samples

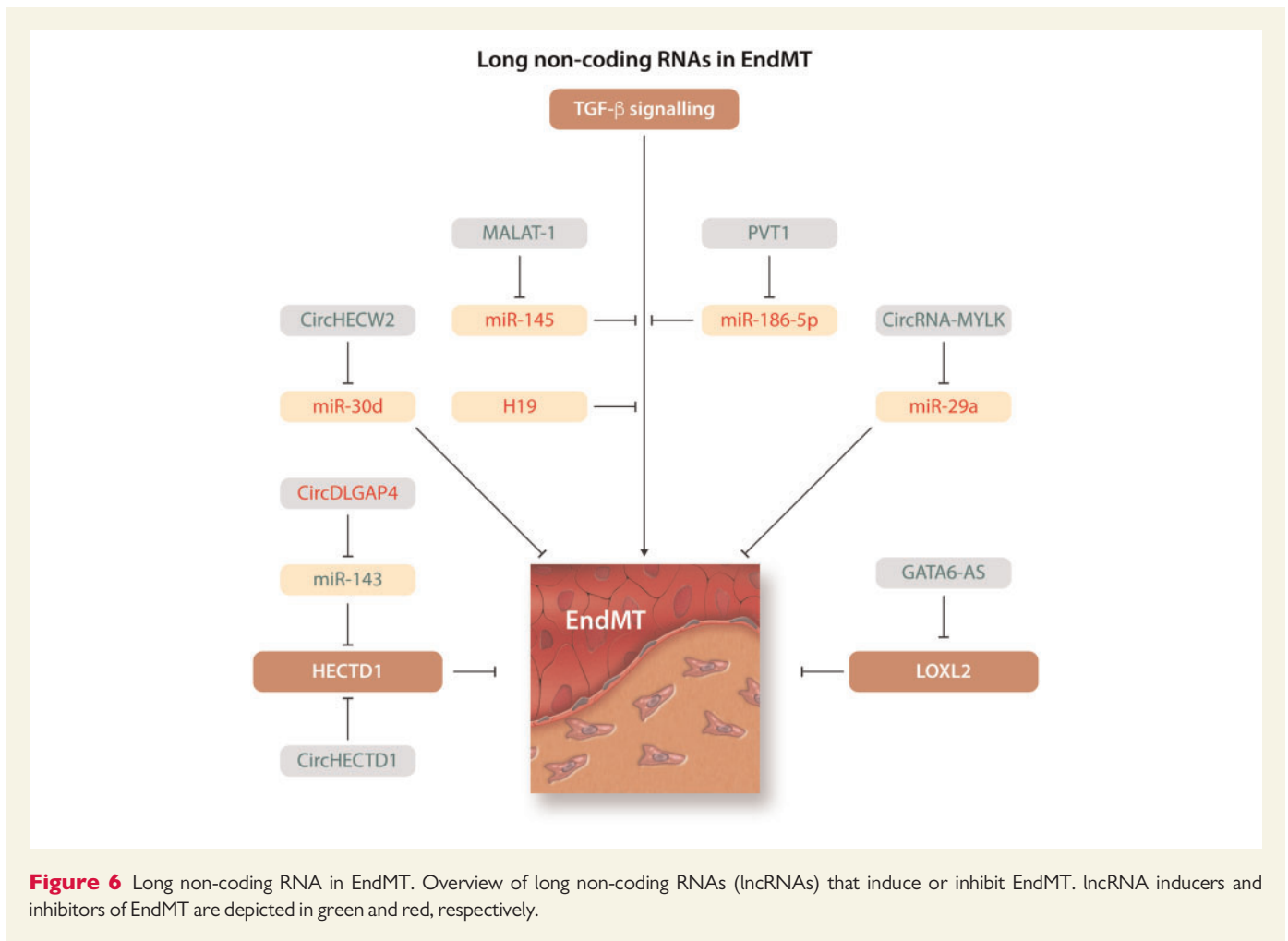
Continued

Table 1 Continued

miRNAs	Biological context	Targets	Experimental model
miR-221	Pathology—Tumour angiogenesis	ZEB2	<i>In vitro</i> —HUVECs ¹⁰²
miR-302c	Pathology—Hepatocellular carcinoma	MTDH	<i>In vitro</i> —Human hepatocellular carcinoma cell line and HUVECs ⁹⁹
	Pathology—Corneal blindness	?	<i>In vivo</i> —Ectopic transplant mouse model
miR-424/503	Pathology—Pulmonary hypertension	?	<i>In vitro</i> —Human corneal endothelial cells ¹⁰⁰
miR-483	Pathology—Aortic valve calcification	?	<i>In vitro</i> —Human pulmonary artery endothelial cells ⁹⁶
	Pathology—Kawasaki disease	CTGF	<i>In vitro</i> —Human aortic valve endothelial cells ⁹⁰
			<i>In vivo</i> —Porcine aortic valve leaflets
			<i>In vitro</i> —Lung endothelial cells from endothelial KLF4 transgenic mice and HUVECs ⁹¹
			<i>In vivo</i> —Sera from Kawasaki disease patients
miR-494	Pathology—Preeclampsia	VEGF	<i>In vitro</i> —HUVECs ¹⁰³
miR-497	Pathology—Diabetic Nephropathy	ROCK1/2	<i>In vitro</i> —Human renal glomerular endothelial cells ¹⁰⁵
			<i>In vivo</i> —Diabetic rats
miR-532	Pathology—Myocardial Infarction	prss23	<i>In vitro</i> —Mouse cardiac endothelial cells ⁹⁸
			<i>In vivo</i> —Mouse model of myocardial infarction
Let-7 family	Pathology—Neointima formation	?	<i>In vitro</i> —Primary mouse endothelial cells ⁵⁸
			<i>In vivo</i> —Mouse arterial transplant model and human coronary arteries
	Pathology—Inflammation	?	<i>In vitro</i> —HUVECs and human retinal endothelial cells ⁹⁵
	Pathology—Diabetes-related kidney fibrosis	?	<i>In vitro</i> —Human dermal microvascular endothelial cells and HUVECs ^{108,109}
			<i>In vivo</i> —Fibrotic diabetic kidney disease mouse model
Differential effects on EndMT			
miR-20a	Development—Outflow tract cushion development	Vegfa	<i>In vivo</i> —Mouse embryos ¹²³
			<i>Ex vivo</i> —Outflow tract explant culture
	Pathology—Molecular characterization	TGFBR1/2, SARA	<i>In vitro</i> —HUVECs ¹²⁴
miR-126	Pathology—Tumour progression	?	<i>In vitro</i> —HUVECs ¹¹⁸
	Pathology—Neointimal hyperplasia	PIK3R2	<i>In vivo</i> —Tumour implantation mouse model
			<i>In vitro</i> —Rat bone marrow-derived endothelial progenitor cells ¹¹⁹
	Development/Pathology—Pulmonary vasculature remodelling	?	<i>In vitro</i> —Rat pulmonary microvascular endothelial cells
			<i>In vivo</i> —Mouse model of persistent pulmonary hypertension of the newborn ¹²⁰
miR-155	Pathology—Molecular characterization	SKI	<i>In vitro</i> —Human coronary artery endothelial cells ¹²¹
	Development—Molecular characterization	?	<i>In vitro</i> —Mouse embryonic endothelial cells ¹²²
miR-200a	Pathology—Cardiac fibrosis	GRB2	<i>In vitro</i> —Human aortic endothelial cells ¹¹⁰
miR-200b	Pathology—Diabetic retinopathy	?	<i>In vitro</i> —Human retinal microvascular cells ¹¹¹
			<i>In vivo</i> —Diabetic mice
	Pathology—Diabetic cardiomyopathy	?	<i>In vitro</i> —Mouse heart endothelial cells ¹¹²
			<i>In vivo</i> —Diabetic mice
	Pathology/Physiology—Angiogenesis	Ets1	<i>In vitro</i> —Human microvascular endothelial cells ¹¹³
miR-200c	Pathology—Aneurysm formation	?	<i>In vitro</i> —EA.hy926 endothelial cells ¹¹⁴
			<i>In vivo</i> —Non-dilated aortas from patients with bicuspid aortic valves
miR-200 family	Development—Vasculogenesis	Flk1, Ets1	<i>In vitro</i> —Mouse embryonic stem cells ¹¹⁶
	Development—Differentiation	?	<i>In vitro</i> —Human embryonic stem cells ¹¹⁷

List of miRNAs regulating EndMT categorized into their inducing, inhibiting or differential role on EndMT. Question mark refers to unknown targets in this context.

AdipoR2, adiponectin receptor 2; ATG5, autophagy related 5; BMPR2, bone morphogenetic protein receptor 2; CDH2, cadherin-2; CTGF, connective tissue growth factor; DPP-4, dipeptidyl peptidase-4; Elk1, ETS transcription factor; Ets1, protein c-ets-1; Flk1, foetal liver kinase 1; GRB2, growth factor receptor-bound protein 2; Has2, hyaluronic acid synthase 2; Icat, beta-catenin-interacting protein; KLF4, kruppel-like factor 4; MAP3K3/7, mitogen-activated protein kinase kinase kinase 3/7; MAPK7, mitogen-activated protein kinase 7; MEF2D, myocyte-specific enhancer factor 2D; MTDH, metadherin; NF- κ B, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrp2, neuropilin 2; PIK3R2, PI3K regulatory subunit p85 beta; PlxnA2, plexin A2; PlxnD1, plexin D1; PPAR γ , peroxisome proliferator-activated receptor γ ; RAC1, ras-related C3 botulinum toxin substrate 1; ROCK, rho-associated, coiled-coil-containing protein kinase; SARA, Smad anchor for receptor activation; SKI, SKI proto-oncogene; SMAD, SMAD family member; TGF β , transforming growth factor beta; TGFBR, transforming growth factor beta receptor; Tmem2, transmembrane protein 2; Twist1, twist-related protein 1; VAV3, guanine nucleotide exchange factor VAV3; VEGF, vascular endothelial growth factor; ZEB2, zinc finger E-box-binding homeobox 2.

**Table 2** Known lncRNAs involved in EndMT

lncRNAs	Biological context	Targets	Experimental model
Inducing EndMT			
GATA6-AS	Development—Angiogenesis	LOXL2	<i>In vitro</i> —HUVECs ¹²⁶
MALAT1	Pathology—Neointimal hyperplasia	miR-145 that targets TGFBR2 and SMAD3	<i>In vitro</i> —Endothelial progenitor cells ¹²⁵
PVT1	Pathology—Prostate cancer	miR-186-5p that targets Twist1	<i>In vitro</i> —Prostate cancer cells ¹²⁷
Inhibiting EndMT			
H19	Pathology—Diabetic retinopathy	TGF-β1 through control of MAPK-ERK1/2 pathway	<i>In vitro</i> —Human retinal endothelial cells ¹²⁸ <i>In vivo</i> —Diabetic retinopathy mouse model and human samples

List of lncRNAs currently known to regulate EndMT categorized into their inducing or inhibiting role on EndMT.

ERK, extracellular signal-regulated kinase; LOXL2, lysyl oxidase homolog 2; MAPK, mitogen-activated protein kinase; SMAD, SMAD family member; TGF-β1, transforming growth factor beta 1; TGFTGFR, transforming growth factor beta receptor; Twist1, twist-related protein 1.

clear that some non-coding RNAs inhibit EndMT such as miR-29, whereas others facilitate or induce EndMT such as miR-21, GATA6-AS, and HECTD1 (Tables 1, 2, and 3). Second, most of these non-coding RNAs have only been described in a single context and we do not know if their roles may differ in other contexts. In addition, different markers for EndMT are used in different studies which might not give us the full pictures of how a certain non-coding RNA affects EndMT. The difference in function of these non-coding RNAs between development and various pathological states

might also be different and needs to be established. Third, non-coding RNAs also interact with other epigenetic modulators such as DNA methylation and histone modifications to affect EndMT.²⁷ These other epigenetic modulators affecting EndMT are not the focus of this review but are described elsewhere.²⁷ Fourth, it is thought that EMT and EndMT occur via similar mechanisms. Importantly, the miRNA signatures between human corneal endothelium tissues and corneal epithelium tissues did not differ much even though the gene signatures were very different.¹³⁸ This might suggest that

Table 3 Known circRNAs involved in EndMT

circRNAs	Biological context	Targets	Experimental model
Inducing EndMT			
CircHECW2	Pathology—Neuroinflammatory disorders	miR-30d that targets ATG5	<i>In vitro</i> —Human brain microvascular endothelial cells ¹³⁰ <i>In vivo</i> —Mouse brain
CircRNA-MYLK	Pathology—Bladder carcinoma	miR-29a that targets VEGFA	<i>In vitro</i> —Human bladder carcinoma cells ¹³¹ <i>In vivo</i> —Mouse xenografts
CircHECTD1	Pathology—Pulmonary disease	HECTD1	<i>In vitro</i> —Mouse microvascular lung cells ¹³⁵ <i>In vivo</i> —Lung silicosis in mouse model and human samples
Inhibiting EndMT			
CircDLGAP4	Pathology—Ischaemic stroke	miR-143 that targets HECTD1	<i>In vivo</i> —Mouse brain ¹³⁶

List of circRNAs regulating EndMT categorized into their inducing or inhibiting role on EndMT.

ATG5, autophagy related 5; HECTD, HECT domain E3 ubiquitin protein ligase; VEGF, vascular endothelial growth factor.

non-coding RNAs function in similar ways in both epithelial and endothelial cells. Of course, we have to establish whether this is similar for other tissues as well. As a further point of consideration, it has been shown that the miRNA signatures of cultured human corneal endothelial cells were very different than those from fresh corneal endothelium tissue.¹³⁸ The same is true for cultured HUVECs when comparing them to freshly isolate tissue-derived umbilical cord human vascular endothelial cells.¹³⁹ Furthermore, the overall miRNA expression also decreased significantly in cultured HUVECs when compared to tissue-derived endothelial cells, and miRNA content appears to be lost during passaging of HUVECs.¹³⁹ The most downregulated miRNA during culturing is miR-126 and the most upregulated miRNA is miR-21.¹³⁹ Also during culturing of HUVECs, the pro-fibrotic miRNAs miR-21-5p and miR-31-5p were upregulated whereas the anti-fibrotic miRNAs miR-126-3p, Let-7 family, and miR-29 family were downregulated, suggesting a transition towards a mesenchymal cell type in aging cells.¹³⁹ As a whole this indicates that the miRNA profile is highly adaptable and that cultured cells may not always represent the best model system for studying miRNA function—proper *in vivo* validation will remain as the gold standard.

It is clear in this emerging field that non-coding RNAs appear to be important players during EndMT. Indeed, as we have reviewed, non-coding RNAs interact with EndMT transcription factors, plus endothelial and mesenchymal genes and signalling pathways, ascribing them a likely pivotal role in regulating this process. As such, they represent promising future clinical targets for modulating EndMT and its accompanied pathologies.^{140,141} As a whole, the regulation of EndMT via non-coding RNAs represents a challenging but promising field with many potential opportunities for future therapeutic clinical translation.

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