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REVIEW

Epigenetic influences on genetically triggered thoracic aortic aneurysm

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Abstract

Genetically triggered thoracic aortic aneurysms (TAAs) account for 30% of all TAAs and can result in early morbidity and mortality in affected individuals. Epigenetic factors are now recognised to influence the phenotype of many genetically triggered conditions and have become an area of interest because of the potential for therapeutic manipulation. Major epigenetic modulators include DNA methylation, histone modification and non-coding RNA. This review examines epigenetic modulators that have been significantly associated with genetically triggered TAAs and their potential utility for translation to clinical practice.

Keywords Thoracic aortic aneurysm · Aortic dilatation · Epigenetics · Non-coding RNA · MicroRNA

Introduction

Thoracic aortic aneurysm (TAA) is a genetically and phenotypically diverse condition characterised by the progressive permanent dilatation of the thoracic aorta, predisposing to aortic rupture and dissection (Goldfinger et al. 2014). TAA is predominantly clinically silent and develops on a background of adverse remodelling of the aortic wall, which may be inherited or acquired.

Genetically triggered TAAs account for up to 30% of all TAAs (Clouse et al. 1998; Albornoz et al. 2006) and are often consequent upon pathological variants in genes encoding key proteins in either vascular smooth muscle cells (VSMCs), the extracellular matrix (ECM) or transforming growth factor-beta (TGF-β) signalling. Non-genetic TAA is usually observed in older patients with comorbidities including hypertension and atherosclerosis (Goldfinger et al. 2014). Genetically triggered TAA is observed in the clinical syndromes of Marfan (MFS), Loeys-Dietz (LDS) and vascular Ehlers-Danlos (vEDS), and also in association with a bicuspid aortic valve (BAV). In other individuals, TAA may occur in the absence of other clinically discernible features (non-syndromal Thoracic Aortic Aneurysm and Dissection (nsTAAD)). Current medical therapy is variably effective at slowing the rate of dilatation, with increasing dimensions conferring an increase in the risk of dissection, which carries a mortality rate of up to 50% (Melvinsdottir et al. 2016). Most patients will ultimately require surgical repair of the aneurysm, with mortality risk ranging from 1 to 5% for elective repair (Kallenbach et al. 2013) and up to 12% for emergency intervention (Goldfinger et al. 2014).

Phenotype variability is well-documented in all forms of genetically triggered TAA and is particularly highlighted in cases where an identical pathogenic gene variant results in different clinical manifestations (De Backer et al. 2007; Loeys 2016). This phenotype variability significantly impacts the ability to predict clinical outcomes, resulting in uncertainty for both clinicians and patients. Therefore, there is a need to develop more sophisticated risk profiling that aims to provide a precision-medicine model for patient management.

Epigenetics: regulators of phenotype

The emerging characterisation of the "epigenome", comprising endogenous mediators that regulate gene expression independent of the DNA sequence, has revolutionised our understanding of health and disease beyond traditional Mendelian

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genetics. "Epigenetics" refers to mechanisms of gene expression regulation by DNA methylation, histone modification and non-coding RNA, such as microRNA (miRNA) and long non-coding RNA (lncRNA) (Tollefsbol 2018). The activity of these mediators is dynamically influenced by environmental stimuli, leading to stable changes in gene expression that are maintained during cell division and can be passed through generations. Accordingly, aberrant epigenetic changes are associated with many disease states (Esteller 2011; Tollefsbol 2018). Characterisation of these changes has resulted in improved understanding of pathological disease mechanisms, as well as the development of disease-specific biological profiles. Importantly, epigenetic mediators have been demonstrated to be modifiable therapeutic targets (Conway et al. 2016), thus providing enormous clinical potential, with the capacity to improve diagnosis, monitoring and treatment of a wide range of diseases.

With epigenetics increasingly recognised in other genetic conditions as modulators of penetrance and expressivity (Feinberg 2007), there is a strong theoretical basis for these mechanisms also occurring in genetically triggered TAA. Detection of aberrant epigenetic changes in TAA will enable a deeper understanding of pathogenesis and may provide explanation for the observed phenotype variation among individuals. In turn, such knowledge may also assist the clinical decision-making process with regard to the optimal time to undertake surgical repair and also provide a stronger mechanistic basis for new drug design towards slowing progression and mitigate the need for surgery. This review provides an update on epigenetic changes found to be associated with genetically triggered TAA, with a summary of progress in translation to clinical practice.

Pathogenesis of thoracic aortic aneurysm

Normal aortic wall architecture

The aortic wall consists of three distinct layers: the outermost adventitia, which contains connective tissue and ECM for tensile support, in addition to local blood and neural supply; the media, which consists of contractile lamellar units comprised of concentric layers of contractile VSMCs interspersed between elastic fibres and surrounded by ECM; and the intima, containing the endothelial surface and subendothelial connective tissue. Collectively, these components enable the aortic wall to tolerate the large changes in pressure that are associated with ventricular contraction, with graded contraction of the VSMCs responsible for reducing the highly pulsatile ventricular flow to a steady, continuous propagation through the vasculature (Belz and Belz 1995). The mechanical properties of the aorta are also maintained through diverse signalling processes within and between the three aortic wall layers, including cell-cell and cell-ECM interactions, mechanotransduction complexes that sense and communicate aortic stretch and responses to external signals, such as neural and hormonal stimuli (Humphrey et al. 2015).

Gene variants

Thoracic aortic aneurysm formation has been linked to variants in a number of genes that are involved in homeostasis of the aortic wall. These include genes that encode proteins responsible for (a) ECM regulation (*FBN1*, *COL3A1*, *LOX*, *MFAP5*, *BGN*), (b) the VSMC contractile apparatus (*MYH11*, *ACTA2*, *MYLK*, *FLNA*, *PRKG1*) or (c) TGF-β signalling (*TGFB2*, *TGFB3*, *TGFBR1*, *TGFBR2*, *SMAD3*) (Verhagen et al. 2018).

Histopathology

Regardless of the gene affected, there is a unifying histopathological change in the aortic wall in genetically triggered TAAs, defined as medial degeneration (Fig. 1) (Halushka et al. 2016). The most prominent feature is the fragmentation, disorganisation and loss of elastic fibres. This has been associated with a pathological imbalance of the molecules that regulate the ECM, primarily the induction of matrix metalloproteinases (MMPs) or reduction of their endogenous regulators, tissue inhibitors of metalloproteinases (TIMPs) (Rabkin 2014). The fragmentation of elastic fibres, along with increased collagen deposition decreases the compliant nature of the aorta, reducing its recoiling ability and increasing wall stiffness (Emmott et al. 2016). In addition, there is loss of VSMCs due to apoptosis or necrosis and focal accumulation of mucoid ECM material, resulting in the loss and/or disruption of aortic contractile units. Together, the loss of structural integrity and mechanical strength in the aortic wall reduces its ability to manage normal haemodynamic loads, consequently predisposing to aortic dissection (Humphrey et al. 2015).

Conditions associated with genetically triggered TAA

Marfan syndrome

Marfan syndrome (MFS) is characterised by multisystem features affecting the cardiovascular, ocular and skeletal systems (Loeys et al. 2010), with an estimated prevalence of 6.5 per 100,000 (Groth et al. 2015). TAA is the most common life-threatening complication in MFS, affecting up to 90% of patients (Hiratzka et al. 2010), with aortic dissection being a major cause of mortality (Faivre et al. 2007).

MFS is caused by pathological variants in *FBN1*, which encodes fibrillin-1, a major ECM glycoprotein in connective

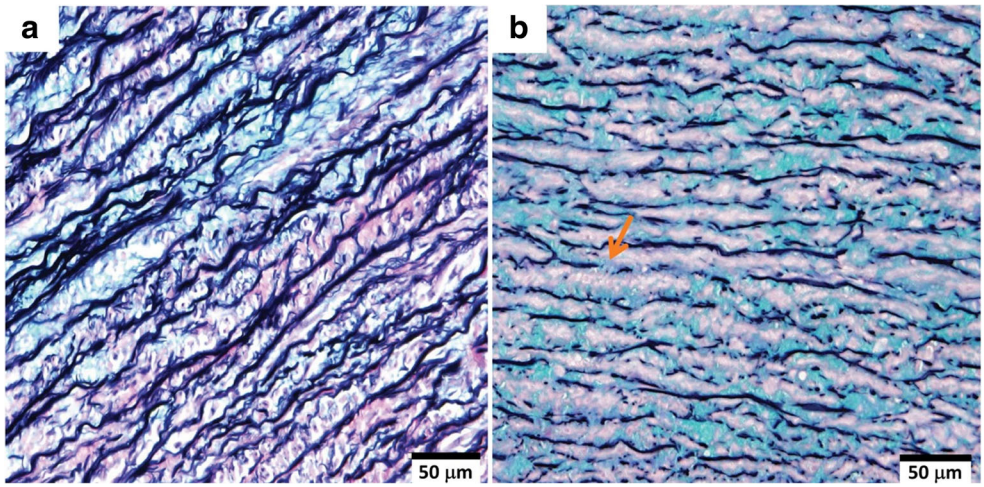

Fig. 1 Histology of the aortic wall medial layer from (**a**) normal aorta and (**b**) a patient with BAV and TAA, stained with Movat pentachrome. The normal aortic media (**a**) has long, intact parallel bands of contractile lamellar units comprising elastic lamellae (black) interspersed with VSMCs (purple), with minimal accumulation of ECM material (blue). In contrast, the BAV-TAA (**b**) media shows severe fragmentation of and loss of elastin fibres (arrow), loss of VSMC nuclei and increased accumulation of mucoid ECM, characteristic of medial degeneration. BAV, bicuspid aortic valve; ECM, extracellular matrix; TAA, thoracic aortic aneurysm; VSMC, vascular smooth muscle cell. Images were provided by M. Emami

tissue distributed throughout the body (Dietz et al. 1991). The underlying mechanism of TAA pathogenesis in MFS remains unclear. Possible mechanisms include the variant fibrillin-1 protein causing an intrinsic impairment of structural integrity or altered force mechanotransduction and adverse remodelling of the aortic wall (Yu and Jeremy 2018). In addition, variants in *FBN1* have been associated with altered TGF-β bioavailability and increased sensitivity to angiotensin II signalling in TAA formation (Yu and Jeremy 2018). While variant *FBN1* is responsible for MFS, there is important phenotypic heterogeneity among individuals who harbour the same *FBN1* variant, including within members of the same family (De Backer et al. 2007; Li et al. 2016b), indicating a role for other genetic or epigenetic mechanisms in determining phenotype.

Loeys-Dietz syndrome

Loeys-Dietz syndrome (LDS) has widespread manifestations in the craniofacial, neurocognitive, skeletal and cardiovascular systems and is characterised by extensive vascular abnormalities including arterial tortuosity and TAA or dissection (Loeys et al. 2010). The prevalence is unknown but appears to be less than MFS (Loeys and Dietz 2008). Aggressive thoracic aortic disease is the most clinically significant event, affecting over 95% of patients (Loeys et al. 2006). LDS is caused by pathological variants in TGF-β signalling pathway genes: the TGF-β ligands (*TGFB2* and *TGFB3*), its receptors (*TGFBR1* and *TGFBR2*) or its key intracellular signalling mediators Smad2 and Smad3 (*SMAD2* and *SMAD3*) (Schepers et al. 2018).

Vascular Ehlers-Danlos syndrome

The vascular type of Ehlers-Danlos syndrome (vEDS) is characterised by a generalised vasculature and integumentary tissue fragility, with cardinal features of thin, translucent skin, easy bruising, characteristic facial appearance and ruptures of the arterial system, intestines or uterus (Beighton et al. 1998). vEDS is a rare subtype of Ehlers-Danlos syndrome, with estimated prevalence between 1:50,000 and 1:200,000 (Germain 2007). It is caused by pathological variants in *COL3A1* that encodes the type III collagen alpha chain, which is highly expressed in the arterial system and hollow organs (Beighton et al. 1998). Aortic dissection or rupture is the leading cause of death, with a median survival of 50 years (Germain 2007).

Bicuspid aortic valve

Bicuspid aortic valve (BAV) is the most common congenital cardiac abnormality, characterised by abnormal fusion of the normally tri-leaflet aortic valve cusps which produces a valve with two cusps that are usually asymmetrical (Osler 1886). It affects 1–2% of the population with a male predominance (Siu and Silversides 2010). TAA is the most common non-valvular comorbidity in BAV, occurring in up to 45% of affected individuals (Tzemos et al. 2008; Siu and Silversides 2010).

The aetiology of BAV is unknown, but it is likely polygenetic, with causative genes identified in less than 4% of cases (Garg et al. 2005; McKellar et al. 2007). Causative gene discovery is further complicated by significant variability in penetrance and associated non-valvular manifestations (Andelfinger et al. 2016). Susceptibility towards TAA in

BAV also remains poorly understood. The two predominant theories that aim to explain the increased prevalence are the "haemodynamic theory" and the "genetic theory". The "haemodynamic theory" broadly suggests that TAA arises from a background of increased aortic wall stress consequent on turbulent blood flow from the malformed BAV (Sievers et al. 2016; Roman et al. 2017). The "genetic theory" suggests that underlying gene variants are responsible for the increased predisposition to both BAV and BAV-TAA (Prakash et al. 2014; Andelfinger et al. 2016). The relative contributions of genetics and haemodynamics remain unclear, but they are likely interrelated in a complex pathological process. Overall, the risk of aortic dissection is very low (< 1% 10-year dissection risk) compared to that of MFS (~ 7.9% per 10 years) and nsTAAD (~ 3.6% per 10 years) (Tzemos et al. 2008; Michelena et al. 2011; Sherrah et al. 2016); however, it is still approximately eight times higher than the general population (Michelena et al. 2011).

Non-syndromal thoracic aortic aneurysm and dissection

Non-syndromal thoracic aortic aneurysm and dissection (nsTAAD) is characterised by an inherited predisposition to TAA and dissection, without any other physical features (Milewicz et al. 2013). As TAA is often asymptomatic, presentation usually occurs at a later age than the syndromic forms, with surgical repair most commonly performed at age 51–60, compared to MFS at age 31–40 (Robertson et al. 2016). Calculated 10-year mortality due to dissection is approximately 7.8% for patients under clinical surveillance; however, with the inclusion of undiagnosed patients, true mortality is likely much higher and possibly up to 50% (Melvinsdottir et al. 2016). Due to the asymptomatic nature and absence of external physical signs, the population incidence is unknown; however, up to 16% of patients undergoing aortic surgery have characteristic pathology consistent with nsTAAD (Robertson et al. 2016). Additionally, up to 50% of nsTAAD probands have a first-degree relative with TAA (Elefteriades and Farkas 2010; Robertson et al. 2016), suggesting that nsTAAD may be relatively common.

The aetiology of nsTAAD is largely unknown, with causative gene variants identified in only 20% of cases. nsTAAD has an autosomal dominant inheritance pattern with increased male penetrance and variable age of onset and progression (Albornoz et al. 2006; Sherrah et al. 2016). The known affected genes are generally involved in the VSMC contractile apparatus (*ACTA2*, *MYH11*, *MYLK*, *PRKG1*) or TGF-β signalling pathway (*SMAD3*, *TGFBR1*, *TGFBR2*) (Milewicz et al. 2013). As with MFS, there is considerable phenotypic variation in severity of TAA among family members sharing the same causative gene variant (Robertson et al. 2016), indicating a role for other genetic or epigenetic modifiers.

Epigenetics in TAA

MicroRNA

The miRNAs are a class of small (~ 22 nucleotide) highly conserved non-coding RNA molecules that modulate gene expression by inhibiting mRNA translation to maintain homeostasis in a variety of physiological processes (Bartel 2004). They dynamically regulate over 60% of human protein-coding genes, in a manner that is highly tissue- and context-dependent (Ha and Kim 2014). Dysregulated miRNA expression is widely implicated in a variety of disease processes (Gommans and Berezikov 2012).

The synthesis and maturation process of miRNA has been extensively covered elsewhere (Ha and Kim 2014). Briefly, miRNA genes are transcribed from intra- or inter-genic regions of the genome and undergo a number of cleavage and maturation steps in the nucleus before export to the cytoplasm, and incorporation with the RNA-induced silencing complex (RISC) (Bartel 2004). The miRNA sequence guides the RISC complex to target mRNA transcripts by imperfect sequence complementarity between the miRNA and the 3'UTR of the target mRNA (Lewis et al. 2005). The target mRNA is then cleaved or translationally repressed, resulting in reduced protein product (Bartel 2004).

Importantly, the imperfect sequence recognition enables each miRNA to regulate the expression of multiple mRNA genes. This gives miRNAs the capability to extensively modify gene expression profiles and dynamically regulate homeostasis. However, despite their functional redundancy, it has become apparent that changes in the expression of a single miRNA and the consequence on protein expression are an important factor in the pathogenesis of many disease states (Gommans and Berezikov 2012).

Several different mechanisms and mediators regulate the expression of miRNAs. These include miRNA transcription being influenced by host gene expression in cases where the promoter sequence is shared, or alternatively miRNA expression may be altered by epigenetic mechanisms, transcription factors and non-coding RNA (Gulyaeva and Kushlinskiy 2016). Genomic variants that result in changes to either mediators of miRNA transcription or other proteins involved in miRNA biogenesis may also affect miRNA function (Gulyaeva and Kushlinskiy 2016). Exogenous factors, such as environmental carcinogens, diet, smoking and alcohol consumption, and endogenous factors, including stress, hormones and changes to the tissue microenvironment, such as hypoxia, have also been shown to induce changes in miRNA expression (Gulyaeva and Kushlinskiy 2016). Single-nucleotide polymorphisms in the miRNA promoter can also modulate miRNA expression (Hrdlickova et al. 2014 and have been documented to be influential in several disease contexts (Yue et al. 2018).

Dysregulated miRNA expression is implicated in vascular pathologies such as atherosclerosis, hypertension, TAA and abdominal aortic aneurysm (AAA) (De Lucia et al. 2017; Li and Maegdefessel 2017), with emerging evidence that it also contributes to genetically triggered TAA. There are relatively few studies focusing on genetically triggered TAA, which is reflective of their low incidence combined with the inherently difficult nature of aortic tissue sample acquisition. The predominant miRNAs that have been associated with genetically triggered TAA to date are the miR-29 family, miR-143/145 and miR-17.

miR-29 family

The miR-29 family, comprising miR-29a, miR-29b and miR-29c, has been the most extensively studied miRNA in genetically triggered TAA (Table 1). The family is transcribed from two independent bi-cistronic clusters as miR-29a/miR-29b1 and miR-29b2/miR-29c, with miR-29b1 and miR-29b2 identical in sequence (Boon et al. 2011). They are considered important regulators of ECM homeostasis, with gene targets in elastin (*ELN*) (van Rooij et al. 2008), several collagens and MMPs (Liu et al. 2010).

miR-29a An increase in miR-29a expression was observed in BAV-TAA tissue compared to controls (Ikonomidis et al. 2013; Albinsson et al. 2017), with demonstrated regional differences in miRNA expression. It was also shown to be increased in the aortic concavity (inner curvature) but decreased in the convexity (outer curvature) (Albinsson et al. 2017), which is typically under greater stress (Barker et al. 2012). These data suggest that miR-29a plays a role in adapting the aortic wall to the haemodynamic stress that arises from the BAV (Albinsson et al. 2017). In non-genetic TAA, miR-29a was decreased in aortic tissue obtained during prophylactic replacement surgery both before (Jones et al. 2011) and after dissection (Liao et al. 2011). Further studies using cultured human aortic VSMCs have demonstrated that miR-29a is a direct regulator of MMP-2 expression (Jones et al. 2011), indicating that its primary role may be to regulate ECM homeostasis and that this may differ depending on the underlying genetic aetiology and pathological process.

miR-29b An increase in miR-29b was observed in aortic tissue of patients with BAV-TAA (Boon et al. 2011) and non-genetic TAA (Jones et al. 2011); however, it was decreased in aortic tissue from abdominal aortic aneurysms (AAA) (Maegdefessel et al. 2012). A murine MFS ($Fbn1^{C1039G/+}$) model (Merk et al. 2012) and two other mouse models of aortic dilatation (Ang-II/$ApoE^{-/-}$ and $Fibulin\text{-}4^{R/R}$) (Boon et al. 2011) have also demonstrated increased miR-29b expression in aneurysmal aortic tissue. These murine studies showed histological evidence of reduced elastin expression and increased ECM fragmentation and apoptosis. Lending further support to a pathogenic role of miR-29b in aneurysm progression, blockade of miR-29b in vivo was shown to reduce ECM degradation and prevented aneurysmal development in both the MFS mice (Merk et al. 2012) and Ang-II mice (Maegdefessel et al. 2012). Subsequent studies in the MFS mice have shown that while miR-29b blockade prevented TAA formation when administered from birth, it did not reduce aneurysm size once formed (Okamura et al. 2017), indicating a potential limitation in translation to human therapy.

Cell culture studies have demonstrated that miR-29b represses elastin and collagen gene expression and promotes MMP expression, which would result in a reduction of ECM deposition and increased degradation (Chen et al. 2011; Chen et al. 2012). In human aortic VSMCs and adventitial fibroblasts, miR-29b was shown to target *COL1A1* and *COL3A1*, with *ELN* additionally regulated by miR-29b in VSMCs but not fibroblasts (Maegdefessel et al. 2012). Furthermore, miR-29b is selectively modulated by TGF-β1 depending on the cell type. In human aortic fibroblasts (Maegdefessel et al. 2012) and cardiac fibroblasts (van Rooij et al. 2008), TGF-β1 reduces miR-29b and increases collagen and elastin gene expression, altogether promoting a fibrotic response that is well-established for TGF-β. However, in aortic VSMCs, TGF-β1 has no effect on miR-29b levels (Maegdefessel et al. 2012; Merk et al. 2012).

The discordance in miR-29b expression among different tissue samples suggests that it may be differentially regulated depending on aneurysm context. It is increased in TAA associated with MFS, non-genetic TAA and BAV, while it is decreased in AAA. This may be due to the inherent differences in the tissue at the different locations or differences in the underlying pathogenesis. There is a general observation of elevated TGF-β in genetically triggered TAA as well as in AAA; however, AAA is associated with more significant tissue fibrosis. This may mean that fibroblasts have a reduced role in TAAs and a greater role in AAA, and this may dictate the cell-specific effects of TGF-β and overall miR-29b expression in the aorta.

miR-29c miR-29c was shown to be increased in BAV-TAA aortic tissue (Licholai et al. 2016; Albinsson et al. 2017), in addition to the aortic tissue taken from a murine TAA model ($Fibulin\text{-}4^{R/R}$) (Boon et al. 2011). Conversely, miR-29c was decreased in acute thoracic aortic dissection tissue samples, similarly to miR-29a (Liao et al. 2011).

Overall, the miR-29 family appears to adopt a cell- and tissue-specific role in ECM regulation. There is a trend towards increased expression in TAAs of different aetiology, which overall favours degradation of the ECM. This is likely mediated through multiple transcriptional programmes that require further elucidation as to their relevance to genetically

Table 1 miR-29 family expression in aortic pathology

Condition	Expression change	Species	Cell/tissue	Effects	Reference
miR-29a					
BAV-TAA	↑ CCV vs control CCV ↑ CCV vs CVX (within-patient)	Human	AscAo wall	Regional miRNA expression changes induced by haemodynamic stress	Albinsson et al. (2017)
BAV-TAA	↑	Human	AscAo wall	/	Ikonomidis et al. (2013)
TAA	↑	Mouse (*Fibulin-4$^{R/R}$*)	AscAo wall	/	Boon et al. (2011)
Non-genetic TAA	↓	Human	AscAo wall In vitro aortic VSMCs	↑ MMP-2	Jones et al. (2011)
TAD	↓	Human	AscAo wall	↑ collagen and fibrosis	Liao et al. (2011)
miR-29b					
BAV-TAA	↑	Human	AscAo wall	/	Boon et al. (2011)
Non-genetic TAA	↑	Human	AscAo wall	/	Boon et al. (2011)
MFS	↑	Mouse (*Fbn1$^{C1039G/+}$*)	AscAo wall	↑ MMP-2, ↓ elastin; ↑ elastin fragmentation, ↑ apoptosis Induced by TGF-β1	Merk et al. (2012) Okamura et al. (2017)
TAA	↑	Mouse (*Fibulin-4$^{R/R}$*)	AscAo wall	/	Boon et al. (2011)
Normal aorta	↑ with ageing	Mouse	Aorta (region undefined)	Gradual repression of ECM genes sensitises aorta to aneurysm with increasing age	Boon et al. (2011)
Atherosclerosis	↑ by ox-LDL	Human Mouse (HFD)	In vitro aortic VSMCs	↑ MMP-2, ↑ MMP-9	Chen et al. (2011) Chen et al. (2012)
AAA	↑	Mouse (Ang-II)	Abdo Ao wall	↓ elastin, ↓ collagen; ↑ ECM fragmentation	Boon et al. (2011)
AAA	↓	Human	Abdo Ao wall	↑ elastin, ↑ collagens (*COL1A1*, *COL3A1*, *COL5A1*)	Maegdefessel et al. (2012)
AAA	↓	2 x mouse (*PPE-C57BL/6*) and (AngII-*ApoE$^{-/-}$*)	Abdo Ao wall	↑ elastin, ↑ collagens; ↑ fibrosis; ↑ AAA diameter	Maegdefessel et al. (2012)
Aortic VSMCs	miR-29b inhibition	Human	In vitro aortic VSMC	miR-29b inhibition: ↑ elastin, ↑ collagens (*COL1A1*, *COL3A1*)	Maegdefessel et al. (2012)
Aortic adventitial fibroblasts	miR-29b inhibition	Human	In vitro aortic VSMC	miR-29b inhibition: ↑ collagens (*COL1A1*, *COL3A1*) miR-29b ↓ by TGF-β1	Maegdefessel et al. (2012)
miR-29c					
BAV-TAA	↑ CCV vs control CCV	Human	Asc Ao wall	Regional miRNA expression changes induced by haemodynamic stress	Albinsson et al. (2017)
Non-genetic TAA, BAV-TAA	↑	Human	AscAo wall	↓ collagens, ↓ elastin; ↑ MMP-9	Licholai et al. (2016)
TAA	↑	Mouse (*Fibulin-4$^{R/R}$*)	AscAo wall	/	Boon et al. (2011)
TAD	↓	Human	AscAo wall	↑ collagen and fibrosis	Liao et al. (2011)

AAA, abdominal aortic aneurysm; Abdo, abdominal; AngII, angiotensin II; Ao, aortic; ApoE, apolipoprotein E; AscAo, ascending aortic; BAV, bicuspid aortic valve; CCV, aortic concavity; CVX, aortic convexity; COL1A1, collagen 1 alpha-1 chain; COL3A1, collagen 3 alpha-1 chain; COL5A1, collagen 5 alpha-1 chain; ECM, extracellular matrix; Fbn1, fibrillin-1; HFD, high fat diet; LCA, left coronary artery; MFS, Marfan syndrome; MI, myocardial infarction; MMP, matrix metalloproteinase; ox-LDL, oxidized low-density lipoprotein; PPE, porcine pancreatic elastase; TAA, thoracic aortic aneurysm; TAD, thoracic aortic dissection; TGF-β, transforming growth factor-beta; VSMC, vascular smooth muscle cell

triggered TAA pathogenesis. Combined, these studies illustrate the important role of the miR-29 family in ECM regulation and its potential for therapeutic modulation.

miR-143/145

miR-143 and miR-145 are two of the best characterised cardiovascular-related miRNAs, which play an essential role in VSMC differentiation and phenotype (Boettger et al. 2009; Cordes et al. 2009). They are transcribed together from a large primary pri-miR-143/145 bi-cistronic gene cluster that is then enzymatically cleaved into miR-143 and miR-145 (Rangrez et al. 2011). They are under transcriptional control by many SMC transcription factors, including TGF-β, in a feed-forward mechanism that maintains VSMCs in a contractile phenotype (Cordes et al. 2009; Boucher et al. 2011). They are also influenced by local environment changes, such as vascular injury, which often represses their transcription in favour of ECM repair (Liu et al. 2013b).

In the normal aortic wall, VSMCs are a phenotypically diverse population, ranging along a spectrum from contractile/differentiated to synthetic/dedifferentiated (Rensen et al. 2007). The majority of aortic VSMCs are contractile, characterised by the expression of a contractile gene profile, and are predominantly composed of actin and myosin filaments. Synthetic VSMCs are usually a minor population in the aortic wall and primarily function in maintenance and repair by promoting expression of ECM genes, such as collagen and elastin (Rensen et al. 2007). miR-143/145 collaboratively maintain contractile VSMC populations in the aorta, with miR-145 promoting contractile gene expression and miR-143 suppressing synthetic gene expression (Cordes et al. 2009).

Loss of miR-143/145 is associated with impaired aortic contractility and is associated with TAA regardless of the underlying aetiology (Elia et al. 2009; Albinsson et al. 2017), in addition to other conditions of vascular damage and disease (Liu et al. 2013b; Quiat and Olson 2013). Decreased miR-143/145 expression was observed in the aortic wall of patients with BAV-TAA that had mild aortic dilatation (< 4.5 cm), in correlation with immunohistochemical evidence of reduced contractile gene expression (Alajbegovic et al. 2017). Additional work has demonstrated that there is decreased miR-143 but increased miR-145 expression together with increased miR-29a in BAV-TAA aortic tissue, and this was in conjunction with a plasma profile showing increased miR-133a and miR-145 and altered MMP/TIMP expression (Ikonomidis et al. 2013). The mechanism of the divergence between miR-143/145 expression is not currently understood, but it may be the result of differing post-translational modifications or differences in miRNA stability.

Other work in BAV-TAA has identified regional differences in miRNA expression, with decreased miR-143 in aortic tissue obtained from the convex compared to concave regions, indicating that haemodynamic changes from the malformed valve may influence local miRNA expression (Albinsson et al. 2017). BAVs with a right-left cusp fusion pattern are significantly associated with increased aortic wall shear stress in the aortic convexity (Barker et al. 2012); therefore, stress-induced degenerative changes may be associated with loss of miR-143/145 expression, which further propagates loss of function that accompanies aortic dilatation.

Murine models of TAA ($ApoE^{-/-}$ and transverse aortic constriction) are concordant with the human tissue studies, showing that miR-143/145 are decreased in TAA (Elia et al. 2009). miR-143/145 knockout rodent models exhibit histological evidence of dedifferentiated VSMCs, reduced medial density, inflammation, fibrosis and a loss of myogenic tone (Xin et al. 2009; Holmberg et al. 2018).

Human cell culture studies have demonstrated the therapeutic potential of miR-145 to protect against vascular damage. In aortic VSMCs, pre-treatment with a miR-145 mimic has been shown to prevent inflammation-induced vascular injury through modulation of CD40 (Li et al. 2016a) and tumour necrosis factor-alpha (TNF-α) (Guo et al. 2016). Activation of CD40 receptors on VSMCs and endothelial cells triggers inflammatory cascades that lead to vascular diseases, such as atherosclerosis (Song et al. 2012). Increased miR-145 expression was shown to suppress CD40 activation and interrupt VSMC proliferation and dedifferentiation in response to platelet-derived growth factor-BB (PDGF-BB) (Li et al. 2016a) and TNFα (Guo et al. 2016), which are both upregulated in early vascular damage. In addition, simultaneous blockade of miR-145 with aspirin treatment abolished the protective effect of aspirin and reversed the associated gene expression profiles (Guo et al. 2016). miR-145 may therefore mediate part of the anti-inflammatory effects of aspirin treatment by blocking TNF-α-mediated activation of CD40.

Despite the wealth of research into miR-143 and miR-145 in vascular disease, there is still a relative paucity of studies in genetically triggered TAA. However, the current studies highlight these miRNA as likely key players in the pathological VSMC dedifferentiation that is commonly observed in genetically triggered TAA.

miR-17

The miR-17/92 cluster, comprising miR-17, miR-18a, miR-19a, miR-19b-1, miR-20a and miR-92a-1, has been extensively characterised in many aspects of human development and disease, with dysregulation in cardiovascular disease well established (Mogilyansky and Rigoutsos 2013).

Increased miR-17 has been observed in BAV-TAA aortic tissue compared to control (Wu et al. 2016). Within individual BAV-TAA patients, miR-17 was increased in less dilated (< 4 cm diameter) aortic tissue segments compared to severely

dilated (> 4 cm diameter) segments (Wu et al. 2016), indicating that miR-17 may contribute to early TAA development, but as the aorta dilates this effect diminishes (Wu et al. 2016). Furthermore, increased miR-17 levels correlated with decreased levels of TIMP-1, -2 and -3 and increased MMP-2 levels, indicating that the role of miR-17 is likely to be in the regulation of MMP-mediated ECM degradation.

miR-17/92 expression has not been investigated in MFS or LDS; however, murine palatogenesis studies indicate that the cluster might contribute to palatal manifestations in addition to TAA in these conditions (Wang et al. 2013; Cao et al. 2016; Ries et al. 2017). The cause of palatal deformity is unknown, but it is associated with aberrant TGF-β signalling (Iwata et al. 2011). miR-17/92 has been identified to be essential for normal mouse palatogenesis, with inhibition of the cluster in vivo resulting in palatal deformity (Ries et al. 2017). Decreased miR-17 is accompanied by increased expression of TGF-β receptors and decreased TGF-β ligand in maxillary tissue (Ries et al. 2017).

miR-17 contributes to ECM maintenance by targeting *TIMP1* and *TIMP2* in human VSMCs, which are repressors of the matrix degrading enzyme, MMP-2 (Wu et al. 2016). Additional miR-17 gene targets identified in non-vascular cell lines include *TIMP3* (Yang et al. 2013), *TGFBR1* and *TGFBR2* (Ries et al. 2017), and the anti-proliferative bone morphogenic protein receptor-2 (*BMPR2*) (Luo et al. 2014). Other work has described a TGF-β-Smad3-miR-17/92-BMPR2 axis in the promotion of TGF-β-mediated VSMC proliferation (Luo et al. 2014). Increased miR-17/92 and Smad3 have been identified in carotid artery restenosis tissue, which is characterised by excessive TGF-β signalling and VSMC proliferation. Subsequent studies have shown that TGF-β is able to induce miR-17/92 transcription via Smad3 binding at Smad-binding elements in the miRNA promoter, and *BMPR2* has been identified as a miR-17/92 target (Luo et al. 2014).

Overall, miR-17/92 may contribute to TAA development by MMP/TIMP-mediated degradation of the aortic ECM, in addition to TGF-β-mediated pathological remodelling and VSMC proliferation.

Circulating miRNA

The identification of changes in miRNA levels in the circulation represents an alternative method of disease investigation, due to their potential utility as relatively non-invasive biomarkers that are economical to measure (Mitchell et al. 2008). miRNAs are stably expressed in plasma and serum and are resistant to degradation by endogenous RNAses (Chen et al. 2008). Their expression within the circulation has been shown to correlate with different physiological states (Gilad et al. 2008) and has led to the development of disease-specific miRNA expression profiles (Mitchell et al. 2008). Development of a similar circulating miRNA profile for TAA may lead to improvements in diagnosis and assist in both prognosis and optimising decisions regarding timing of surgical intervention.

miRNA extracted from whole blood of a small MFS cohort identified 11 miRNAs with significantly different expression compared to a control cohort (Abu-Halima et al. 2018). Significant correlations were seen between increased left ventricular end-diastolic dimension and increased levels of miR-151-5p, miR-24, miR-30e, miR-324-5p, miR-500b, miR-502-3p and miR-627. In addition, miR-331-3p was significantly increased in MFS without mitral valve prolapse (MVP) compared to MFS with MVP (Abu-Halima et al. 2018).

A small number of studies have examined circulating miRNAs in BAV-TAA cohorts. A global miRNA expression profile identified significantly decreased expression of miR-122 and miR-486 and significantly increased expression of miR-130a in plasma compared to a control group (Martinez-Micaelo et al. 2017). Subsequent pathway analysis identified TGF-β signalling as the most enriched pathway, with 32 possible miRNA-gene interactions. Within the BAV cohort, miR-718 expression had a significant inverse correlation with aortic diameter, with predicted gene targets in vascular remodelling and the focal adhesion pathway.

A targeted study evaluating a panel of six miRNAs in a BAV-TAA cohort identified miR-133a and miR-145 to be significantly decreased in the plasma compared to control (Ikonomidis et al. 2013), although these data were not corroborated subsequently (Martinez-Micaelo et al. 2017). The variance may reflect differences in methodology or the known biological heterogeneity that makes BAV-TAA so difficult to study. This is reflected in a more recent study in which the expression of seven miRNAs was analysed according to distinct BAV phenotypic subgroups; those with aortic root dilatation and concomitant insufficiency versus BAV with severe aortic stenosis (Girdauskas et al. 2018a). Within the root dilatation subgroup, circulating miR-17 and 106a were significantly increased in the less dilated (< 50 mm) compared to severely dilated (> 50 mm) aortas; however, when compared overall to the aortic stenosis cohort, significant differences were observed in all seven miRNAs (miR-17, miR-16a, miR19a, miR-20a, miR-21, miR-106a and miR-145) (Girdauskas et al. 2018b). While the lack of a control cohort in this study limits further interpretation, it is clear that there are likely different miRNA profiles associated with different BAV phenotypes, and this should be factored in to future experimental design in BAV studies.

Overall, the development of circulating miRNA profiles in genetically triggered TAAs is still in preliminary stages. More observational studies using larger and well-defined cohorts are required to identify candidate miRNAs that can act as biomarkers of the disease state. In addition, longitudinal studies

of the same patient group may provide miRNAs that can be used as markers of TAA progression.

miRNAs in other cardiovascular studies

In addition to studies detailing the role of miRNAs in TAA, there has been extensive characterisation of miRNAs in other cardiovascular pathologies that have been reviewed elsewhere (De Lucia et al. 2017; Li and Maegdefessel 2017). While there are important differences in molecular pathogenesis limiting direct comparison and inference, these studies are informative in terms of understanding miRNA-mRNA regulatory relationships that affect the vasculature. In addition, other studies have characterised the normal function of miRNAs within the vasculature, for example in endothelial cells and VSMCs (Ballantyne et al. 2016), molecules involved in ECM regulation (Hu et al. 2016) and in major signalling pathways, such as TGF-β (Climent et al. 2015; Forte et al. 2016). The relevance of these miRNAs with respect to genetically triggered TAA is yet to be investigated.

Long non-coding RNA

Long non-coding RNAs (lncRNAs) have emerged in the last decade as a large and functionally diverse class of non-protein-coding RNA at least 200 nt in length, which regulate gene expression at the transcriptional and post-transcriptional levels (Engreitz et al. 2016). Research on lncRNA is still emerging, with broad estimations as to the number of lncRNAs ranging from 20,000 to 100,000 (Kopp and Mendell 2018), but very few having been biologically characterised. One of the best established is Xist (X inactive specific transcript), which orchestrates X chromosome inactivation through highly ordered interactions with chromatin scaffolding proteins and targeted binding to X chromosomal DNA, where it mediates transcriptional silencing along the entirety of the chromosome (Wutz et al. 2002). Characterisation of lncRNAs in vascular biology and disease has rapidly progressed, with a number of extensive reviews available (Li and Maegdefessel 2017; Leeper and Maegdefessel 2018; Simion et al. 2018).

MALAT1

MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) is highly expressed in endothelial cells (Simion et al. 2018) and was recently implicated in genetically triggered TAA, with increased expression linked to pathological VSMC dedifferentiation in the aneurysmal aortic wall (Cardenas et al. 2018). In aortic tissue from MFS, nsTAAD and non-genetic TAA, increased expression of *MALAT1*, the epigenetic repressor histone deacetylase 9 (HDAC9), and the chromatin-remodelling enzyme, BRG1, was observed when compared to normal aortic tissue. These expression changes were also observed in two mutant VSMC cell lines that represent genetically triggered TAA ($TGFBR2^{G357W}$ and $ACTA2^{R179H}$) and were correlated with decreased contractile gene expression (Cardenas et al. 2018).

MALAT1 is essential to transport BRG1 and HDAC9 into the nucleus, where the promoters of contractile genes are methylated by HDAC9, resulting in pathological VSMC dedifferentiation (Cardenas et al. 2018). These data have been confirmed in a MFS mouse model ($Fbn1^{C1039G/+}$) where deletion of either *MALAT1* or *HDAC9* impaired aneurysm formation (Cardenas et al. 2018). These deletions correlated with increased contractile gene expression and decreases in elastin fragmentation, MMP activity, TGF-β signalling and VSMC proliferation.

The cause of the observed upregulation of *MALAT1* in genetically triggered TAA tissue is unknown. Its expression can be modulated by factors including hypoxia, p53 transcription factor mutations, miRNA and polymorphisms within the *MALAT1* gene (Simion et al. 2018). It may also be influenced by the pathological context. For example, *MALAT1* was decreased in human aortic and coronary VSMCs cultured in "ECM stiffening" conditions reflective of arterial disease, and *MALAT1* knockdown limited stiffness-induced VSMC proliferation and migration (Yu et al. 2018). Overall, these studies suggest that *MALAT1* may play an important role in the regulation of vascular homeostasis.

HOTAIR

Decreased *HOTAIR* expression has been observed in calcified BAV aortic leaflets, and this was inversely correlated with the expression of two key calcification genes, *ALPL*, encoding alkaline phosphatase, and *BMP2*, encoding bone morphogenic protein 2 (Carrion et al. 2014). *HOTAIR* epigenetically regulates gene expression by promoting histone H3K27 methylation (Gupta et al. 2010).

Decreased *HOTAIR* expression has also been identified in the aortic tissue of a small non-genetic TAA cohort, which was negatively correlated with aortic diameter (Guo et al. 2017). Knockdown in human aortic VSMCs resulted in decreased expression of collagens I and III and increased apoptosis, indicating *HOTAIR* is a potential mediator of ECM regulation in the aorta (Guo et al. 2017).

HOTAIR expression is down-regulated by the WNT/β-catenin pathway in cultured human aortic valvular interstitial cells subjected to cyclic mechanical stretch (Carrion et al. 2014). Increased WNT/β-catenin signalling is implicated in MFS (Chopra et al. 2017) and in other aneurysm formation and vascular pathology in general (Mill and George 2012), implicating *HOTAIR* as another mediator of pathological WNT/β-catenin signalling, thus potentially driving aortic and valvular pathology in genetically triggered TAA.

HIF1A-AS1

HIF1A-AS1 (hypoxia inducible factor 1 alpha antisense RNA 1) is implicated in TAA in association with BRG1-mediated VSMC apoptosis (Wang et al. 2015). BRG1 is a major regulator of vascular development through chromatin remodelling (Griffin et al. 2008), and its expression was increased in aortic tissue of a non-genetic TAA cohort (Wang et al. 2015). In human aortic VSMCs, BRG1 knockdown resulted in VSMC apoptosis and reduced proliferation, which was mediated through *HIF1A-AS1* (Wang et al. 2015). *HIF1A-AS1* expression in TAA tissue has not been investigated; however, increased levels were detected in the serum of a non-genetic TAA cohort (Zhao et al. 2014), indicating a potential role for this lncRNA in aneurysm pathogenesis.

DNA methylation

DNA methylation is a physiological process that involves the addition of methyl groups to DNA, which results in repression of gene transcription without changing the DNA sequence (Curradi et al. 2002). It is a somatically heritable trait that is also influenced by environment. Both cytosine and adenine nucleotides can be methylated; however, DNA methylation is found almost exclusively in CpG dinucleotides, which are segments of DNA sequence comprising a cytosine followed by a guanine nucleotide in a 5′ to 3′ direction with a phosphate in between (Curradi et al. 2002). In mammals, approximately 70–80% of all CpG cytosines are methylated (Jabbari and Bernardi 2004). Certain CpG regions within the DNA, known as CpG islands, serve as regulatory units of transcription. These islands are typically 200–1500 base pairs long, with 50% located in the promoter regions of constitutively active genes, and 25% within regions that serve as alternative promoters (Larsen et al. 1992). These CpG islands typically are methylated at very low levels, thus allowing the expression of the resident gene (Curradi et al. 2002).

Tissue-specific, genome-wide DNA methylation profiling performed in aortic tissue from BAV-TAA has identified nine novel genes with significant differential methylation and differential gene expression, when compared to TAA tissue without a known genetic trigger (Shah et al. 2015). The strongest finding was the hypermethylation and decreased expression of *PTPN22* in BAV-TAA tissue. *PTPN22* (protein tyrosine phosphatase, non-receptor type 22) has an important role in regulating immune responses, with decreases in its encoded protein, lymphoid tyrosine phosphatase (Lyp), shown to increase T cell signalling (Burn et al. 2011). Activated T cells in the aortic wall can lead to VSMC apoptosis and degradation of the ECM (He et al. 2008); thus, hypermethylation of *PTPN22* in the aorta may contribute to medial degeneration.

Genes previously associated with TAA, including *ACTA2*, and genes associated with cardiovascular development, such as *GATA4*, were shown to be differentially methylated but not differentially expressed in BAV-TAA (Shah et al. 2015). As DNA methylation is important for embryological development, it has been suggested that altered DNA methylation in these genes may contribute to abnormalities in aortic valve development and aortic cell migration during embryogenesis and, therefore, may not necessarily be altering gene expression at the time of tissue analysis.

Epigenetic mechanisms may also contribute to the altered MMP expression that is observed in genetically triggered TAA. There are extensive CpG islands throughout *MMP9* (Chernov and Strongin 2011), and hypomethylation of the *MMP9* promoter has been correlated with increased *MMP9* transcriptional activity in lymphoma cells (Chicoine et al. 2002). *MMP3* gene expression was increased in a colon cancer cell line after dual knockout of key DNA methyltransferase genes (*DNMT1* and *DNMT3b*) (Couillard et al. 2006). In human VSMCs, miR-29b has been shown to increase *MMP2* and *MMP9* expression through the suppression of the DNA methyltransferase genes, *DNMT3a* and *DNMT3b* (Takada et al. 2009; Chen et al. 2011).

The TET2 DNA demethylase is highly expressed in VSMCs and is an important regulator of VSMC phenotype (Liu et al. 2013a). Decreased TET2 expression has been identified to occur in proliferative vascular pathologies, such as atherosclerosis, and knockdown in human VSMCs resulted in loss of contractile phenotype and increased expression of synthetic VSMC markers (Liu et al. 2013a). Overexpression of *TET2* was shown to be sufficient to induce the contractile phenotype, and local injection into rat arteries prevented the intimal hyperplasia that is associated with pathological VSMC switching (Liu et al. 2013a). The role of TET2 in TAA development is a current area of investigation.

Histone modifications

Histones are positively charged proteins that associate with DNA to form nucleosomes, the basic structural unit of chromatin. Nucleosomes contain a small length of DNA wrapped tightly around eight core histone proteins: two dimers of H2A/H2B and two dimers of H3/H4 (Luger et al. 1997). Each core histone protein contains a modifiable amino acid tail. Various post-transcriptional modifications occur at the N-termini of these tails, including acetylation, methylation, phosphorylation and ubiquitination (Kouzarides 2007). These modifications can alter DNA-histone interactions, ultimately changing the chromatin structure and modulating the accessibility of transcriptional machinery to DNA binding sites to produce changes in gene transcription (Webster et al. 2013).

Two well-characterised histone modifications associated with transcriptional activation are H3 acetylation at Lys 9 (H3K9) and H3 methylation at Lys 4 (H3K4) (Verdone et al. 2005; Greer and Shi 2012). Increased acetylation and

methylation at these specific histone residues in the *SMAD2* promoter was associated with increased Smad2 protein in VSMCs isolated from aortic tissue samples from individuals with MFS, BAV and non-genetic TAA (Gomez et al. 2011). *SMAD2* overexpression in these VSMCs was subsequently shown to be modulated by histone acetyltransferases (p300/PCAF) (Gomez et al. 2013). Thus, the dysregulated TGF-β signalling that is frequently observed in genetically triggered TAA may be contributed to by aberrant histone modulations affecting *SMAD2* transcription.

Histone acetylation is also able to modulate VSMC phenotype by altering chromatin accessibility and SMC transcription factor binding (Liu et al. 2014). Contractile VSMC gene expression is induced by the binding of myocardin/serum response factor (SRF) to CArG ($CC(A/T)_6GG$) DNA sequence elements in SMC gene promoter regions (McDonald et al. 2006), and the binding capacity of SRF to CArG-elements has been shown to rely heavily on preceding histone hyperacetylation (Qiu and Li 2002).

Aberrant histone modifications affecting VSMCs in TAA may be a result of the altered biomechanical environment that accompanies aortic dilatation. It has been suggested that the increased wall strain and altered mechanotransduction cascades result in changes to VSMC chromatin state that subsequently alters downstream gene transcription profiles (Chen et al. 2013; Michel et al. 2018). Altered histone acetylation has been observed in AAA, with increased expression of histone acetyltransferases in human AAA tissue compared to normal aortic tissue (Han et al. 2016); however, this has not yet been explored in TAA.

Epigenetics as a therapeutic target

The use of non-coding RNAs as therapeutic molecules is in the early stages of development, with many currently in preclinical stages but few having reached clinical trials. Miravirsen, a miR-122 inhibitor for the treatment of chronic hepatitis C infection, has progressed to phase 2a studies and has demonstrated efficacy with few moderate-severe adverse events (Janssen et al. 2013). A small number of miRNA therapeutics are in phase 1 studies, mostly for the treatment of various cancers (Mellis and Caporali 2018); however, one trial was suspended due to multiple immune-related severe adverse events (Beg et al. 2017; Chakraborty et al. 2017).

An injectable hyaluronan-based hydrogel enabling targeted miRNA delivery to the heart has demonstrated preclinical efficacy in a murine model of myocardial infarction (MI). miR-29b that had been incorporated into the hydrogel and injected into the MI border zone demonstrated beneficial ECM changes and maintenance of cardiac function compared to control, decreasing the area of myocardial fibrosis (Monaghan et al. 2018). Similarly, administration of miR-302 to promote cardiomyocyte proliferation post-MI demonstrated local and sustained proliferation for two weeks after a single injection, indicating this method may have long-acting therapeutic potential (Wang et al. 2017).

In AAA, preclinical efficacy has been established for miR-126 in suppressing inflammation associated with aneurysm development. Using a dual-targeted approach in a murine Ang-II model, miR-126 conjugated with a VCAM-1-specific antibody was incorporated into ultrasound-guided microbubbles, which were subsequently delivered to inflamed endothelial cells of the AAA in weekly intravenous injections (Wang et al. 2018). VCAM-1 adhesion molecules are upregulated in AAA, thus binding allowed the targeted release of miR-126 to the affected endothelium to exert its anti-inflammatory effects. Amelioration of aneurysmal growth was observed after a period of four weeks.

Delivery of miRNA therapeutics to the aortic tissue in genetically triggered TAA may be more challenging than in AAA, given that the pathology in TAA predominantly occurs in the media, as opposed to the intima in AAA, in which intravenous agents benefit from having direct access. This may be resolved with novel techniques, such as a hydrogel-based injection, that will undoubtedly continue to emerge over the coming years.

Therapeutics aimed at methylation and histone-based modifications is complicated by their non-specific effects, which typically result in broad changes in gene expression (Yang et al. 2014). A number of DNA methyltransferase inhibitors (DNMTs) and histone deacetylase inhibitors (HDACi) are either in use or in clinical trials for cancer treatment (Jones et al. 2016) and are being explored for the treatment of various cardiovascular diseases; however, this has not progressed beyond preclinical studies (Schiattarella et al. 2018).

Future directions

Our understanding of the role of epigenetics in the pathophysiology of complex genetic diseases continues to expand at a rapid pace. With respect to genetically triggered TAA, epigenetics may provide insights into the observed phenotypic variation and, importantly, may assist in the development of personalised clinical management. Epigenetic mediators, such as miRNAs, are effector biological molecules whose expression can be altered through targeted mechanisms, presenting new opportunities for therapeutic strategies. However, large cohort studies capable of yielding robust clinical correlates to identify potential targets are still required. The mechanisms by which epigenetic mediators are regulated remain poorly understood and warrant further investigation. This would subsequently enable better understanding of the possible causative role of epigenetics in pathogenesis and strengthen the basis for exploring epigenetics-based therapeutics. These future

research aims would significantly benefit from international multi-site collaborations and the establishment of a large international database and biobank.

Conclusion

Genetically triggered TAA is a complex condition with phenotypic heterogeneity that results in early morbidity and mortality in affected individuals. Epigenetic changes appear to play an important role in the pathogenesis and phenotypic heterogeneity of TAA and may represent feasible targets for future therapy.

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Compliance with ethical standards

Conflict of interest Stefanie Portelli declares that she has no conflict of interest. Elizabeth Robertson declares that she has no conflict of interest. Cassandra Malecki declares that she has no conflict of interest. Kiersten Liddy declares that she has no conflict of interest. Brett Hambly declares that he has no conflict of interest. Richmond Jeremy declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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