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Review

microRNAs in bicuspid aortic valve associated aortopathy: Recent advances and future perspectives

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ABSTRACT

The risk of acute aortic events in patients with bicuspid aortic valve (BAV) constitutes a medical concern in terms of timing and surgical decision.

During the past years, there has been a growing interest in the potential of microRNAs (miRNAs) as crucial epigenetic factors in multiple cellular processes associated with BAV aortopathy. Nevertheless, there are still challenges that need to be overcome before miRNAs could enter clinical practice, and further validation studies in larger and well-defined BAV cohorts are now required.

This review aims at providing a comprehensive overview of the available data on the expression profiles and function of specific miRNAs in BAV aortopathy, evaluating miRNA signatures as potential molecular markers of disease. We also discuss the role of other novel classes of non-coding RNAs, including long non-coding RNAs and circular RNAs, in BAV-associated aortopathy, mainly regarding their possible implementation as diagnostic and prognostic markers.

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Contents

Introduction

Bicuspid aortic valve (BAV) is the most common congenital cardiac malformation with a prevalence of 1–2% in the general population [1].

Although BAV may remain asymptomatic for a lifetime, the natural history of BAV disease often results in severe cardiac complications related to the valve and/or the aorta [2]. Specifically, severe aortic stenosis and regurgitation are the most common complications of BAV. Moreover, 20 to 40% of individuals with BAV manifest a progressive dilatation of the ascending aorta, with an approximately 8 times higher risk of dissection and rupture than in the general population [3,4].

Currently, cardiac surgery is considered the gold standard for aortic dilatation treatment [5]. Aortic diameter, in combination with family history of disease, root phenotype, and presence of coarctation, represents the main clinical risk indication for elective surgical intervention [6]. Nevertheless, the American College of Cardiology/American Heart Association guidelines still have a controversial

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position regarding recommendations of surgical resection based on the aortic size between 5.0 and 5.5 cm [7]. Indeed, clinical studies have highlighted the limited value of such aortic dimensions, excluding about 50% of patients having an aortic diameter under 5.0 cm from prophylactic replacement of the ascending aorta [8–10].

As a consequence, there is an urgent need to develop additional reliable molecular predictors able to monitor BAV progression and guide practice decisions. Additionally, a more detailed comprehension of the molecular mechanisms underlying BAV aortopathy is required to fully assess the risk and to develop novel therapeutic strategies [11].

During the past years, microRNAs (miRNAs) have emerged as central regulators of heart development and promising biomarkers in a wide range of cardiovascular diseases [12,13]. Thanks to their ability to target thousands of mRNA molecules, miRNAs have a strong impact on gene regulation and functional pathways, controlling the expression of key components of signal transduction at multiple levels. Therefore, the possibility of measuring their levels using non-invasive methods has opened up a new avenue in the diagnosis, prognosis, and treatment of complex diseases.

This review discusses the current knowledge regarding the involvement of miRNAs in the development of BAV aortopathy, considering their potential role as biomarkers in the light of BAV patients' management. We also discuss how the recent interest in other classes of non-coding RNAs has opened exciting research and diagnostic perspectives in this field.

microRNA biogenesis and function

miRNAs are generated by the cleavage of precursor hairpins in two sequential processing events. In the nucleus, miRNAs are transcribed as long primary miRNA transcripts (pri-miRNAs), subsequently cleaved by the RNase III enzyme Drosha into a shorter precursor-miRNA (pre-miRNA) hairpin [14,15]. The karyopherin XPO5 (Exportin-5) mediates the export of such hairpin into the cytoplasm, where it is further processed into an unstable, ~22 nucleotides miRNA duplex structure by the RNase III protein Dicer [16]. Only the mature miRNA is incorporated into the RNA-induced silencing complex (RISC) in order to direct the mRNA target silencing.

miRNAs act as post-transcriptional repressors of gene expression primarily by an imperfect base pairing with the mRNA target in a sequence-specific manner in the cell cytoplasm, although a non-canonical function in the nucleus has been suggested [17,18]. Canonically, miRNAs bind to the 3′ untranslated regions of their target mRNAs by a "seed" region which is positioned at the 5′ end of the miRNA [16]. Variances in the complementary degree between miRNA:mRNA duplex enable a single miRNA to target multiple mRNAs, whereas a single gene can be modulated by several miRNAs [16]. High complementarity is generally required for the target mRNA cleavage through the activity of Argonaute-2 enzyme. A partial complementarity seems to induce translation repression or mRNA instability, even if some investigations revealed that some miRNAs are able to stimulate mRNA translation [19].

Interestingly, the discovery of a significant amount of stable miRNAs in the extracellular environment, such as serum, plasma, and seminal and follicular fluid, has added another level of complexity in the regulation of the genome [20]. Selectively exported into the extracellular space, circulating miRNAs are incorporated into extracellular vesicles (exosomes, microvesicles) or lipoproteins that protect miRNAs from degradation [21]. The unique expression signatures of such miRNAs have been associated with different pathological outcomes, including cardiovascular diseases, highlighting their potential use as novel non-invasive biomarkers of disease (Fig. 1) [22].

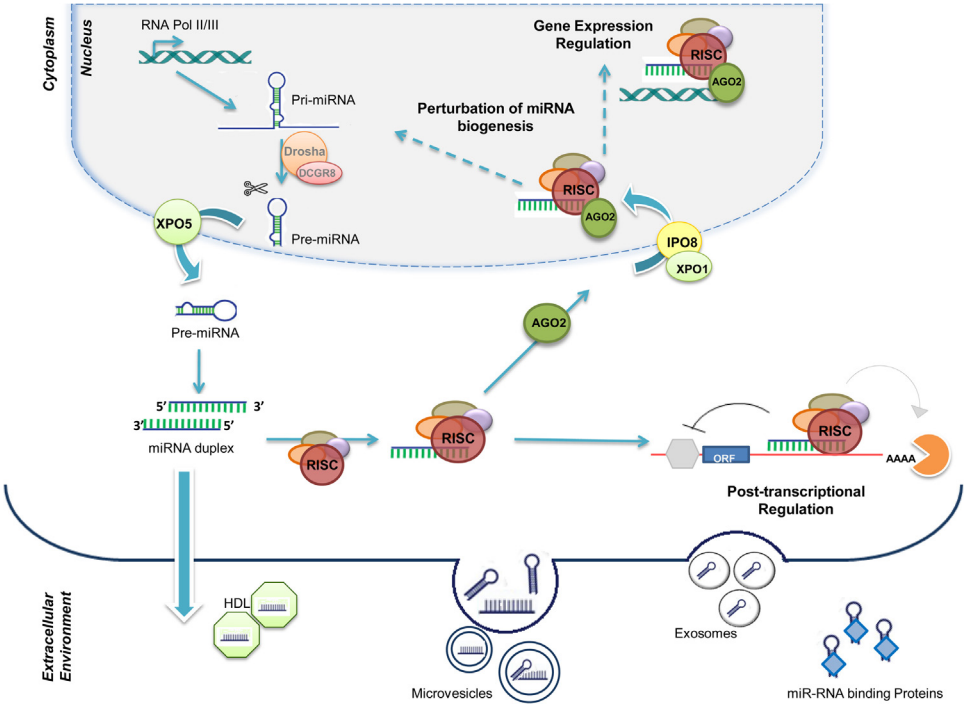

Fig. 1. miRNA biogenesis, function and release in the extracellular environment. In the conventional miRNA biogenesis pathway, miRNAs are exported out of the nucleus by the specific nuclear transport receptor Exportin 5 (XPO5) and further processed by Dicer to the mature miRNA duplex in the cytoplasm, in order to silence target gene expression by inhibiting protein translation or degrading mRNA molecules. Additionally, miRNAs can be selectively incorporated into exosomes or microvescicles, or coupled with protein or lipid-based carriers, and released into the extracellular environment in order to modulate cell function and intercellular communications.
Alternatively, some miRNAs, once exported to the cytoplasm, may combine with AGO2 proteins to form a complex, which is imported into the nucleus via Exportin 1 (XPO1) and Importin 8 (IPO8).

microRNA signature in BAV-associated aortopathy

Much debate exists about the underlying pathogenesis of BAV aortopathy. Beyond the genetic bases of BAV, a role for hemodynamic forces in the disease development has been also recognized, whereby an abnormal cups formation during embryogenesis has led to disturbed flow and shear stress [23]. In this context, miRNAs have been demonstrated to fill the gap between genetic and hemodynamic factors, regulating gene expression and responding to environmental changes at a post-transcriptional level [24]. Indeed, different miRNAs, such as miR-181b, have been shown to be shear-sensitive in aortic valve endothelial cells, potentially triggering valve diseases [25–27].

Nevertheless, studies reliably correlating genetic, epigenetic, and hemodynamic factors in the onset and progression of BAV aortopathy are lacking. Recently, a complex network of transcriptional and epigenetic mechanisms governing NOTCH1 haploinsufficiency has been observed in BAV [28]. The authors demonstrated the impact of heterozygous nonsense mutations in NOTCH1 on H3K27 acetylation, affecting the transcriptional regulatory mechanisms of genes involved in the calcification of the aortic valves [28].

Interestingly, Girdauskas et al. [29], have examined the association between the presence of rare genetic variants and specific miRNAs in BAV aortopathy. Although a significantly lower expression of blood miR-145 in the subgroup with NOTCH1 variants has been identified, direct evidence for the proposed genetic pathway remains to be demonstrated [29].

The potential implication of genetic variants within miRNA-related genes on miRNA expression and function needs to be addressed since it may reveal new insights into the complex regulatory networks related with the onset and progression of BAV aortopathy as well as identify clinically useful biomarkers.

To date, most miRNA-related research has been focused on vascular remodeling and pathogenesis of aortic aneurysm (AA) formation, excluding patients with BAV [30–35].

Recently, a microarray screening firstly demonstrated the presence of differentially expressed miRNAs between convex and concave portions of dilated aortas in BAV patients [36]. The authors demonstrated that the presence of BAV altered specific miRNA expression profiles in mildly dilated AAs both in the aortic convexity vs. concavity within the same cohort of patients, and in dilated AAs vs. control aortas, having potential implications for vascular cell phenotype, mechanosensing, and remodeling [36]. An additional in silico prediction analysis suggested that altered expression of such miRNAs might affect mechanotransduction pathways especially in BAV convexity, with particular reference to TGF-β1, Hippo, and PI3K/Akt/FoxO pathways [36].

This is consistent with previous findings that identified such unique profile of signaling pathways controlling extracellular matrix (ECM) composition within the aortic convexity of BAV patients [37–39]. Indeed, degenerative lesions within the vessel wall involving ECM fragmentation adjacent to areas of smooth muscle cell necrosis may reduce the structural integrity of the aorta in response to shear stress and disturbed blood flow, resulting in progressive aortic dilatation [40].

Specifically, the imbalance between matrix metalloproteinases (MMPs) and their inhibitors tissue inhibitors of metalloproteinases (TIMPs) is of critical importance in aneurysm formation in BAV patients, modulating the ECM turnover [41,42]. Interestingly, different signatures of these enzymes have been observed in different aortic regions in BAV, underlying the impact of hemodynamic factors in the pathogenesis of BAV and/or ascending aortic aneurysms [43].

Such difference has been recently supported by elevated expression levels of miR-17 cluster (miR-18a, miR-19a/b) and other miRNAs with the same seed sequence (miR-17, miR-20a/b, miR-106a/b, miR-93) in less dilated aortic regions as compared to severely dilated regions from BAV patients [44]. Of particular interest was the finding that upregulation of those miRNAs decreased the expression of TIMPs and thereby increased MMP2 activity, thus contributing to ECM degradation and aortic dilatation [44].

Moreover, miR-195 has emerged as a promising miRNA involved in the early calcification of bicuspid aortic valves [45]. Du and colleagues have recently demonstrated a significant downregulation of miR-195 in BAV leaflets compared with that in tricuspid aortic valve (TAV), which were inversely correlated with a higher mRNA expression of SMAD7 in both human and porcine valve interstitial cells. Moreover, the authors examined the functional changes between BAVs and TAVs, showing a higher expression of MMP2 and MMP9 in BAV leaflets, indicating a disorder in the extracellular matrix of BAV leaflets, likely inducing valvular calcification [45].

Recently, Maleki et al. [46] identified a miR-200-dependent process of vascular remodeling in the ascending aorta of BAV patients prior to aortic dilatation by using a combination of systems biology approach, quantitative real time PCR (qRT-PCR), and chromatin immunoprecipitation (CHIP) analyses. Specifically, the authors demonstrated a significantly lower expression of miR-200c and a simultaneous upregulation of the targets ZEB1/ZEB2 in non-dilated BAV aorta compared to non-dilated TAV. These findings firstly suggested a new mechanism of vascular remodeling through EndMT/EMT based on a miR200c/ZEBs negative feedback loop in the aortic wall of BAV patients prior to aneurysm [46].

Ikonomidis et al. [47] have identified a unique molecular signature in both aortic and plasma samples from BAV patients, including miRNAs involved in the regulation of ECM composition (miR-1, miR-21, and miR-143) [47]. Interestingly, the combination of circulating biomarkers, such as those characterized by Ikonomidis in thoracic aortic aneurysms (TAAs) associated with BAV (MMP-2, TIMP-2, miR-143, miR-133a, and miR-145), may help to screen different clinical phenotypes and could represent significant predictors of aortic dissection and aneurysm in BAV patients [47]. Nevertheless, miRNA regulation of specific ECM proteases in BAV aortopathy requires further investigations.

Actin polymerization is important to maintain a contractile phenotype of smooth muscle cells, resisting mechanical stress due to the blood flow and thus preserving the structural integrity of the vascular wall [48]. Several mutations in actin and other contractile genes have been associated with arterial aneurysms and dissections, causing destabilization of the actin filaments [49]. By integrating information from multiple qRT-PCR based miRNA arrays, Alajbegovic and colleagues [50] identified a group of five miRNAs (miR-1, miR-22, miR-143, miR-145, and miR-378a), that were sensitive to actin polymerization and MRTF-A overexpression in human aortic smooth muscle cells (HASMCs). Specifically, except for miR-22 and miR-378a, the expression of the other actin/MRTF-A-sensitive miRNAs resulted in significantly reduced dilated aortic tissue from BAV patients compared to TAV, together with a reduced actin polymerization [50]. This study suggested that the destabilization of actin filaments and the concomitant altered transcriptional regulation of specific miRNAs in smooth muscle cells may be involved in the development and progression of aortic aneurysms in BAV patients, thus paving the way for future therapeutic intervention.

Recently, the first study of miRNome expression profiles in TAA specimens from BAV and TAV patients has been performed by our group [51]. We have identified 12 miRNAs differentially expressed in BAV compared to TAV patients, which are known to modulate the expression of gene pathways governing valve development and function. Above them, miR-424-3p and miR-3688-3p were confirmed to be downregulated in BAV patients [51].

Although miRNA expression profiles in TAA samples from BAV patients have provided new insight into molecular mechanisms of BAV and associated aortopathy, determination of circulating miRNAs may add more information on the originating tissue, serving as stable and highly specific biomarkers of disease.

To date, specific signatures of circulating miRNAs have been identified in plasma samples of BAV patients. Specifically, expression levels of circulating miR-122, miR-130a, and miR-486 differed significantly between BAV and TAV with and/or without aortic dilatation, probably affecting the TGF-β1 signaling pathway [52]. Furthermore, the expression of miR-718 in plasma was significantly inversely correlated to the aortic diameter, thus serving as an independent predictor of aortic dilation [52].

The same authors have also investigated the impact of post-transcriptional regulation on endothelial damage caused by the disturbed flow in the ascending aorta of BAV patients. Using a bioinformatics approach, the authors identified a cluster of highly co-expressed circulating miRNAs located at the 14q32 that modulate various signaling pathways involved in BAV-related pathophysiological processes, including endothelial damage [53].

The aforementioned miR-17 and miR-106a, belonging to the miR-17 cluster, recently showed higher expression levels in blood samples of BAV patients with less dilated versus severely dilated aorta, thus correlating with the aortopathy severity and the risk of adverse aortic events in BAV [54]. Although it was a preliminary study, it confirmed the importance of such a cluster in the BAV-associated aortopathy, highlighting the potential of circulating miRNAs as potential attractive biomarkers of disease.

Recent research by Gallo et al. [55] identified for the first time exosomal circulating miRNAs that may predict the severity of aortic size and valve morphology differently. Specifically, the authors demonstrated that the expression levels of miR-126, miR-15b, miR-195, miR-221, miR-24, miR-30b, and miR-320a was modulated by the severely-dilated versus the less-dilated aorta. Interestingly, the aortic valve morphology significantly affected the expression of miR-133a, miR-155, miR-320a, miR-34a, miR-494, and the plasma levels of other biomarkers associated with the disease (i.e. TGF-β, MMP-3, MMP-9, TIMP-4). Further target prediction analysis demonstrated the involvement of those miRNAs in the regulation of the TGF-β pathway, thus suggesting a role in the pathogenesis of BAV aortopathy [55].

An overview of dysregulated miRNAs potentially associated with BAV and associated aortopathy is reported in Table 1. Although all these studies indicate a significant role of miRNAs in the aortic wall pathogenesis of BAV disease, miRNA signatures are far from a routine clinical application. Potential confounding factors, small or moderately sized studies and the use of different unstandardized methodologies may explain the huge

Table 1
Putative miRNAs associated with BAV aortopathy.

miRNAs	Validated miRNAs	Origin	Method	Findings	Ref.
84 miRNAs	–	Aortic tissue	Microarray qRT-PCR	• Differentially expressed miRNAs in TAV and BAV patients compared to controls • Differentially expressed miRNAs between aortic concavity and convexity in patients only	[36]
21 miRNAs	16 miRNAs related to miR-17 cluster	Aortic tissue	Microarray qRT-qPCR	• Regulation of matrix degradation in progressive aortic dilation in BAV-associated aortic aneurysm	[44]
miR-26a miR-30b miR-195 miR-486 miR-139 miR-192	miR-195	Aortic tissue	qRT-PCR	• miR-195 is downregulated more in stenotic aortic leaflets from BAV patients compared with that from patients with TAV. • miR-195 downregulation is associated with valvular calcification via targeting SMAD7	[45]
miR-200 family	miR-200 family	aortic tissue	qRT-PCR ChIP analyses	• A miR-200-dependent process of EndMT/EMT in ascending aortas of BAV patients prior to aneurysm development	[46]
miR-1 miR-21 miR-143	–	Aortic tissue; plasma sample	qRT-PCR	• Differential plasma profiles of MMPs, TIMPs, and miRNAs in ascending TAA specimens from patients with BAV and TAV	[45]
miR-1 miR-22 miR-143 miR-145 miR-378a	miR-1 miR-22 miR-143 miR-145 miR-378a	Aortic tissue	qRT-PCR	• A decrease in actin/MRTF-regulated miRNAs in the aorta from patients with mild aortic dilations as compared to donors	[50]
12 miRNAs	miR-424-3p miR-3688-3p	Aortic tissue	miRNome	• Specific dysregulated miRNAs in BAV patients as compared to TAV affecting different target genes and pathways linked to BAV and aneurysm formation	[51]
miR-122 miR-130a miR-486 miR-718	miR-122 miR-130a miR-486 miR-718	Plasma sample	Microarray qRT-PCR	• New molecular marker associated with BAV and aortic dilation mainly through the activation of TGF-β1 pathway and vascular remodeling	[52]
131 miRNAs	19 miRNAs located in the 14q32 locus	Plasma sample	Microarray qRT-PCR	• Endothelial damage responsible for BAV disease	[53]
11 miRNAs	miR-17 miR-106a	Blood sample	qRT-PCR	• Differentially expressed miRNAs in patients with severely dilated aorta versus patients with less dilated aorta	[54]
47 miRNAs	miR-155 miR-34a miR-320a miR-133a miR-494	Blood sample	qRT-PCR	• Aortic valve morphology differently modulates miRNA analytes and protein proteolytic activity with alterations in the expression level of validated miRNAs and the plasma measurements of TGF-β MMP-3, MMP-9, TIMP-4	[55]

qRT-PCR: quantitative real time PCR; TAV: tricuspid aortic valve; BAV: bicuspid aortic valve; CHIP: chromatin immunoprecipitation; EndMT: endothelial mesenchymal transition; EMT: epithelial mesenchymal transition.

data heterogeneity and inconsistency among studies, underling the need for additional research to increase test accuracy and assess the exact roles of miRNAs in BAV-associated aortopathy.

Novel potential biomarkers: long non-coding RNAs and circular RNAs

With the development of high-throughput sequencing technologies, there is an increasing interest in novel classes of non-coding RNAs (ncRNAs) as crucial players in the regulation of disease-relevant genes. In particular, long non-coding RNAs (lncRNAs) and circular RNAs (circRNAs) are emerging as important regulators and potential biomarkers in a wide range of cardiovascular diseases [56–58].

lncRNAs are non-protein coding transcripts with a length of more than 200 nucleotides and account for a large proportion of the non-coding transcriptome [59]. CircRNAs are a specific subtype of lncRNAs generated from exonic or intronic sequences, which are conserved across species, and show tissue-specific expression patterns. Because circRNAs form a covalently closed loop, they are free of exonuclease-mediated degradation and more stable than linear RNAs [60], constituting a huge advantage from a clinical point of view. Similar to lncRNAs, circRNAs can act as miRNA 'sponges' to regulate gene expression in distinct cardiovascular physio-pathological processes. Both non-coding RNAs are reported to play essential roles in a large variety of biological functions, including transcriptional regulation of gene expression, epigenetic regulation, cellular differentiation, proliferation, and apoptosis.

Although the research into circRNAs and lncRNAs is still at an early stage in the field of aortopathy, it is becoming even more important.

It is only recently that circRNAs have been shown to be dysregulated in human thoracic aortic dissection (TAD) [61]. Following gene ontology enrichment analysis and construction of the circRNA-miRNA co-expression network, dysregulated circRNAs resulted to be involved in the pathological processes of TAD. Interestingly, the upregulated hsa_circRNA_101238 has been found to inhibit the expression of hsa-miR-320a by acting as miRNA sponge, and in turn, increasing the expression of several TAD-associated genes, mainly MMP9 [61].

HIF1 alpha-antisense RNA 1 was the first reported lncRNA found to play a key role in TAA pathogenesis [61]. The expression of HIF1A-AS1 has been shown to be regulated by Brahma-related gene 1, whose levels are elevated in TAAs. In addition, the suppression of HIF1A-AS1 resulted in reduced apoptosis and increased proliferation of VSMCs, which may contribute to the pathogenesis of BAV-associated TAA [62,63].

More recently, Li et al. [64] profiled differential expression of lncRNAs between TAA and normal aortic samples by microarray analysis. Through different filtering steps, the authors selected two TAA-related lncRNAs (RP11-465L10.10 and CTD-2184D3.5) and elaborated their relationship with protein-coding genes, thus providing insights into the research of novel biomarkers and therapeutic targets for TAA [64].

Moreover, a transcriptomic profiling on both lncRNAs and mRNAs was also performed in sporadic TAA by Guo and colleagues [65]. This elegant work revealed the potential of HOX transcript antisense intergenic RNA (HOTAIR), an antisense lncRNA, in the pathogenesis of TAA by impacting on HASMCs apoptosis and extracellular matrix remodeling [65]. This is consistent with previous studies [63,66] which have demonstrated an increased HASMCs apoptosis in the media of ascending aortic aneurysm, leading to a decreased aortic wall elasticity. Hence, HOTAIR knockdown induced early and late apoptosis and reduced cell proliferation, leading to aortic dilation and aneurysm.

Moreover, HOTAIR knockdown induced the suppression of both mRNA and protein levels of collagen types I and III, which are major components of ECM and responsible for the normal tensile strength of the aortic wall. According to previous findings [67,68], this study suggests that a reduction of HOTAIR may weaken the aortic wall by dysregulating collagen, thus causing aortic media remodeling.

These preliminary studies pave the way for future investigations on the potential role of lncRNAs- and circRNAs-pathway regulation in understanding BAV aortopathy mechanisms. Future research is needed to better understand their function and novel potential through their regulation of miRNA-mRNA network.

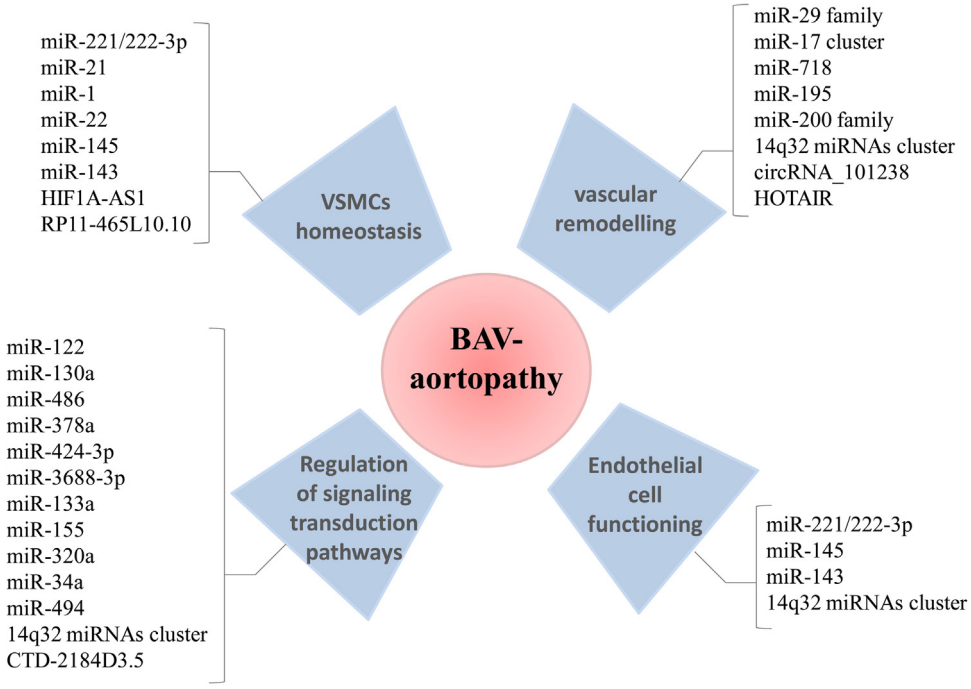

Fig. 2. Non-coding RNAs impacting the hallmarks of bicuspid aortic valve (BAV) aortopathy. The main pathological hallmarks of BAV aortopathy are shown with selected associated ncRNAs that have been linked to the development and progression of disease. VSMC, vascular smooth muscle cell.

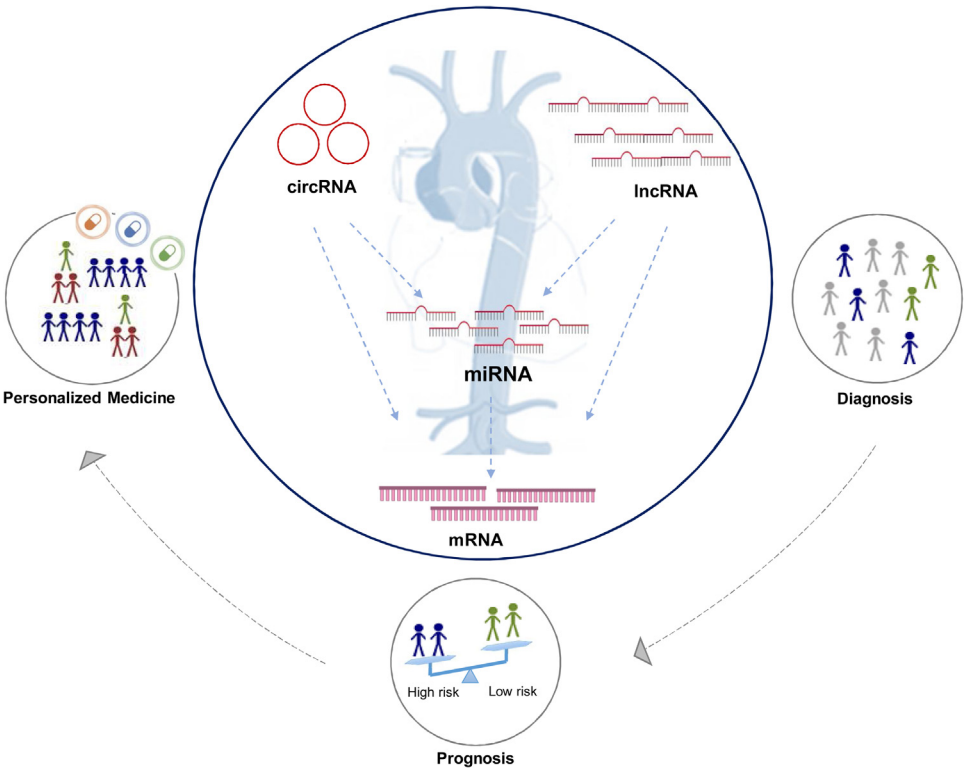

Fig. 3. Clinical potential of non-coding RNAs in bicuspid aortic valve (BAV) aortopathy. Unraveling the significance of ncRNAs in BAV-associated aortopathy may pave the way for new diagnostic tools for earlier detection and better surveillance, and promising therapeutic targets for personalized treatment approaches.

Conclusions and future perspectives

The critical contribution of miRNAs in vascular remodeling as well as aortic aneurysm development and progression has opened up new avenues to exploit their role in the BAV-associated aortopathy. Indeed, initial interesting findings have highlighted the importance of miRNAs in multiple cellular processes associated with BAV aortopathy, suggesting their usefulness as complementary more accurate and cost-efficient disease-related biomarkers (Fig. 2). However, the paucity and the inconsistency of the results obtained up to now underline the huge work that must be done in order to identify an individual miRNA profile that could be used for the determination of risk and progression of disease in patients with BAV aortopathy. These inconsistencies could be explained, in part, by analytical confounders in miRNA detection (e.g. study design, sample collection and handling, data analysis) as well as donor-related factors that could generate artifacts, impairing an accurate quantification. As regarding BAV aortopathy, the phenotypic and genetic heterogeneity present a challenging problem that need to be overcome before miRNAs could enter the clinical practice, and further validation studies in larger and well-defined BAV cohorts are now required. Therefore, at the moment, the use of miRNAs for BAV aortopathy detection is still doubtful.

In this context, the recent identification of a new class of non-coding RNAs such as lncRNAs and circRNAs has opened new perspectives in the development of useful biomarkers for diagnosis, prognosis and new RNA-based disease therapies. Although this new area of interest is still in its infancy, it holds great promise to reveal new cellular and molecular mechanisms with great potential to impact our understanding of the aortic dilatation in BAV patients as well as to reveal novel and reliable predictors of BAV-associated aortopathy (Fig. 2).

In conclusion, the identification of novel molecular signatures may help to define a personalized aortopathy-risk profile in BAV patients, allowing the optimization of each single surgical treatment and therapy intervention (Fig. 3).

Conflicts of interest

The authors declare no financial or other conflicts of interest.

Acknowledgments

None declared.

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