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Micro-RNAs in abdominal aortic aneurysms: insights from animal models and relevance to human disease

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Abstract:

Abdominal aortic aneurysm is a major health concern and may be associated with high rates of mortality linked to acute complications. Diagnosis and treatment are respectively based on imaging and surgical techniques. Drug-based therapies are still mostly ineffective, which highlights a real unmet need. Major pathophysiological mechanisms leading to aneurysm formation involve inflammatory processes, degradation of the extracellular matrix, and loss of smooth muscle cells. However, the precise cellular and molecular pathways are still poorly understood. Recently, microRNAs have emerged as major intracellular players in a wide range of biological processes, and their stability in extracellular medium within microvesicles has led to propose them as mediators of intercellular crosstalk and as potential biomarkers and therapeutic targets in a variety of disease settings. To date, several studies have been performed to address the involvement of micro-RNAs in aneurysm formation and complications. Here we discuss the roles and implications of micro-RNAs in animal models and their relevance to human abdominal aortic aneurysm.

Keywords: Abdominal aortic aneurysm, micro-RNAs, human studies, animal models

Abbreviations:

AAA: abdominal aortic aneurysm

angiotensin II/ apoE-/-: angiotensin II infusion into apolipoprotein E deficient mice

ATLOs : adventitial tertiary lymphoid organs

BLT1: Receptors for leukotriene B4

Chi3l1: chitinase 3-like 1

CTLA4: cytotoxic T-lymphocyte-associated protein 4

ECM: extracellular matrix

IL-3: interleukin-3

miR: micro-RNA

mRNA: messenger RNA

MMP: metalloproteinase

PAD: peripheral arterial disease

PTEN: phosphatase and tensin homolog

RECK: reversion-inducing cysteine-rich protein with kazal motifs

TAA: thoracic aortic aneurysm

TIMP3: tissue inhibitor of metalloproteinase 3

VEGFA: vascular endothelial growth factor A

VSMCs: vascular smooth muscle cells

Introduction

Abdominal aortic aneurysm (AAA) is defined as a focal dilatation of the aorta. Affecting up to 8% of men [1] and 1.3% of women over 65 years old [2], the disease is often asymptomatic but may become life threatening, especially when revealed by an acute rupture [3]. Epidemiological studies highlight the frequent association of AAA with atherosclerosis and some corresponding risk factors [1, 4, 5]. However, the mechanisms underlying AAA formation and progression may differ, particularly with regard to the degree of vascular remodeling in the media layer, and are not completely understood. AAA formation results from a complex process involving vascular and immune cell subsets [6]. Pathological features include adventitial and medial inflammatory cell infiltration associated with a degradation of extracellular matrix (ECM) components, including elastin and collagen, by an array of proteases such as matrix metalloproteinases (MMPs). Impairment of vascular smooth muscle cells (VSMCs) homeostasis favours their (de)differentiation to a secretory phenotype with alteration of migratory, proliferative and survival functions, which may significantly impact vascular remodelling and weaken the aortic wall. Additional alterations of endothelial cell function and formation of an intraluminal thrombus most probably contribute to pathologic aortic wall remodelling and disease progression.

Over the past decades, micro-RNAs (miRs) have emerged as major post-transcriptional regulators of gene expression [7] and have been shown to play key roles in vascular biology and cardiovascular diseases [8-10]. In this context, clinical and experimental studies have been performed to better understand the implications of miRs in AAA pathophysiology.

The aim of our review is to discuss the interest, limits and relevance of models to investigate the roles of miRs in AAA.

Human studies

Clinical relevance

The treatment of AAA is currently based on surgical intervention as no drug has proven yet enough benefit. In practice, the decision to treat relies on a balance between the risk of progression or rupture and the operative risks [4, 5]. However, despite the identification of some risk factors (e.g., large aneurysm diameter, high growth rate, association with female gender, smoking or hypertension), the risk of progression and rupture can still be difficult to predict. While some biomarkers have been reported to be associated with AAA, none of them has proven enough sensitivity or specificity to be used in daily medical practice [11, 12]. Thus, a real need emerged to develop new tools and help clinicians evaluate the risk of progression, rupture and post-therapeutic outcomes.

miRs as potential biomarkers of AAA (Table 1)

In that context, several clinical studies have been set up to identify new biomarkers of AAA (Table 1). The first landmark supporting the idea that miRs could reflect the disease was the observation of a differential miR expression in aneurysmal tissue compared to intact aortic samples. Indeed, some were found upregulated in aneurysmal tissue (miR-21 [13-15], miR-205, 378 [14], miR-126, 20a, 27a, 92a, 221, 222, let-7f, 155, 146a, 223, 124a [15]), others down regulated (miR-133a, 331-3p, 30c-2*, 204 [16], 24, 27b [17]), whereas some (miR-133b, 29b) appeared either increased or decreased [14-16, 18]. Note that the differentially expressed miRs substantially varied between studies. In a few cases, a similar variation of expression was observed for miR-21 and miR-155 in aneurysmal aortic tissue compared to their proper controls [13-15, 19]. In contrast, divergent results were found for miR-29b and miR-133b [14-16, 18], which could be partly explained by differences in methodologies used, including patient population, stage of the disease and technical approaches. While most of the above studies compared miR expression in aorta between patients with or without AAA, one study investigated miR expression in aortic samples exclusively from patients with AAA [19]. Interestingly, the authors found that the composition and the expression of miRs is different

between aortic tissue obtained from the body (at the site of maximum AAA dilatation) and the neck (defined as macroscopically non-dilated aorta), revealing a variation of miR expression within the aneurysmal tissue itself.

In contrast, some investigators studied miR expression in some specific structures present in AAA such as adventitial tertiary lymphoid organs (ATLOs) [20]. As ATLOs are mainly composed of immune cell subsets [21], it is not surprising that the authors observed a differential miR expression in ATLOs compared to VSMCs from non-aneurysmal aorta. Although ATLOs appear to play a role in atherosclerosis and AAA development, they are not systematically observed in atherosclerotic and aneurysmal tissues [20, 22]. Hence, miR expression could reflect the presence of ATLOs and inflammatory processes occurring within adventitial layer during AAA. Besides, these results suggest that cell specific miR expression should be analysed in order to fully understand their role. In another study, some authors addressed this question and found a differential expression of miR-516a-5p and miR-1260 in VSMCs cultured from AAA tissue as compared to VSMCs from non-aneurismal aortas [23].

While the observation of a deregulated miR expression profile in aortic tissue and specific cell subsets during AAA offers perspective to identify new biomarkers of the disease, their potential use in clinical practice is hardly possible using aortic samples as they can only be collected by invasive procedures. The discovery of circulating miRs in different biological fluids, has led to propose them as non-invasive biomarkers in a wide range of pathologies including cardiovascular diseases [24]. Interestingly, a differential miR expression was observed between patients with AAA and controls, both in whole blood [25] and in plasma [17, 25-27].

Some investigators also found a differential miR expression in plasma (miR-15a-3p, 30a-5p) and in serum (miR-155) between patients with AAA and patients with peripheral arterial disease (PAD) [19, 20], suggesting that circulating miRs may display distinct patterns in AAA compared to other cardiovascular diseases. However, in another study investigating other miRs, no difference in miR profile could be detected between patients with AAA and PAD [25], suggesting that changes in the expression of some miRs could be linked to a common pathological process such as atherosclerosis. The identification of similar miR patterns in distinct cardiovascular diseases underlines the need to test their specificity for a potential use in medical practice. Addressing this question in a cohort of 120 individuals, some investigators found that for 3 miRs (miR-191-3p, 455-3p, 1281) investigated, the sensitivity to diagnose AAA varied from 76.7% to 95% and the specificity from 96.7% to 100% [26]. Even

if further investigations are required on larger cohorts, the results are encouraging and may lead to the validation of reliable biomarkers of AAA. Furthermore, circulating miRs could be potential biomarkers of AAA growth, as suggested by the association between aortic diameter and plasma levels of miR-340, 20a, 148a, 125b, 195 [27].

However, only a few studies identified the same disease-related miRs in peripheral blood and results were heterogeneous, as demonstrated by divergent circulating levels for miR-155[15, 19] and miR-29b [15, 25]. The observed difference could be partly explained by the nature of the blood sample used, as the expression of some miRs can vary between plasma and serum [28].

Additional points need also to be more deeply investigated. First, the origin of circulating miRs is not totally elucidated. According to the current knowledge, they could derive from cellular degradation and/or be released through various modes of vesiculation (e.g., microparticles or exosomes) [29]. Besides, circulating miRs measured in blood could come from a wide range of tissues and cell subsets. Even if some miRs misexpressed in aortic samples were also deregulated in the circulation such as miR-20a [15, 27], miR-155 [15, 19], miR-29b [15, 18], and miR-124a, 223, 126, 146a [15], the pathways responsible for their deregulation in aortic samples and blood need to be further investigated and potential links identified. Also, the role of differentially expressed miRs remains to be established in order to better appreciate their relevance as biomarkers or causal factors in AAA pathophysiology.

Roles of miRs in the pathophysiology of human AAA

Intracellular miRs mainly regulate gene expression through binding to their target messenger RNA (mRNA), inhibiting their translation or inducing their degradation [7]. Several studies identified predicted targets of deregulated miRs using bioinformatics approach and many of them appear to be involved in biological pathways relevant to AAA development such as inflammation, ECM remodelling, apoptosis or cell proliferation [16, 23, 25].

Some investigators provided direct *in vitro* experimental evidence for regulation of the theoretical targets by the identified miRs, and further addressed gene expression or protein levels of the potential miR targets *in vivo*. Interestingly, a correlation was found between some deregulated miRs in aneurysmal aorta and expression of their putative targets involved in inflammation, as demonstrated by the opposite variation between miR-155 and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) expression [19], miR-24 and chitinase 3 like 1

(Chi3l1) [17], miR-146a, 124, 223, 126 and inflammatory cytokines [15] or miR-516-5p and levels of IL-3 and VEGFA secreted by VSMCs [23]. Besides, several studies observed a decrease of some miRs (miR-1260, 29b) in aneurysmal aorta, in association with significant changes in protein or gene expression of ECM components such as collagen [18, 23]. Thus, clinical studies revealed concomitant deregulation of some miRs and their putative targets, some of them being key-players in mechanisms involved in AAA formation, including inflammation, fibrosis or ECM remodelling, suggesting potentially causal roles of miRs in the pathophysiology of human AAA.

Limits of clinical studies

Clinical studies have highlighted the potential implication of miR-regulated processes in the pathophysiology of AAA. However, comparison of the results among different studies reveals a low overlap of differentially regulated miRs. Thus, several considerations should be taken into account when designing clinical studies in order to minimize potential bias in data collection, analysis and interpretation.

The first point relates to the population studied and the inclusion criteria. Pathophysiological characteristics including age, sex, presence of comorbidities or associated cardiovascular risk factors may impact on miR expression [30-33] and act as confounding elements. Such factors should be clearly identified and controlled for during study design and in the final statistical analysis.

Additional considerations relate to sample collection. First, human aortic tissue can only be collected from patients who undergo a surgical intervention and as a consequence, analysis of aortic miR expression was most often performed on small cohorts. Obtaining proper aortic controls can even be more challenging. Studies used aortic samples from different origin: 1) patients undergoing liver transplantation or surgical valve repair [15, 23], 2) organ-donors [13, 17, 18, 20], 3) cadaveric donors [16, 25]. However, although miRs are highly stable in both tissues and biological fluids [34, 35], their stability in post-mortem conditions is not well known [36]. While in the majority of studies aortic control samples were taken from the abdominal aorta, one study obtained samples from the ascending aorta [15]. Note that in physiological conditions, thoracic and abdominal aortas are subjected to different hemodynamic loads, differ in histological and biochemical composition, and in the embryologic origin of their VSMCs [37, 38]. In pathological

setting, AAA and thoracic aortic aneurysm (TAA) are characterized by distinct epidemiological patterns and aetiological factors [39]. While a few miRs deregulated in AAA such as miR-21, miR-29b, and miR-133a were also differentially expressed in TAA compared to controls [13-15, 18, 40-43], specific miRs other than those identified in AAA could be identified in TAA, including differential expression of miR-1, 29a, 143, 145, and 486-5p [40-42, 44]. As such, it is highly plausible that the pattern of miR expression may differ between abdominal and thoracic aorta, both in health and disease. Thus, appropriate control samples should come from the same aortic region. In other studies, some investigators did not collect aortic samples from patients without AAA and made direct comparisons between miRs expressed in AAA body and in neck [19]. The use of the neck as control to study miR expression presents the advantage to be perfectly matched with the samples obtained from the body of the AAA. However, histological analysis revealed that even if biopsies from the neck show relative preservation of intimal and medial structures, the morphology is not completely normal, as demonstrated by inflammatory cells infiltrated in the adventitial layer [45]. As a consequence, it is possible that miR expression in the neck of AAA differ from intact non aneurysmal aorta.

Compared to aortic tissues, blood samples have the advantage to be easier to collect, allowing to study larger cohorts of patients with AAA. Nevertheless, circulating miR profile may differ depending on the nature of the sample [28] and can be altered by treatments commonly prescribed to patients with AAA such as anticoagulants, antiplatelets [46], antidiabetic agents [47], or cholesterol lowering drugs [48]. Also, the numerous methods used for miR detection and data normalization can potentially impact on miR profile [49, 50].

As a conclusion, clinical studies on miRs in AAA offer interesting opportunities to explore their potential use as biomarkers and to study their role in AAA pathophysiology. However, the methodology used differed between the studies, making difficult any direct comparison between the results. Besides, clinical studies present several limitations in addressing the precise cellular and molecular effects of miRs in AAA development. First, aortic samples can only be taken from patients who have surgical indication, which means that they have advanced or complicated lesions. This is a major limitation to the study of the role of miRs in the early stages of AAA development. Second, while some correlations between miRs and their putative targets have been identified, it is difficult to determine if miR deregulation has a causal effect on AAA development, or on the contrary results from a compensatory mechanism to regulate it. Hence, the need to use animal models, which allow detailed mechanistic approaches to the disease process.

Animal studies

Animal models of AAA used to study miRs

Several experimental models of AAA have been developed over the past decades including genetic, surgical and chemically induced models in rodents or larger animals [51-53]. Surgical models directly induce mechanical injury within the arterial wall leading to AAA but require technically difficult procedures and expose the animals to prolonged anaesthesia. In that context, due to their efficiency to induce AAA, their relative technical simplicity and low cost, studies on miRs have been exclusively performed on chemically induced AAA in rodents (Table 2).

Angiotensin II infusion into apolipoprotein E deficient mice (angiotensin II/ apoE-/-) has been, to date, the model the most commonly used to study miRs in AAA [13, 14, 17, 18, 27, 54]. This model usually induces AAA located in the suprarenal aorta within 28 days and reproduces some of the major features observed in AAA pathophysiology such as medial degradation with ECM remodelling, inflammation and association with atherosclerosis [55, 56]. While hyperlipidaemia increases incidence, it is not required for the induction of AAA. As a consequence, some investigators used angiotensin II infusion in C57BL/6 mice [43]. Another commonly used chemically induced model of AAA is the elastase perfusion model. The technique requires a surgical intervention to introduce a catheter in the infrarenal aorta and perfuse pancreatic porcine elastase for 5 to 15 minutes [57]. The perfusion of elastase induces a destruction of medial elastic tissue and inflammation, leading to infrarenal AAA formation within 14 days [58]. At last, some investigators used a third model in rats, consisting in application of calcium chloride in combination with collagenase on the adventitial layer of the aorta between the renal branches and the iliac bifurcation [59]. Calcium chloride application provokes calcium deposition throughout the media, disruption of ECM and induction of inflammatory responses, leading to infrarenal AAA development between 2 or 4 weeks [60].

Roles of miRs in AAA pathophysiology and as therapeutic tools

The studies performed so far in animal models have provided interesting results. First, differential miR expression between aneurysmal and control aorta was observed in all the models described above [13, 14, 17, 18, 27, 43, 54, 59]. Furthermore, several studies reported inverse

correlations between miR levels and the expression of their putative targets, involved in key mechanisms of AAA pathophysiology. As an example, miR-29b expression was decreased in aneurysmal aortic wall and negatively correlated with collagen and elastin gene expression [18], which strongly suggested a role of miR-29b in ECM remodelling. In another study, a decrease of miR-24 was observed concomitantly with an increase of its putative target Chi3l1, pinpointing the potential role of miR-24 in inflammation [17]. Similarly, a decrease of miR-26a in aneurysmal aortic tissue was reported to occur in parallel to an increase of several predicted targets, including genes involved in cell cycling, apoptosis, proliferation, migration or cytokine production [54].

While correlation between miR expression and their predicted targets can be studied in both human and animal models, animal studies offer the great possibility to modulate miR expression and study its direct impact on the disease process. miR modulation consists in overexpressing a miR using a miR-mimic or inhibiting its expression with an antago-miR. miR-mimics are double stranded oligonucleotides in which one of the strand is the mature miR sequence. In the cell, miR biogenesis machinery recognizes the miR-mimic and processes it, delivering the mature miR strand and leading to its overexpression [61]. In contrast, antago-miRs are synthetic single-stranded RNA analogues complementary to miRs, which hybridize specifically with the target miR, inhibiting its effect [62].

Modulating miR expression allows for better understanding of their roles. Several studies highlighted the potential contribution of miRs to AAA development. As an example, miR-712 and its homolog miR-205 were increased concomitantly with: 1) a decrease of two MMP inhibitors TIMP3 (tissue inhibitor of metalloproteinase 3) and RECK (Reversion-inducing cysteine-rich protein with kazal motifs), and 2) an increase of MMP activity in the abdominal aortic endothelium of angiotensin II-treated apoE-/- mice compared to controls [14]. Silencing miR-712 or miR-205 reversed the downregulation of TIMP3 and RECK, decreased MMP activity and elastin fragmentation, preventing AAA formation. Hence, miR-712 and miR-205 misexpression seems to play a causal effect in aortic endothelium promoting AAA progression through targeting key molecules involved in ECM remodelling.

Another study found a decrease of miR-24 in AAA tissue compared to non-diseased aorta, both in the elastase and angiotensin II/ apoE-/- models [17]. In the elastase model, miR-24 inhibition using intraperitoneal injection of lenti-anti-miR-24 increased aortic dilatation and incidence of rupture, concomitantly with an increase of Chi3l1. In contrast, miR-24

overexpression using a lenti-premiR-24 injection revealed opposite effects on Chi3l1 expression, decreased macrophage infiltration and attenuated AAA dilatation. Similar results were found in angiotensin II/ apoE-/- model, suggesting that miR-24 contributes to AAA development through the regulation of inflammatory processes via targeting Chi3l1.

While the above studies revealed a contributing role of some miRs to AAA formation, others have suggested protective or compensatory roles for a few miRs. For example, miR-29b expression was decreased in infrarenal aortic tissue of both elastase and angiotensin II/ apoE-/- models, concomitantly with an increase of collagen and elastin [18]. Further inhibition of miR-29b through anti-miR-29b injection boosted the increase of collagen and elastin gene expression resulting in a significant reduction of AAA expansion. Another study corroborated this finding and reported a decrease of aortic diameter and enhanced survival after systemic inhibition of miR-29b in angiotensin II/ apoE-/- mice [27]. Overexpressing miR-29b through administration of premiR-29b showed opposite effects, increasing AAA development [18]. These results indicate that the decrease of miR-29b observed in aortic tissue may result from a compensatory mechanism to counter-regulate AAA expansion. In another study, miR-21 was found increased in AAA tissue of elastase and angiotensin II/ apoE-/- models [13]. miR-21 overexpression increased cell proliferation and decreased apoptosis in the aortic wall, associated with a reduction of PTEN protein (phosphatase and tensin homolog) and activation of AKT which led to protection against AAA expansion. In contrast, antago-miR-21 injection increased PTEN expression and promoted AAA growth. Hence, miR-21 upregulation observed in AAA tissue appears to be a physiological response elicited to counter-regulate and prevent AAA expansion.

Thus, several miRs have been identified as causally linked to the development and complication of AAA in animal models, offering the perspective to use miRs as therapeutic tools and targets. Nevertheless, further studies in animal models need to be performed before potential applications can be translated to the clinical arena. First, the sustainability of the biological effect of miR modulation needs to be further investigated as several studies observed a steady decrease of the biological effect during the 28-day follow up period [13, 18]. Second, the delivery and uptake of miR modulators need to be well characterized in order to improve selective targeting and limit potential side effects. Analysis of aortic localisation and effects of some miR modulators after systemic injection suggested that they were almost exclusively limited to the site of injury in the elastase model [13, 17, 18]. However, their uptake in the aortic wall in other AAA models needs to be further characterized. Systemic

administration of some miR modulators of miR-29b or miR-21 was associated with biological effects in other organs such as the heart, the liver or the kidney [13, 18, 27], which may induce substantial side effects. Hence, local administration could be a step forward to limit undesirable effects. Interestingly, some investigators studied *in vivo* local inhibition of some miRs using an aortic isograft model [27]. The technique consists in harvesting the aorta, incubating it with miR modulators, then re-implanting it into the carotid artery of recipient mice. While the technique resulted in effective local modulation of miR expression, it can't be extrapolated for a potential use as therapeutic tool in patients as it requires aortic tissue removal and re-implantation. In practice, surgical intervention physically excludes the aneurysm using a synthetic prosthesis [4, 5]. Thus, investigating local modulation of miR expression through drug administration via the prosthesis would be of great interest. To date, experiments using stent grafts for surgical repair of AAA have been mostly performed on big animals such as dogs and pigs given the larger size of the aorta [63, 64]. Nevertheless, graft implantation in smaller animals like rats has been developed [65] and performed in animals with induced AAA [66]. In this context, a recent study addressed the feasibility of local miR modulation using an anti-miR-21-coated stent [67]. To investigate the role of miRs in in-stent restenosis, the authors used a model in which balloon-injured and stented human internal mammary arteries were anastomosed to the abdominal aorta of Rowett nude rats. Interestingly, anti-miR-21–coated stents reduced in-stent restenosis compared to bare metal stents and screening for off-target effects did not reveal any miR-21 expression changes in kidney, liver, heart, or lung. Hence, stent graft implantation in murine models as well as the development of stents coated with miR modulators offer great perspectives for the testing and translation of miR-based therapeutic strategies to combat AAA.

Impact of risk factors on miRs and consequences for AAA development

While in human studies presence of associated risk factors may potentially confound results interpretation, animal studies offer the advantage of a homogeneous background and the possibility to investigate the contribution of individual risk factors to miR expression and AAA development. Among the risk factors of AAA, smoking is considered as one of the most important [4, 5]. As previously mentioned, miR-21 expression is increased in the aneurysmal aortic segments of both elastase and angiotensin II mouse models [13]. Interestingly, subcutaneous nicotine administration during 60 days boosted the increase of miR-21 in both

models. The overall results of the study suggested that miR-21 upregulation was a physiological protective response to prevent AAA expansion.

The impact of aging has also been addressed. Investigators reported an increase of miR-29b expression in aortic tissue of aged compared to young mice [43]. miR-29b expression is decreased in the aneurysmal aorta of both elastase and angiotensin II/ apoE-/- models, and its downregulation seems to play a protective role on AAA formation [18]. Interestingly, an increase of miR-29b expression was observed in the aneurysmal aorta of aged wild type mice infused with angiotensin II [43]. Further, inhibition of miR-29b expression in this model prevented the increase of aortic diameter induced by angiotensin II. These results suggest that down regulation of miR-29b could result from a physiological response to prevent AAA expansion, and that aging could, at least partly, contribute to AAA development through an impairment of that mechanism. Hence, animal models offer the possibility to better understand the direct roles of miRs and the molecular mechanisms that link risk factors identified in epidemiological studies to AAA development.

miRs in other forms of aortic aneurysm

Aortic aneurysm may also involve the thoracic aorta. TAA develop frequently at younger ages, and are often linked to hereditary influence and display distinct pathological features compared to AAA[39]. Only a few studies investigated miR expression in animal models of TAA: fibulin-$4^{R/R}$ knockdown and Fbn1 (C1039G/+) Marfan mouse model [43, 68]. miRs differentially expressed within the aneurysmal aortic wall during TAA differed from those identified in AAA, as demonstrated by changes in expression of miR-29a and miR-29c in TAA, whereas no variation was observed for miR-21 [43, 68]. In contrast to AAA animal models, miR-29b expression was found increased in thoracic aneurysmal aortas [43, 68], and played a causal role as demonstrated by the prevention of early aneurysm development induced after blockade of miR-29b in Marfan mice [68].

Relevance of animal models of AAA

Animal models provide interesting opportunities to study miRs in AAA. However, it is necessary to identify the potential confounding factors that may alter miR expression profile and downstream miR effects in each model. For example, hypertension, which develops in

response to infusion of angiotensin II [69] may be associated with modification of miR expression in the aorta [70] and may therefore account, at least in part, for changes of miR profile observed in angiotensin II-infused mice. The use of apoE-/- mice, which develop hypercholesterolemia and atherosclerosic lesions [71] may also be a confounding factor given the potential direct impact of atherosclerotic changes on miR expression profile in the aortic wall [72]. Thus, data reproducibility between different animal models of AAA is worth of evaluation.

A few studies investigated the same miRs in different models of AAA. Interestingly, consistent results of miR expression in aneurysmal aorta were found between different models for miR-21 and miR-29b [13, 14, 18, 54] and their effects on the disease process were reproducible [13, 18]. Note that while a decrease of miR-29b was observed in aortic tissue in both elastase and angiotensin II/ apoE-/- models [18], one study reported an increase of its expression in the aorta of 18-month wild type C57BL/6 mice infused with angiotensin II [43]. However, that difference may be attributed to the impact of aging on the aorta. The concordance of results between different animal models suggests that the identified miRs are strongly linked to the pathophysiology of AAA, and less to the direct effect of AAA-inducing agents or to the presence of associated disease states such as hypertension, hypercholesterolemia or atherosclerosis. Nevertheless, it is possible that divergences between animal models are underestimated due to the fact that inconsistent results among different models in the same study are less likely to be published.

The relevance of animal models to human AAA pathophysiology also needs to be addressed.

The mouse models described above all reproduce some of the human features of AAA such as higher incidence in males than in females. They also mimic some of the histological characteristics of human lesions including medial degradation, ECM remodelling, and inflammatory responses [55, 58, 60]. However, each model has its own specificities, which may make it differ from the human disease.

Infusion of angiotensin II in ApoE-/- is associated with the development of atherosclerosis, hypertension and hypercholesterolemia, which are risk factors frequently identified in human AAA [4, 5]. Death related to aortic ruptures can be observed in the model [73], allowing to study the mechanism leading to this complication. Nevertheless, aneurysm formation tends to occur in the suprarenal aorta, while the most frequent location of AAA in humans is infrarenal. In addition, rupture observed in that animal model is favoured by the rapid development of

medial dissection associated with the formation of transmural thrombi and haematomas [73, 74] In human AAA, medial dissection is not common, in contrast to rupture, and thrombi usually appear in the intraluminal part of the aorta [75]. Compared to angiotensin II infusion, elastase perfusion model has the advantage to directly induce AAA in the infrarenal aorta [57, 58]. However, incidence of rupture is rare. The calcium chloride model does not lead to thrombus formation or rupture, which are major features of the human disease.

Dissimilarities of each of the models with the human disease may question the translational relevance of the results obtained in mice. Interestingly, some miRs identified in animal studies were also observed to be dysregulated in humans (Table 2). This is for example the case of miR-21 [13-15, 54], mir-221, miR-146a and miR-222 [15, 59], miR-24 [17], or miR-205 [14] which were similarly modulated in aneurysmal tissue of mice and humans.

However, the expression of other miRs revealed more heterogeneous results. This was the case for miR-133b, which was increased in endothelial cells of murine aneurysmal aorta [14], but was reported to be either increased [14] or decreased [16] in human aneurysmal aorta compared to normal aortic tissue. Note that in the animal study, investigators only used the endothelial part of the aorta, while in both human studies the whole wall tissue was processed to extract RNAs. Heterogeneous results were also found for miR-29b, which was decreased in two different animal models [18], while its expression was either decreased [18] or increased [15] in human aneurysmal aorta. miR-195 was increased in the aorta of angiotensin II/ apoE-/- mice, but when its circulating level was investigated in human plasma, an inverse association was found between miR-195 and the presence of AAA [27]. Note that the origin of the biological samples used to determine miR-195 expression differed, which may explain the opposite variation of this miR between mice and humans.

Thus, even if animal models are useful to study the role of miRs in AAA development, dissimilarities of expression were found for some miRs between humans and animals, which can be attributed to differences in methodology and/or pathophysiological features. Moreover, even if miRs are well conserved, their regulation may differ among species, which may account for some of the observed differences [76].

Future directions

We believe that a few issues should be taken into account in future work. Most studies addressed the expression of a limited number of miRs and followed a hypothesis-driven approach. As changes in miRs do not occur in isolation, it should be useful to follow, at least in pilot studies, an unbiased discovery-driven approach looking at all existing miRs with the aim to account for the formidable complexity of the disease and try to identify novel miR signatures (combinations of miRs). This may not only improve the sensitivity and specificity of circulating miRs as potential biomarkers of AAA but may also inform about the de-regulation of selective and previously unsuspected biological pathways involved in the disease.

In both clinical and experimental studies addressing vascular miR expression, the role of miRs was mainly investigated in whole aortic tissue samples. To fully understand their complex role in AAA pathophysiology, it would be useful to specifically study miR expression in the different structures of the aortic wall and in specific cell subsets. The use of laser capture microdissection which allows for the extraction of very precise tissue structures and cells [77] could be very useful in that purpose. This can be coupled with advanced flow cytometry-based cell sorting and RNA sequencing techniques to gain deeper insight into the expression profile and biological pathways altered in specific locations and specific cell types, both systemically and within the aneurysmal wall.

Finally, the low level of consistency among the small clinical studies underlines the need to perform multicentre studies on larger cohorts of patients, providing enough power to detect a significant result after adjustment for multiple covariates and confounding factors. In this regard, longitudinal studies would be a great asset to investigate miR variation, roles and implications during disease progression. It could offer the opportunity to explore miRs as biomarkers of progression and risk of rupture, but also as biomarkers of therapeutic outcome.

Conclusion

Human and animal studies show that distinct miRs are differentially expressed within the aneurysmal wall (Figure 1) and may play causal roles in AAA formation (Figure 2). Basic experimental and translational approaches present advantages and limitations, but may complement each other. Human studies offer the possibility of identifying selective miRs as potential biomarkers and players in AAA pathophysiology. However, the investigation of

their precise roles may be limited and impacted by several confounding factors. Hence, the need to standardize study design and methods used, at least for miR analysis. Animals are more suitable to mechanistic studies and interventional approaches, which should allow for a better comprehension of the precise roles of miRs in AAA pathophysiology. There is an immense need to develop better animal models that more closely reproduce the human pathophysiological features of AAA. New models of the disease have been developed over the past few years [53], some of which have led to a reconsideration of the role of major pathways in disease pathogenesis [78]. However, we believe that the current models are highly perfectible and research should still be directed towards this aim. Graft implantation in rodents as well as coated stent technology are under development and would offer new opportunities to test therapeutic strategies based on miR modulation. No doubt that major advances in the understanding of miR functions and regulations are to be expected and could offer innovative perspectives to identify, treat and improve the daily management of patients with AAA.

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Contribution statements

All authors confirmed that they have contributed to the intellectual content of this article and have approved the final version.

	Population	Samples	Main miRs identified	Reference
AAA	Screening study - 5 patients with infrarenal AAA undergoing elective surgical repair - 5 controls from cadaveric donors Validation study - 36 patients with AAA undergoing surgical repair (25 elective, 11 ruptured) - 7 controls from cadaveric donors	Aorta: full thickness aortic wall	- Differential miR expression between patients with AAA and controls: → Decreased: miR-133b, 133a, 331-3p, 30c-2*, 204	Pahl et al, 2012 [16]
	- 15 patients with infrarenal large AAA (59 to 68 mm) undergoing surgical repair - 5 controls from organ donors	Aorta: full thickness aortic wall	- Differential miR expression between patients with AAA and controls : → Decreased: miR-29b	Maegdefess el et al, 2012 [18]
	- 13 patients with large AAA (57 to 68 mm) undergoing surgical repair (8 active smokers, 5 non-smokers) - 5 controls from organ donors	Aorta: full thickness aortic wall	- Differential miR expression between patients with AAA and controls : → Increased: miR-21	Maegdefess el et al, 2012 [13]
	- 5 patients with AAA who underwent surgery - 4 controls from patients without AAA	Aorta: formalin-fixed paraffin-embedded sections	- Differential miR expression between patients with AAA and controls : → Increased: miR-205, 21, 133b, 378	Kim et al, 2014 [14]
	Screening study - 6 patients with AAA undergoing surgical open repair - 6 patients from men undergoing liver transplantation Validation study - 10 patients with AAA undergoing surgical open repair (6 elective AAA, 4 ruptured AAA) - 10 controls	Aorta: medial wall dissected from luminal thrombus and adventitia, culture of vascular smooth muscle cells (VSMCs)	- Differential miR expression between patients with AAA and controls: → Increased: miR-516a-5p → Decreased: miR-1260	Cheuk et al, 2014 [23]
	- 10 patients with large infrarenal AAA (>50mm) undergoing surgical open repair	Aorta: full thickness of the aortic wall - Body biopsy: at the site of maximum AAA dilatation - Neck biopsy: at the site of macroscopically non-dilated AAA neck	- miRs found exclusively in AAA body: miR-96, 9, 105, 33 - miRs found exclusively in AAA neck: miR-189, 151, 154, 154*, 153, 188, 147, 183, 199b - Differential miR expression between AAA body and AAA neck: → Increased: miR-155, 28, 30a-3p, 150, 302b*, 93, 99a	Biros et al, 2014 [19]
	- 10 patients with large infrarenal AAA (>50mm) undergoing surgical open repair - 10 patients with peripheral arterial disease (PAD) as controls	Serum	- miRs found exclusively in patients with AAA, not in controls : miR-220, 10a, 23b - Differential miR level between patients with AAA and patients with PAD: → Increased: miR-155	
	Initial study - 19 patients with infrarenal AAA undergoing surgical open repair - 10 controls from cadaveric donors Validation study - 22 patients with AAA infrarenal undergoing surgical open repair - 17 controls from cadaveric donors - 3 patients with aorto-occlusive disease	Aorta: full thickness of the aortic wall	- No significant difference observed between patients with AAA and controls	Stather et al, 2015 [25]
	Initial study - 15 patients with large AAA (>54mm) - 10 healthy controls Validation study - 40 patients with small AAA (30-54mm)	Whole blood	- Differential miR level between patients with AAA and controls: → Decreased: miR-15a, 196b - Differential miR level between patients with PAD and controls: → Decreased: miR-let-7e, 15a, 196b	

	- 40 patients with large AAA (>54mm) - 40 patients with AAA who had surgical repair (samples collected 6 months after open or endovascular repair) - 40 patients with PAD - 40 healthy controls		→ Increased: miR-411	
	- 36 patients with small AAA (30-54mm) - 36 patients with large AAA (>54mm) - 35 patients who had AAA repair (samples collected 6 months after open or endovascular repair) - 35 patients with PAD - 28 healthy controls	Plasma	- Differential miR level between patients with AAA and controls: → Decreased: miR-196b but significance not maintained after binary logistic regression	
	- 20 patients with infrarenal AAA undergoing surgical open repair - 14 controls from organ donors	Aorta: whole tissue or extraction of areas rich in adventitial tertiary lymphoid organs (ATLOs)	- Differential miR expression in ATLOs compared to VSMCs from non-aneurysmal aorta: → Increased: 489-3p → Decreased: miR-15a-3p, 30a-5p	Spear et al, 2015 [20]
	- 24 patients with AAA requiring surgery (> 50 mm or increasing more than 10 mm during the past 6 months), - 18 patients with PAD	Plasma	- Differential miR level between patients with AAA and patients with PAD: → Decreased: miR-15a-3p, 30a-5p	
	- 22 patients with infrarenal large AAA (52 to 115 mm) undergoing elective surgical open repair - 14 controls from organ donors	Aorta: full thickness aortic wall	- Differential miR expression between patients with AAA and controls : → Decreased: miR-24, 27b	Maegdefess el et al, 2014 [17]
	- 54 patients with small AAA (45-67 mm) - 51 patients with large AAA (69-150 mm) - 22 patients with peripheral vascular occlusive disease (PVOD) - 52 controls	Plasma	- Differential miR level between patients with AAA and controls: → Decreased: miR-24 - Differential miR level between patients with AAA and patients with PVOD: → Decreased: miR-24	
	- 13 patients with infrarenal AAA undergoing elective surgical open repair - 7 controls from ascending normal aorta from patients undergoing aortic valve replacement surgery	Aorta: full thickness aortic wall	- Differential miR expression between patients with AAA and controls : → Increased: miR-126, 20a, 27a, 92a, 221, 222, let-7f, 21, 155, 146a, 223, 124a, 29b	Kin et al, 2012 [15]
	- 23 patients with AAA - 17 patients with coronary artery disease (CAD) - 12 healthy volunteers	Plasma	- Differential miR level between AAA and controls: → Decreased: miR-124a, 29b, 155, 223, 126, 146a - Differential miR expression in plasma between AAA and CAD: → Decreased: miR-124a, 29b, 155, 223, 21	
	<u>Screening study</u> - 10 patients with AAA - 10 healthy controls <u>Validation study</u> - 60 patients with AAA - 60 healthy controls	Plasma	- Differential miR level between patients with AAA and controls: → Increased: miR-191-3p, 455-3p, 1281	Zhang et al, 2015 [26]
	- 32 patients with AAA - 41 controls	Plasma	- Inverse association of miR level with the presence of AAA and the aortic diameter: miR-195	Zampetaki et al, 2015 [27]
TAA	- 30 patients with ascending TAA + tricuspid aortic valves (TAV) undergoing surgery - 10 controls from ascending non aneurysmal aorta of heart donors and coronary artery bypass graft patients	Aorta: full thickness aortic wall	- Differential miR expression between patients with TAA + TAV and controls : → Decreased: miRs -1, 21, 29a, 133a, 486-5p	Jones et al, 2011 [40]
	- 10 patients with ascending TAA + tricuspid aortic valves (TAV) undergoing surgery	Aorta: full thickness aortic wall	- Differential miR expression between patients with TAA + TAV and controls :	Pei et al, 2015 [44]

	- 10 controls patients from non-aneurysmal aortas of coronary artery bypass graft patients		→ Increased: miR-145	
	- 10 patients with non-familial non syndromic ascending TAA undergoing surgery - 10 controls from ascending non aneurysmal aorta of heart transplant recipients	Aorta: full thickness aortic wall	- Differential miR expression between patients with TAA and controls : → 99 differentially expressed miRs identified by microarray	Patuzzo et al, 2012 [42]
	- 79 patients with TAA + bicuspid aortic valve (BAV) undergoing surgery - 30 patients with TAA + tricuspid aortic valve (TAV) undergoing surgery - 33 controls from non-aneurysmal aorta of coronary bypass surgery patients	- Aorta: full thickness aortic wall	- Differential miR expression between patients with TAA + BAV, with TAA + TAV and controls : → Increased: miR-29b	Boon et al, 2011 [43]
	- 21 patients with ascending TAA + bicuspid aortic valves (BAV) undergoing surgery - 21 patients with ascending TAA + tricuspid aortic valve (TAV) undergoing surgery - 10 controls from non-aneurysmal ascending aorta of heart transplant donors or recipients	Aorta: full thickness aortic wall	- Differential miR expression between patients with TAA + BAV and controls : → Decreased: miR-143 → Increased: miR-29a, 145 - Differential miR expression between patients with TAA + TAV and controls : → Decreased: miR-1, 133a, 143 → Increased: miR-21 - Differential miR expression between patients with TAA + TAV and patients with TAA + BAV → Decreased: miR-1 → Increased: miR-21	Ikonomidis et al, 2013 [41]
		Plasma	- Differential miR level between patients with TAA + BAV and controls : → Decreased: miR-145 → Increased: miR-133a - Differential miR expression between patients with TAA + TAV and controls : → Decreased: miR-21, 29a, 143 → Increased: 133a	

Table 1: Summary of human studies on miRs in aortic aneurysms.

Legends: AAA: abdominal aortic aneurysm

BAV: bicuspid aortic valves

CAD: coronary artery disease

PAD: peripheral arterial disease

PVOD: peripheral vascular occlusive disease

TAA: thoracic aortic aneurysm

TAV: tricuspid aortic valves

VSMCs: vascular smooth muscle cells

	Animal models	Main miRs dysregulated in animal models validated by qPCR	miRs observed in animal models also dysregulated in patients with aortic aneurysm
AAA	Angiotensin II infusion in apoE-/- mice	- Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: [13]: miR-21 [27]: miR-195 → Decreased: [18]: miR-29b [17]: miR-24 [54]: miR-26a - Differential miR expression between endothelium of aneurysmal aorta and controls: → Increased: [14]: miR-21, 1, 133b, 207, 378, 1957, 712, 205 → Decreased: [14]: miR-1952, 1249 - Differential miR expression in plasma between mice with AAA and controls: → Decreased: [17]: miR-24	- Differential miR expression between abdominal aneurysmal whole aortic tissue and controls: → Increased: [13]: miR-21 [15]: miR-21, 146a, 29b, 221, 222 [14]: miR-21, 133b → Decreased: [18]: miR-29b [17]: miR-24 [16]: miR-133b - Differential miR level in plasma between patients with AAA and controls: → Decreased: [17]: miR-24 [27]: miR-195 [15]: miR-29b - Differential miR level in plasma between patients with AAA and coronary artery disease: → Decreased: [15]: miR-29b
	Angiotensin II infusion in C57BL/6 mice	Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: [43]: miR-29b	
	Elastase perfusion in ApoE-/- mice	Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: [54]: miR-21 → Decreased: [54]: miR-26a	
	Elastase perfusion in C57BL/6 mice	Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: [13]: miR-21 → Decreased: [18]: miR-29b [17]: miR-24 - Differential miR level in plasma between mice with AAA and controls: → Decreased: [17]: miR-24	
	Calcium chloride and collagenase application in Sprague-Dawley rats	Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: [59]: miR-19a, 19b,132, 221	
TAA	Fibulin-4(R/R) knockdown mice	Differential miR expression between aneurysmal aortic tissue and controls: [39-44, 68] → Increased: miR-29a, 29b, 29c [43]	- Differential miR expression between aneurysmal whole aortic tissue (from patients with TAA + BAV) and controls: → Increased: miR-29a [41]
	Fbn1(C1039G/+) mice	Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: miR-29b [68]	- Differential miR expression between patients with TAA and controls : → Increased: miR-29b [42]

Table 2: Main miRs identified in animal models of aortic aneurysms and comparison with miRs. dysregulated in human

Legends: AAA: abdominal aortic aneurysm

BAV: bicuspid aortic valve

ApoE-/-: apolipoprotein E deficient mice

qPCR: quantitative polymerase chain reaction

TAA: thoracic aortic aneurysm

TAV: tricuspid aortic valve

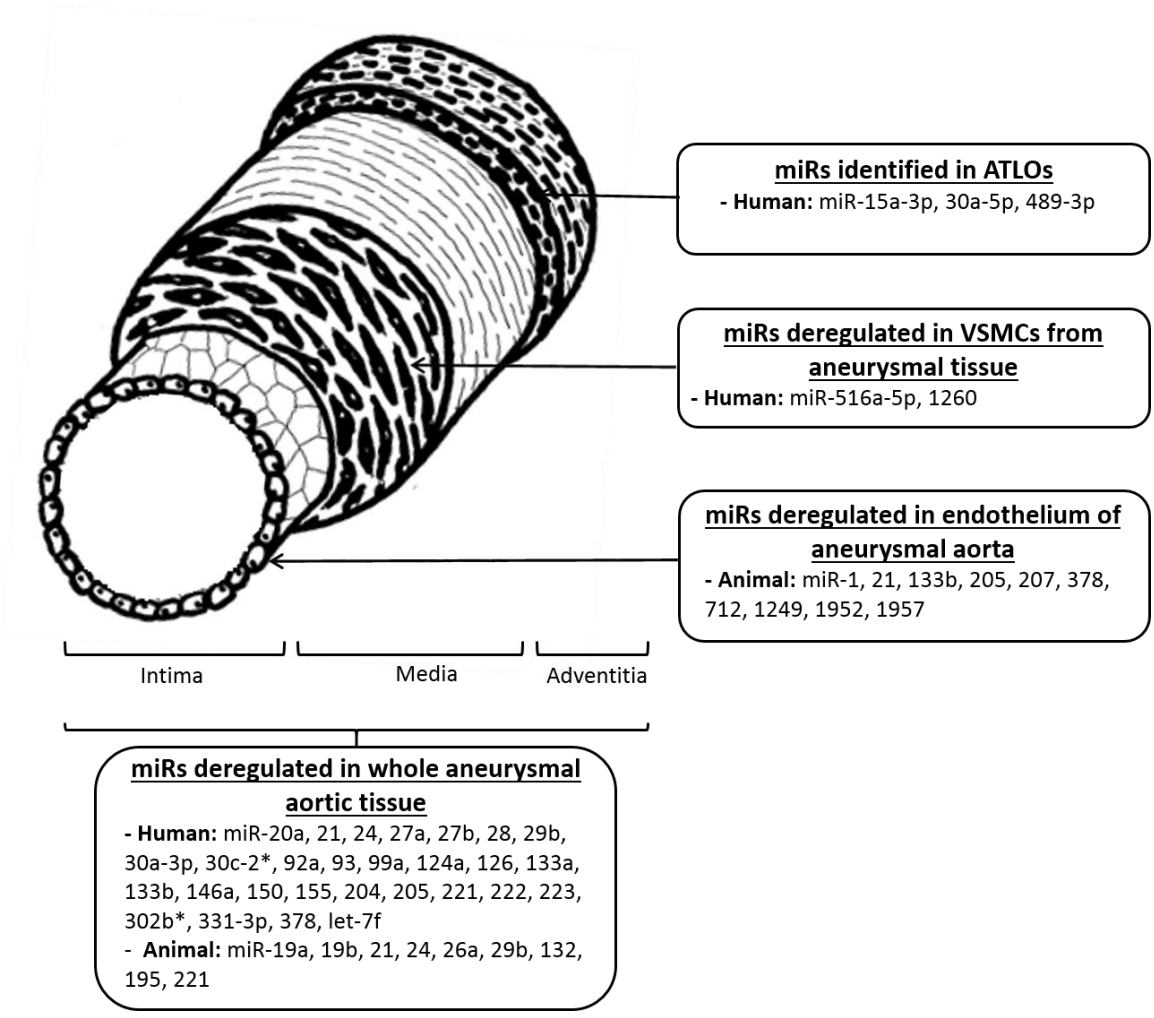

Figure 1: Main miRs identified in aortic structures during AAA.

Legends: AAA: abdominal aortic aneurysm

ATLOs : adventitial tertiary lymphoid organs

VSMCs: vascular smooth muscle cells

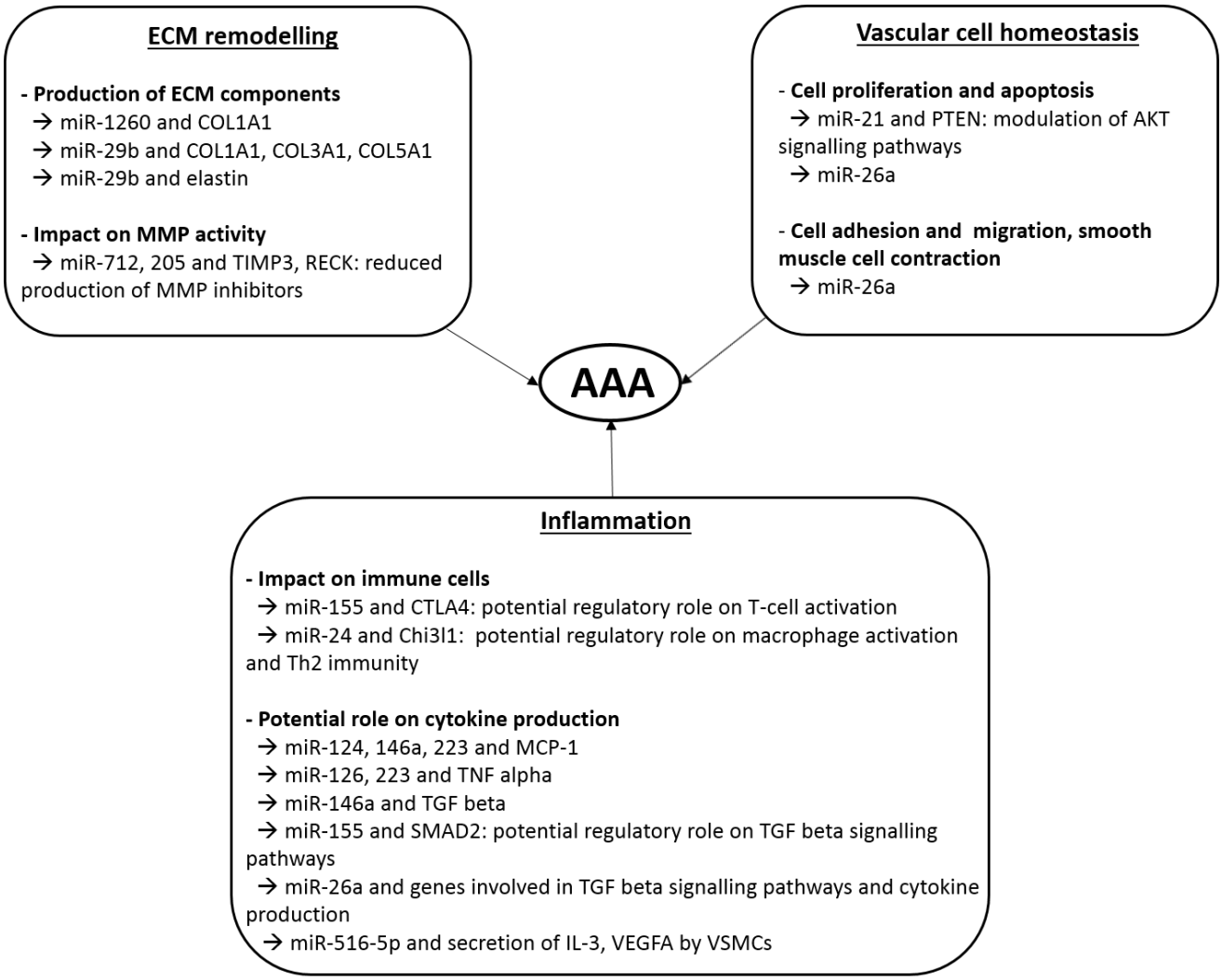

Figure 2: Implication of miRs in pathogenic pathways of AAA.

Legends: AAA: abdominal aortic aneurysm

Chi3l1: chitinase 3-like 1

COL1A1: collagen type 1, alpha 1

COL3A1: collagen type 3, alpha 1

COL5A1: collagen type 5, alpha 1

CTLA4: cytotoxic T-lymphocyte-associated protein

ECM: extracellular matrix

IL-3: interleukin-3

MCP-1: monocyte chemoattractant protein-1

MMP: metalloproteinase

PTEN: phosphatase and tensin homolog

RECK: reversion-inducing cysteine-rich protein with kazal motifs

TGF beta: transforming growth factor beta

Th2: T helper lymphocyte, type 2

TIMP3: tissue inhibitor of metalloproteinase 3

TNF alpha: tumor necrosis factor alpha

VEGFA: vascular endothelial growth factor A

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Micro-RNAs in abdominal aortic aneurysms: insights from animal models and relevance to human disease

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	Population	Samples	Main miRs identified	Reference
AAA	Screening study - 5 patients with infrarenal AAA undergoing elective surgical repair - 5 controls from cadaveric donors Validation study - 36 patients with AAA undergoing surgical repair (25 elective, 11 ruptured) - 7 controls from cadaveric donors	Aorta: full thickness aortic wall	- Differential miR expression between patients with AAA and controls: → Decreased: miR-133b, 133a, 331-3p, 30c-2*, 204	Pahl et al, 2012 [16]
	- 15 patients with infrarenal large AAA (59 to 68 mm) undergoing surgical repair - 5 controls from organ donors	Aorta: full thickness aortic wall	- Differential miR expression between patients with AAA and controls : → Decreased: miR-29b	Maegdefess el et al, 2012 [18]
	- 13 patients with large AAA (57 to 68 mm) undergoing surgical repair (8 active smokers, 5 non-smokers) - 5 controls from organ donors	Aorta: full thickness aortic wall	- Differential miR expression between patients with AAA and controls : → Increased: miR-21	Maegdefess el et al, 2012 [13]
	- 5 patients with AAA who underwent surgery - 4 controls from patients without AAA	Aorta: formalin-fixed paraffin-embedded sections	- Differential miR expression between patients with AAA and controls : → Increased: miR-205, 21, 133b, 378	Kim et al, 2014 [14]
	Screening study - 6 patients with AAA undergoing surgical open repair - 6 patients from men undergoing liver transplantation Validation study - 10 patients with AAA undergoing surgical open repair (6 elective AAA, 4 ruptured AAA) - 10 controls	Aorta: medial wall dissected from luminal thrombus and adventitia, culture of vascular smooth muscle cells (VSMCs)	- Differential miR expression between patients with AAA and controls: → Increased: miR-516a-5p → Decreased: miR-1260	Cheuk et al, 2014 [23]
	- 10 patients with large infrarenal AAA (>50mm) undergoing surgical open repair	Aorta: full thickness of the aortic wall - Body biopsy: at the site of maximum AAA dilatation - Neck biopsy: at the site of macroscopically non-dilated AAA neck	- miRs found exclusively in AAA body: miR-96, 9, 105, 33 - miRs found exclusively in AAA neck: miR-189, 151, 154, 154*, 153, 188, 147, 183, 199b - Differential miR expression between AAA body and AAA neck: → Increased: miR-155, 28, 30a-3p, 150, 302b*, 93, 99a	Biros et al, 2014 [19]
	- 10 patients with large infrarenal AAA (>50mm) undergoing surgical open repair - 10 patients with peripheral arterial disease (PAD) as controls	Serum	- miRs found exclusively in patients with AAA, not in controls : miR-220, 10a, 23b - Differential miR level between patients with AAA and patients with PAD: → Increased: miR-155	
	Initial study - 19 patients with infrarenal AAA undergoing surgical open repair - 10 controls from cadaveric donors Validation study - 22 patients with AAA infrarenal undergoing surgical open repair - 17 controls from cadaveric donors - 3 patients with aorto-occlusive disease	Aorta: full thickness of the aortic wall	- No significant difference observed between patients with AAA and controls	Stather et al, 2015 [25]
	Initial study - 15 patients with large AAA (>54mm) - 10 healthy controls Validation study - 40 patients with small AAA (30-54mm)	Whole blood	- Differential miR level between patients with AAA and controls: → Decreased: miR-15a, 196b - Differential miR level between patients with PAD and controls: → Decreased: miR-let-7e, 15a, 196b	

	- 40 patients with large AAA (>54mm) - 40 patients with AAA who had surgical repair (samples collected 6 months after open or endovascular repair) - 40 patients with PAD - 40 healthy controls		→ Increased: miR-411	
	- 36 patients with small AAA (30-54mm) - 36 patients with large AAA (>54mm) - 35 patients who had AAA repair (samples collected 6 months after open or endovascular repair) - 35 patients with PAD - 28 healthy controls	Plasma	- Differential miR level between patients with AAA and controls: → Decreased: miR-196b but significance not maintained after binary logistic regression	
	- 20 patients with infrarenal AAA undergoing surgical open repair - 14 controls from organ donors	Aorta: whole tissue or extraction of areas rich in adventitial tertiary lymphoid organs (ATLOs)	- Differential miR expression in ATLOs compared to VSMCs from non-aneurysmal aorta: → Increased: 489-3p → Decreased: miR-15a-3p, 30a-5p	Spear et al, 2015 [20]
	- 24 patients with AAA requiring surgery (> 50 mm or increasing more than 10 mm during the past 6 months), - 18 patients with PAD	Plasma	- Differential miR level between patients with AAA and patients with PAD: → Decreased: miR-15a-3p, 30a-5p	
	- 22 patients with infrarenal large AAA (52 to 115 mm) undergoing elective surgical open repair - 14 controls from organ donors	Aorta: full thickness aortic wall	- Differential miR expression between patients with AAA and controls : → Decreased: miR-24, 27b	Maegdefess el et al, 2014 [17]
	- 54 patients with small AAA (45-67 mm) - 51 patients with large AAA (69-150 mm) - 22 patients with peripheral vascular occlusive disease (PVOD) - 52 controls	Plasma	- Differential miR level between patients with AAA and controls: → Decreased: miR-24 - Differential miR level between patients with AAA and patients with PVOD: → Decreased: miR-24	
	- 13 patients with infrarenal AAA undergoing elective surgical open repair - 7 controls from ascending normal aorta from patients undergoing aortic valve replacement surgery	Aorta: full thickness aortic wall	- Differential miR expression between patients with AAA and controls : → Increased: miR-126, 20a, 27a, 92a, 221, 222, let-7f, 21, 155, 146a, 223, 124a, 29b	Kin et al, 2012 [15]
	- 23 patients with AAA - 17 patients with coronary artery disease (CAD) - 12 healthy volunteers	Plasma	- Differential miR level between AAA and controls: → Decreased: miR-124a, 29b, 155, 223, 126, 146a - Differential miR expression in plasma between AAA and CAD: → Decreased: miR-124a, 29b, 155, 223, 21	
	<u>Screening study</u> - 10 patients with AAA - 10 healthy controls <u>Validation study</u> - 60 patients with AAA - 60 healthy controls	Plasma	- Differential miR level between patients with AAA and controls: → Increased: miR-191-3p, 455-3p, 1281	Zhang et al, 2015 [26]
	- 32 patients with AAA - 41 controls	Plasma	- Inverse association of miR level with the presence of AAA and the aortic diameter: miR-195	Zampetaki et al, 2015 [27]
TAA	- 30 patients with ascending TAA + tricuspid aortic valves (TAV) undergoing surgery - 10 controls from ascending non aneurysmal aorta of heart donors and coronary artery bypass graft patients	Aorta: full thickness aortic wall	- Differential miR expression between patients with TAA + TAV and controls : → Decreased: miRs -1, 21, 29a, 133a, 486-5p	Jones et al, 2011 [40]
	- 10 patients with ascending TAA + tricuspid aortic valves (TAV) undergoing surgery	Aorta: full thickness aortic wall	- Differential miR expression between patients with TAA + TAV and controls :	Pei et al, 2015 [44]

	- 10 controls patients from non-aneurysmal aortas of coronary artery bypass graft patients		→ Increased: miR-145	
	- 10 patients with non-familial non syndromic ascending TAA undergoing surgery - 10 controls from ascending non aneurysmal aorta of heart transplant recipients	Aorta: full thickness aortic wall	- Differential miR expression between patients with TAA and controls : → 99 differentially expressed miRs identified by microarray	Patuzzo et al, 2012 [42]
	- 79 patients with TAA + bicuspid aortic valve (BAV) undergoing surgery - 30 patients with TAA + tricuspid aortic valve (TAV) undergoing surgery - 33 controls from non-aneurysmal aorta of coronary bypass surgery patients	- Aorta: full thickness aortic wall	- Differential miR expression between patients with TAA + BAV, with TAA + TAV and controls : → Increased: miR-29b	Boon et al, 2011 [43]
	- 21 patients with ascending TAA + bicuspid aortic valves (BAV) undergoing surgery - 21 patients with ascending TAA + tricuspid aortic valve (TAV) undergoing surgery - 10 controls from non-aneurysmal ascending aorta of heart transplant donors or recipients	Aorta: full thickness aortic wall	- Differential miR expression between patients with TAA + BAV and controls : → Decreased: miR-143 → Increased: miR-29a, 145 - Differential miR expression between patients with TAA + TAV and controls : → Decreased: miR-1, 133a, 143 → Increased: miR-21 - Differential miR expression between patients with TAA + TAV and patients with TAA + BAV → Decreased: miR-1 → Increased: miR-21	Ikonomidis et al, 2013 [41]
		Plasma	- Differential miR level between patients with TAA + BAV and controls : → Decreased: miR-145 → Increased: miR-133a - Differential miR expression between patients with TAA + TAV and controls : → Decreased: miR-21, 29a, 143 → Increased: 133a	

Table 1: Summary of human studies on miRs in aortic aneurysms.

Legends: AAA: abdominal aortic aneurysm

BAV: bicuspid aortic valves

CAD: coronary artery disease

PAD: peripheral arterial disease

PVOD: peripheral vascular occlusive disease

TAA: thoracic aortic aneurysm

TAV: tricuspid aortic valves

VSMCs: vascular smooth muscle cells

	Animal models	Main miRs dysregulated in animal models validated by qPCR	miRs observed in animal models also dysregulated in patients with aortic aneurysm
AAA	Angiotensin II infusion in apoE-/- mice	- Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: [13]: miR-21 [27]: miR-195 → Decreased: [18]: miR-29b [17]: miR-24 [54]: miR-26a - Differential miR expression between endothelium of aneurysmal aorta and controls: → Increased: [14]: miR-21, 1, 133b, 207, 378, 1957, 712, 205 → Decreased: [14]: miR-1952, 1249 - Differential miR expression in plasma between mice with AAA and controls: → Decreased: [17]: miR-24	- Differential miR expression between abdominal aneurysmal whole aortic tissue and controls: → Increased: [13]: miR-21 [15]: miR-21, 146a, 29b, 221, 222 [14]: miR-21, 133b → Decreased: [18]: miR-29b [17]: miR-24 [16]: miR-133b - Differential miR level in plasma between patients with AAA and controls: → Decreased: [17]: miR-24 [27]: miR-195 [15]: miR-29b - Differential miR level in plasma between patients with AAA and coronary artery disease: → Decreased: [15]: miR-29b
	Angiotensin II infusion in C57BL/6 mice	Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: [43]: miR-29b	
	Elastase perfusion in ApoE-/- mice	Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: [54]: miR-21 → Decreased: [54]: miR-26a	
	Elastase perfusion in C57BL/6 mice	Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: [13]: miR-21 → Decreased: [18]: miR-29b [17]: miR-24 - Differential miR level in plasma between mice with AAA and controls: → Decreased: [17]: miR-24	
	Calcium chloride and collagenase application in Sprague-Dawley rats	Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: [59]: miR-19a, 19b,132, 221	
TAA	Fibulin-4(R/R) knockdown mice	Differential miR expression between aneurysmal aortic tissue and controls: [39-44, 68] → Increased: miR-29a, 29b, 29c [43]	- Differential miR expression between aneurysmal whole aortic tissue (from patients with TAA + BAV) and controls: → Increased: miR-29a [41]
	Fbn1(C1039G/+) mice	Differential miR expression between aneurysmal whole aortic tissue and controls: → Increased: miR-29b [68]	- Differential miR expression between patients with TAA and controls : → Increased: miR-29b [42]

Table 2: Main miRs identified in animal models of aortic aneurysms and comparison with miRs. dysregulated in human

Legends: AAA: abdominal aortic aneurysm

BAV: bicuspid aortic valve

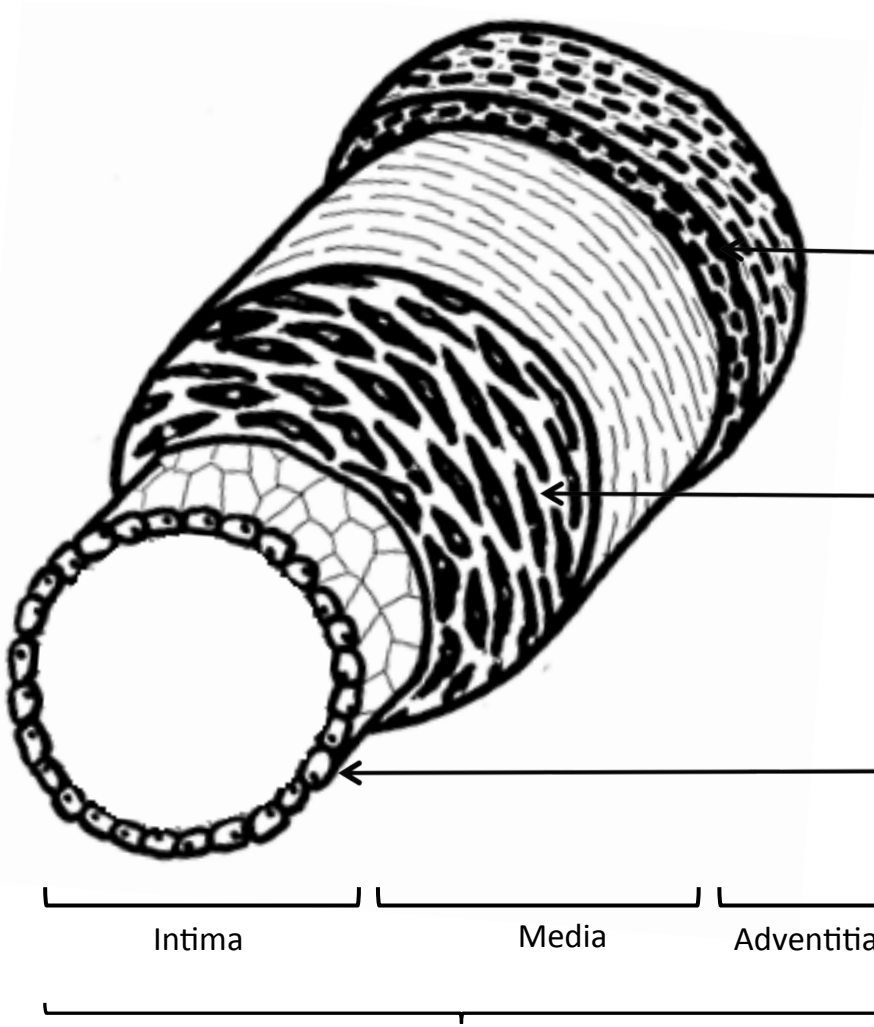
ApoE-/-: apolipoprotein E deficient mice

qPCR: quantitative polymerase chain reaction

TAA: thoracic aortic aneurysm

TAV: tricuspid aortic valve

Figure 1



<u>miRs identified in ATLOs</u>

- Human: miR-15a-3p, 30a-5p, 489-3p

<u>miRs deregulated in VSMCs from aneurysmal tissue</u>

- Human: miR-516a-5p, 1260

<u>miRs deregulated in endothelium of aneurysmal aorta</u>

- Animal: miR-1, 21, 133b, 205, 207, 378, 712, 1249, 1952, 1957

<u>miRs deregulated in whole aneurysmal aortic tissue</u>

- Human: miR-20a, 21, 24, 27a, 27b, 28, 29b, 30a-3p, 30c-2*, 92a, 93, 99a, 124a, 126, 133a, 133b, 146a, 150, 155, 204, 205, 221, 222, 223, 302b*, 331-3p, 378, let-7f

- **Animal:** miR-19a, 19b, 21, 24, 26a, 29b, 132, 195, 221

Figure 1

Figure 2

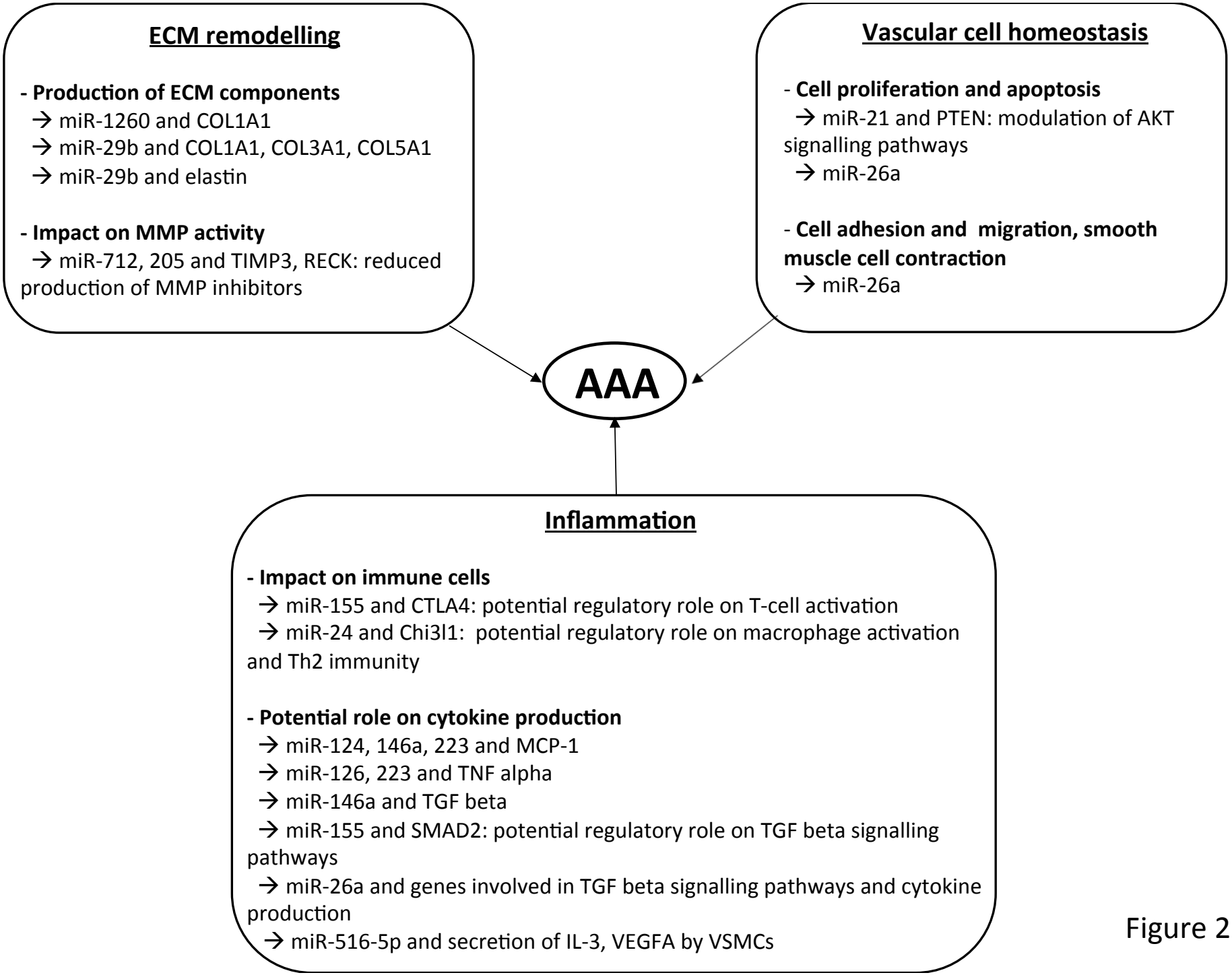


Figure 2