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Dianna M. Milewicz

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Commentary

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MicroRNAs, fibrotic remodeling, and aortic aneurysms

Dianna M. Milewicz

Division of Medical Genetics, Department of Internal Medicine, The University of Texas Health Science Center at Houston, Houston, Texas, USA.

Aortic aneurysms are a common clinical condition that can cause death due to aortic dissection or rupture. The association between aortic aneurysm pathogenesis and altered TGF- β signaling has been the subject of numerous investigations. Recently, a TGF- β -responsive microRNA (miR), miR-29, has been identified to play a role in cellular phenotypic modulation during aortic development and aging. In this issue of *JCI*, Maegdefessel and colleagues demonstrate that decreasing the levels of miR-29b in the aortic wall can attenuate aortic aneurysm progression in two different mouse models of abdominal aortic aneurysms. This study highlights the relevance of miR-29b in aortic disease but also raises questions about its specific role.

The major disease affecting the aorta is the aneurysm, an abnormal widening or ballooning of an artery due to weakness in its wall. The most common location for aneurysms is the infrarenal segment of the aorta, and these are referred to as abdominal aortic aneurysms (AAAs). AAAs primarily affect men over the age of 65 years, and risk factors for AAAs include cigarette smoking, atherosclerosis, hypertension, and a family history of AAA (1). Although the epidemiology and heritability of the disease are well described, the relationship between the risk factors and the aneurysm formation is not well understood. For example, despite the atherosclerotic risk factors and concurrent risk for coronary artery disease in patients with AAAs, it is debated whether atherosclerotic processes directly contribute to aneurysm development or whether atherosclerosis in the aortic wall is merely a “bystander” condition. Other pathologic features of AAAs include chronic transmural inflammation, degradation of elastic fibers, and loss of SMCs (1).

The second most common location for aortic aneurysms is the ascending thoracic aorta, and these aneurysms present at a younger age and affect approximately twice as many men as women (2). Hypertension and congenital bicuspid aortic valve (BAV) are risk factors for thoracic aortic aneurysms (TAAs), and a genetic predisposition also contributes prominently to the etiol-

ogy. Marfan syndrome (MFS) predisposes to TAAs and is caused by mutations in *FBN1*, which encodes fibrillin-1, a component of elastin-associated microfibrils (3). Studies of an established mouse model of MFS have suggested that defects in *FBN1* lead to promiscuous activation of TGF- β as it is released from stores in the microfibrils (4). Other syndromes predisposing individuals to TAA caused by mutations in either the TGF- β receptor type I or II genes (*TGFBR1* or *TGFBR2*, respectively) or *SMAD3* have also implicated excessive activation of TGF- β signaling in aortic disease (5–7). TAAs inherited in families without syndromic features are due to mutations in genes encoding proteins involved in SMC contraction, including the SMC-specific isoforms of α -actin (*ACTA2*) and myosin heavy chain (*MYH11*), along with the kinase that controls SMC contraction (*MYLK*) (8–10). The aortic pathology of TAAs is characterized by elastic fiber fragmentation and loss, proteoglycan accumulation, and either focal or diffuse regions of SMC loss.

MicroRNA-29 and fibrosis

Individual microRNAs (miRs) can target numerous mRNAs and have been referred to as the “micromanagers” of cellular gene expression (11); they can orchestrate gene expression profiles associated with phenotypic changes in cells and disease progression. The miR-29 family of miRs contains 3 members (miR-29a, miR-29b, and miR-29c) that are encoded by two separate loci, giving rise to bicistronic precursor miRs (miR-29a/b1 and miR-

29b2/c). This family has been demonstrated to target gene transcripts that encode ECM proteins involved in the fibrotic response, including those for type I collagen (*COL1A1* and *COL1A2*), type III collagen (*COL3A1*), fibrillin-1, and elastin (*ELN*) (12), and is known to modulate gene expression during development and aging of the aorta (13, 14) and in the progression of aortic aneurysms (14, 15). Downregulation of miR-29 expression has been identified as part of the fibrotic response associated with liver (16) and kidney (17) fibrosis, fibrotic skin in systemic sclerosis (18), and cardiac fibrosis in response to ischemic insult (12). TGF- β represses miR-29 expression in cardiac fibroblasts, hepatic stellate cells, and dermal fibroblasts, thereby increasing expression of ECM target genes (12, 18, 19). Furthermore, analysis of mouse embryonic fibroblasts deficient in either Smad2 or Smad3 indicates that the TGF- β -driven decrease in miR-29 expression and increase in *Col1a2* and *Col3a1* expression is dependent on Smad3 and not Smad2 (20).

Aortic aneurysms and miR-29b

In this issue of the *JCI*, Maegdefessel et al. demonstrate that modulation of miR-29b expression affects aortic aneurysm progression in two mouse models of the disease (15). AAAs were induced in 10-week-old mice by infusing porcine pancreatic elastase into the infrarenal segment of the aorta for five minutes, which causes extensive destruction of the elastic lamellae and pronounced inflammation in the adventitial regions (21). AAAs were induced in the second model by infusing AngII into 10-week-old *Apoe*^{-/-} mice, which leads to aortic aneurysm formation in the suprarenal aorta, with associated elastin degradation and macrophage accumulation. In both models of aneurysm formation, Maegdefessel and colleagues showed that miR-29b expression was significantly downregulated with aneurysm progression over 21 days, with little to no change in miR-29a and miR-29c (15). Furthermore,

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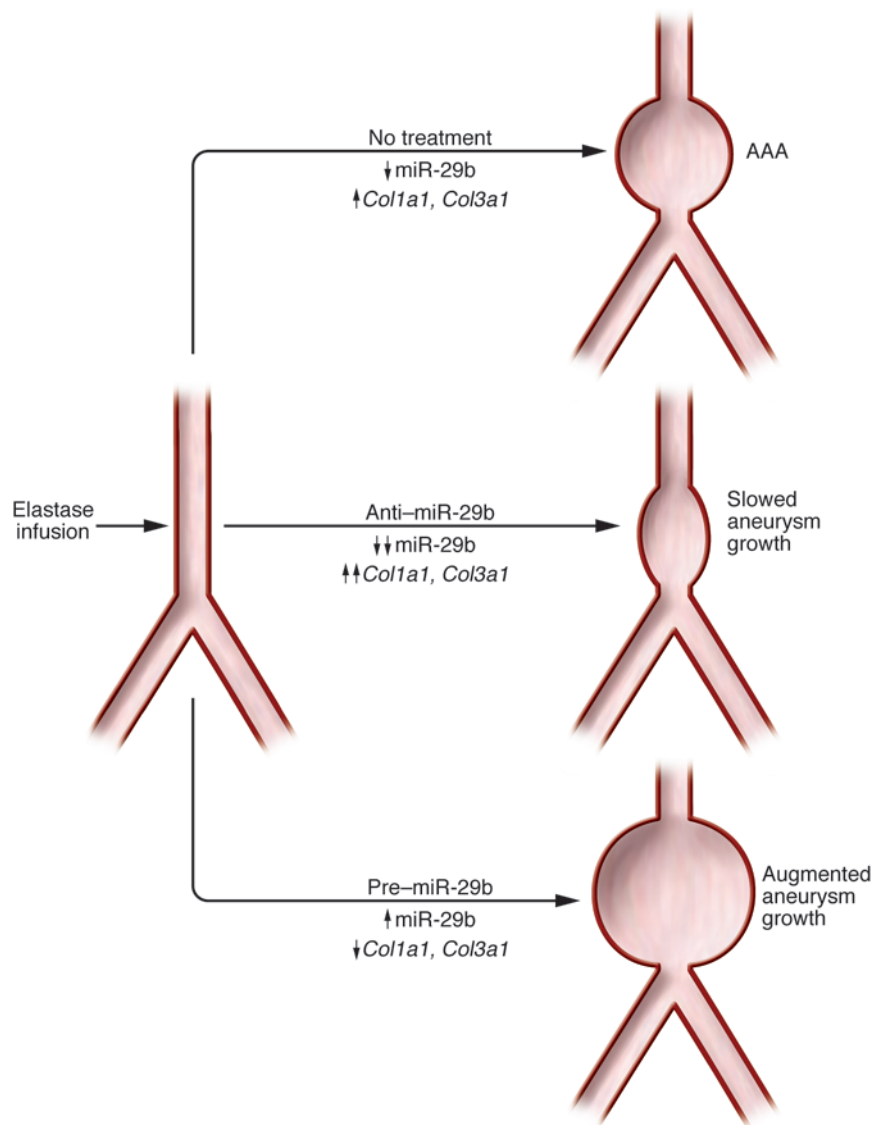


Figure 1

Decreased expression of miR-29b and aortic aneurysm progression. AAAs were induced in 10-week-old mice by infusing porcine pancreatic elastase into the infrarenal segment of the aorta. miR-29b expression was significantly downregulated with aneurysm progression over 21 days, and expression of collagen genes (*Col1a1* and *Col3a1*) increased. Administration of locked nucleic acid anti-miR-29b further decreased miR-29b levels and greatly increased expression of collagen genes, resulting in a reduction in aortic enlargement over time. Increasing the expression of miR-29b using a lentiviral vector (pre-miR-29b) increased expression of miR-29b and decreased collagen gene expression, leading to augmented aortic growth.

expression of *Col1a1* and *Col3a1* increased over time, whereas *Eln* expression was only increased at 14 days in both mouse models. In both aneurysm models, increased expression of miR-29b and decreased collagen gene expression augmented aneurysm growth, whereas inhibition of miR-29b and increased collagen expression slowed aneurysm formation. Taken together, decreasing the expression of miR-29b beyond the

normal decreases that accompany injury to aortic tissue was associated with enhanced expression of several ECM proteins and decreased expansion rates of aortic aneurysms (Figure 1). Expression of miR-29b was also assessed in the aortas of patients with large AAAs compared with that in donor control aortas. Despite the caveat that the control aortas were from substantially younger individuals than those

from the patients with AAA (mean age, 33 years in the controls versus 64 years in the patients), miR-29 expression was decreased and *COL1A1*, *COL3A1*, and *ELN* expression was increased in the AAA aortas compared with that in control aortas.

Maegdefessel and colleagues also provided some initial data to address which cells in the aorta altered expression of miR-29 (15). When adventitial fibroblasts explanted from the aortic arch of human donors were exposed to TGF- β 1, they significantly decreased expression of miR-29b, whereas aortic SMCs did not, implicating adventitial fibroblasts as the cells responsible for the protective fibrotic response in the aorta. Cardiac fibroblasts, and not cardiomyocytes, have similarly been implicated in the fibrotic response in the heart with ischemic insult (12).

The role of miR-29 in aortic aneurysm formation was also recently investigated by Dimmeler and her colleagues but with different results (14). Using a mouse model of thoracic aortic disease, the *FBLN4* hypomorphic mouse, they found that miR-29a, miR-29b, and miR-29c were increased in aortic aneurysms in the mutant mouse. Aortic disease in the *FBLN4* mouse model is associated with evidence of increased TGF- β signaling, including increased nuclear phosphorylated Smad2 (pSmad2) and increased connective tissue growth factor (CTGF) and collagen deposition in the medial and adventitial layer (22). Therefore, it is surprising that miR-29b would be increased rather than decreased in the diseased aorta. Given that Maegdefessel and colleagues identified that adventitial fibroblasts rather than aortic SMCs responded to TGF- β to decrease miR-29b levels (15), the increased expression of the miR-29 family members with the *FBLN4* mouse (14) may be due to the fact that the investigators removed the adventitial layer from the aortic tissues prior to analysis, therefore removing the adventitial fibroblasts. When the expression of the miR-29 family was analyzed in TAA tissues, including aortas from patients with TAAs and BAV, the investigators found increased miR-29b expression compared with that in control tissues (14). However, the methods used to process the human tissues were not provided, and whether the adventitia was removed is not known.

The Dimmeler group also found increased miR-29b expression in the aorta with AngII infusion (14), although their experiments differed from those of Maeg-



defessel et al. in that they used older mice (18 months) and a slightly lower dose of AngII. When miR-29 activity was inhibited, AngII-treated mice displayed increases in ECM gene expression and extraordinary reduction in aorta dilation (14). Therefore, these results correlate with the findings of Maegdefessel et al. in this issue of *JCI*.

miR-29 and missing pieces of the puzzle

From studies of development and aging, miR-29b has emerged as an important modulator of gene expression in the aorta. The studies of Maegdefessel et al. indicate that miR-29b expression is decreased with aortic aneurysm formation in mouse models of AAA, and the associated fibrotic responses attenuates aneurysm formation (15). The aortic wall weakens with increased diameter; therefore, the increased expression of collagens associated with decreased miR-29b expression provides additional tensile strength to the aortic wall. Since TGF- β modulates miR-29b expression, it may be a trophic factor responsible for decreased expression of miR-29b with aneurysm progression. Recently, Wang and colleagues showed greater frequency and severity of aortic disease with AngII infusion in wild-type C57BL/6 mice when TGF- β signaling was blocked using neutralizing antibodies (23). Partial but not complete improvement in the incidence and severity of aortic disease was found after manipulations of the immune response. TGF- β -induced decreases in miR-29b expression, leading to increased ECM protein production, may provide further protective effects of TGF- β . Therefore, the data by Maegdefessel et al. (15) indirectly provide further support for a protective role for TGF- β in these mouse models of aneurysm formation.

At the same time, these are puzzle pieces that do not yet provide a clear picture of the role of TGF- β signaling and miR-29 in thoracic aortic disease. Evidence of excessive TGF- β signaling, based on increased staining for nuclear pSMAD2 in aortic SMCs, has been identified in TAA tissue from patients with genetically triggered thoracic aneurysms and patients with thoracic aneurysms and BAV (4–6, 24). Therefore, the increased levels of miR-29b in a mouse model of TAA and human TAA tissue are difficult to reconcile in the face of evidence of increased TGF- β signaling. However, as mentioned above, the decrease in miR-29b could simply be due to not including the adventitial fibroblasts in the analyses, since

these cells decrease expression of miR-29b more robustly in response to TGF- β than aortic SMCs.

An alternative explanation for increased pSMAD2 immunostaining in aortic tissues accompanied by increased miR-29b levels is a lack of response of aortic cells to TGF- β , leading to increased TGF- β production to compensate for this resistance. Relevant to this speculation is the fact that the *TGFBR1* and *TGFBR2* mutations causing thoracic aortic disease such as Loeys-Dietz syndrome (LDS) fall in the kinase domain of these receptors, and a subset of the mutations have been shown to disrupt kinase function critical for TGF- β signaling (25). Furthermore, some patients with thoracic aortic disease have frameshift mutations in *SMAD3* predicted to cause haploinsufficiency (6, 7). Could the increased nuclear pSMAD2 immunostaining observed in the aortas of patients with *SMAD3* mutations reflect increased shunting of TGF- β signaling through SMAD2 rather than SMAD3? Previous studies have indicated that decreased miR-29b levels in response to TGF- β are dependent on SMAD3 rather than SMAD2 (20), therefore the increased SMAD2 signaling would not compensate for the loss of SMAD3 signaling. It is also important to note that patients with *TGFBR1*, *TGFBR2*, or *SMAD3* mutations have thin, translucent skin, with visible veins and scars that are atrophic and wide, findings that are suggestive of decreased expression of ECM proteins in the skin and poor myofibroblast contraction of scars and consistent with loss of TGF- β signaling. Incomplete transformation of dermal fibroblasts from patients with *TGFBR2* mutations to myofibroblasts with exposure to TGF- β 1 has been demonstrated, further supporting a loss of SMAD3 signaling in these cells (25).

The differential response of adventitial fibroblasts compared with that of SMCs shown by Maegdefessel and colleagues also raises important questions as to the specific roles of SMCs and adventitial fibroblasts in aortic disease progression. It has been shown that adventitial fibroblasts constitutively secrete numerous proinflammatory cytokines, particularly IL-6 and MCP-1, and expression of these genes is further upregulated in response to AngII stimulation (26). AngII also stimulates adventitial fibroblasts to recruit monocytes, and the recruited monocytes further activate fibroblast proliferation, adventitial thickening, and additional cytokine production (26).

Investigating the role of TGF- β and miR-29 in this adventitial inflammatory and fibrotic response with AngII infusion is critical for our understanding of aortic disease progression.

Finally, miR-29 expression is altered with skeletal muscle differentiation; miR-29 expression is suppressed in myoblasts but increases during differentiation to facilitate myogenesis (27). Studies have identified that the adventitia contains progenitor cells that can differentiate into SMCs (28). Could miR-29 expression similarly be involved in the differentiation of the adventitial progenitor cells to SMCs in the ascending thoracic aorta with aneurysm progression? Although a glimpse of the role of TGF- β and miR-29 is revealed as pieces of the puzzle are identified, there is much more research to be done before this picture will be complete.

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Address correspondence to: Dianna M. Milewicz, The University of Texas Medical School at Houston, 6431 Fannin, 6.100, Houston, Texas 77030, USA. Phone: 713.500.6715; Fax: 713.500.0693; E-mail: dianna.m.milewicz@uth.tmc.edu.

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Preserving postischemic reperfusion in the kidney: a role for extracellular adenosine

Joel M. Weinberg¹ and Manjeri A. Venkatachalam²

¹Division of Nephrology, Department of Internal Medicine, Veterans Affairs Ann Arbor Healthcare System and University of Michigan, Ann Arbor, Michigan, USA. ²Department of Pathology, University of Texas Health Science Center (UTHSC) at San Antonio, San Antonio, Texas, USA.

Several adenosine receptor subtypes on endothelial, epithelial, mesangial, and inflammatory cells have been implicated in ischemic acute kidney injury, a life-threatening condition that frequently complicates the care of hospitalized patients. In this issue of the *JCI*, Grenz and coworkers provide novel insight into how preservation of postischemic renal perfusion by endothelial cell adenosine A2B receptors is antagonized by adenosine reuptake into proximal tubule cells by equilibrative nucleotide transporter 1, which can be inhibited by dipyridamole. The work suggests that adenosine A2B receptor agonists and inhibition of equilibrative nucleoside transporters by dipyridamole may have therapeutic potential in ischemic acute kidney injury, a condition for which there are currently no specific therapeutic interventions.

Acute kidney injury (AKI) is a life-threatening condition that frequently complicates the care of hospitalized patients. It is clinically defined by an abrupt reduction in renal clearance function (as typically measured by an increase in serum creatinine) and, when associated with structural damage to the renal parenchyma, can be prolonged, require dialysis support prior to recovery,

and eventuate in chronic kidney disease even if recovery from the acute insult occurs (1, 2). While AKI can have multiple etiologies, ischemia is common to many of them (1) and predictably occurs in settings such as coronary bypass, where up to 7%–10% of patients develop AKI, which has been associated with 40%–70% mortality in the smaller cohort that requires dialysis and with increased subsequent long-term mortality even if dialysis is not needed (3).

Despite intensive research efforts and identification of multiple approaches effective in experimental models, there are no

specific therapeutic interventions for treatment of AKI other than renal replacement therapy. Several targeted therapies have been tested in the clinic, but thus far all have failed (4). Both a better understanding of the underlying pathogenesis and new therapeutic targets continue to be needed. In this issue of the *JCI*, Grenz et al. elucidate a role for the purine nucleoside adenosine in protecting mouse kidneys from ischemic AKI by preserving postischemic blood flow (5). If the phenomena observed in mice hold true in humans, these data have important therapeutic implications, as they suggest that modulating adenosine levels via effects on adenosine A2B receptor (Adora2b) on endothelial cells might be of benefit in individuals with AKI.

Generation of adenosine during AKI and its multiple potential effects

Changes in purine nucleotide metabolism are a hallmark of tissue oxygen deprivation (6). Within cells, failure to maintain ATP production due to limitation of oxidative phosphorylation and compensatory

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