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Commentary

Navigating toward gene therapy in Marfan syndrome: A hope for halting aortic aneurysm

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The aorta serves as the artery tasked with blood distribution from the heart to the distal parts of the human body. Aneurysm is one of the most common chronic conditions affecting the aorta and often ends in acute events, such as dissection and rupture (giving rise to severe internal bleeding, often resulting in sudden death), ulcers, or hematomas. Aneurysm or aortic dilatation is a pathological expansion that affects the entire aortic wall, with an abnormal increase of at least 50% of normal. Two types of aneurysms and dissections affect the location of the aorta: thoracic (TAAD) and abdominal (AAAD). Their epidemiology is significantly different, the former being more linked to genetic factors and the latter to environmental ones, causing atherosclerosis.[1] A representative example of TAAD is Marfan syndrome. Mutations in the fibrillin-1 gene are responsible for this genetic connective tissue disease, with over 3,000 distinct mutations reported. Its final phenotypical clinical manifestation represents the impact of multi-molecular targets from aberrant cellular signaling (mostly based on transforming growth factor β [TGF-β]), oxidative stress, endothelial dysfunction, and local inflammation, altogether leading to aberrant organization and remodeling of the extracellular matrix of the aortic wall. The identification and functional characterization of affected molecular targets, their interconnection, and genetic modifiers that could explain the wide variability in clinical manifestations (from the mildest to more severe) despite the common genetic mutation are crucial to acquiring current and future aortopathy knowledge for the administration of effective therapeutic agents. However, medical therapies capable of completely halting or even reversing aneurysm formation are not yet available. The work published by Noormalal et al. in a recent issue of *Molecular Therapy Methods and Clinical Development* is based on this primordial aim.[2] The authors study an endoribonuclease, Regnase-1, that cleaves the mRNA of proinflammatory cytokines, such as interleukin-6 (IL-6), IL-12, and IL-18. Regnase-1 is a well-known member of the molecular machinery involved in cancer, as it forms part of the anti-inflammatory molecular machinery of defense.[3] The authors rationalize that this endoribonuclease might be involved in Marfan syndrome aortopathy because (1) its expression is regulated by TGF-β, a crucial aggravating driver of aortopathy,[4] (2) it is a negative regulator of oxidative stress, another known actor in TAAD progression,[5] and (3) its intrinsic anti-inflammatory properties, where the role of inflammation is becoming increasingly more evident in the progression of the aortopathy.[6]

The strategy followed by the authors is the use of adeno-associated viruses (AAVs) to overexpress Regnase-1. They chose AAV serotype 9 because of its preference for major transduction in vascular tissues compared to other serotypes.[7] This lab has a long-standing and ongoing tradition of using AAVs for potential therapeutic proposes.[8–10] The Marfan syndrome model chosen for the study is the fibrillin-1 hypomorphic mgR/mgR mouse, whose aortic aneurysm quickly evolves, usually ending in aortic dissection. Cultured Marfan aortic smooth muscle cells (SMCs) and aortic tissue showed a significant reduction in Regnase-1 protein levels. The transduction of AAV-Regnase-1 in cultured Marfan SMCs caused a significant decrease in the proinflammatory environment induced by interferon γ (IFN-γ). This also occurred in SMCs from patients with Marfan subjected to reparatory surgery. In accordance with this observation, the supernatant isolated from AAV-Regnase-1 transduced cells reduced the migration of macrophages *in vitro*. Notably, the activity of metalloproteinase 9 (MMP9), which, together with MMP2, actively participates in the elastolysis of Marfan aorta, was also reduced without triggering apoptosis, which curiously is not the case for cancer cells. The injection of AAV-Regnase-1 in Marfan mice led to endoribonuclease expression in mural cells of the aortic wall layers.

In comparison with the control, AAV-EGFP, Regnase-1 overexpression reduced the number of elastic breaks, the aorta diameter (measured only histologically, not by ultrasonography), and the presence of proinflammatory markers (IL-6, MCP, and IL-12) in the mgR/mgR aorta. The authors discuss their results within the context of the proinflammatory environment of aortic disease, both at the aortic tissular and circulatory levels, along with the contradictory involvement of TGF-β as an inductor of Regnase-1 expression. Notably, the well-known elevated TGF-β levels observed in aortic tissue and plasma unexpectedly do not increase Regnase-1 expression in Marfan aortic tissue.

Some points require special attention regarding the study design and the general use of AAVs as a transduction tool. One of these is recognized by the authors themselves for the lack of data about the survival rate of animals overexpressing Regnase-1. The endoribonuclease interferes with the progression of the characteristic aortic wall disarray and overexpression of inflammatory cytokines; however, no data are presented about the expected interference in the fatal aortic dissection. This study is

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susceptible to being performed in the mouse model of the disease used (mgR/mgR), which differs from the other popular Marfan mouse model (C1041G/+), as the aneurysm usually ends with the death of the animal by aortic dissection after a certain time. An important point that would be of great interest is whether AAV-Regnase-1 injection in Marfan mice affected circulatory TGF-β plasma levels. The authors only showed the reduced secretion of this cytokine in the supernatant of cultured SMCs submitted to proinflammatory insult with IFN-γ.

Another interesting point to discuss and study in detail is the potential role of the different types of infiltrated and/or (trans) differentiated macrophages in aortopathy progression. Finally, one limitation not discussed in the manuscript as potential translatable medicine is that many individuals harbor antibodies that neutralize AAV transduction. Nonetheless, this disadvantage did not impair approved licensed treatments with AAV-based gene therapy for some rare diseases.[11] This obstacle remains a challenge to overcome for the broader implementation of a generalized AAV-based approach as an efficient and durable therapy. The hope for the future application of genic therapies to Marfan syndrome and related diseases is getting closer to AAV locally targeted to specific vascular cells,[12] locally applied utilizing biomimetic polymer,[13] and/or the CRISPER-Cas technologies.[14] However, in the meantime, classical pharmacological-based therapy requires improvements to halt, if not at least mitigate, the path toward the always risky surgical intervention.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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