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Aging, Smooth Muscle Vitality, and Aortic Integrity

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Abstract

Advances in medical genetics and imaging have resulted in a significant increase in the number of diagnosed thoracic aortic aneurysms. Recent findings establish a link between diminished nicotinamide phosphoribosyltransferase (NAMPT) and compromised smooth muscle cell vitality in aortic dilatation. These findings have myriad implications given the diverse roles of NAMPT, which is central to the production of nicotinamide adenine dinucleotide (NAD^+), and thus ATP production, as well the activity of multiple NAD^+ consuming proteins. Given its central role in vascular cell vitality and thus matrix integrity, further attention should be directed to the NAMPT-NAD^+ control system in thoracic aortopathy.

Keywords

actomyosin activity; apoptosis; ATP; mechanotransduction; inflammation

Aging is a primary risk factor for many cardiovascular diseases. The manifold effects of vascular aging result, in part, from adverse phenotypic changes to endothelial cells and vascular smooth muscle cells (VSMCs), increased inflammation, and associated changes in extracellular matrix (ECM) composition, structure, and mechanical properties[1]. These aging-related changes progress in different ways throughout the vasculature. Aging of the aorta manifests at the vessel level as a gradual dilatation and structural stiffening, which impact the global hemodynamics and local wall mechanics. In particular, a stiffer aorta increases the pulse wave velocity, which can augment the pulse pressure in the proximal (thoracic) aorta and increase mechanical stress. Increases in mechanical loading are typically sensed by the VSMCs and result in changes in gene expression and downstream gene products that affect the composition and structure of the aortic wall, thus establishing a feedback loop connecting mechanical loading, cell function, and structural integrity[2].

In addition to genetic mutations and uncontrolled hypertension, aging is an important risk factor for the development of thoracic aortic aneurysms (TAAs). Defined as a 50% or greater dilatation of the aorta, these lesions are characterized histopathologically by damaged elastic

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fibers, compromised smooth muscle function, pooled mucoid material, and remodeled collagen fibers[2], all of which are seen to a lesser degree in aortic aging[1]. It seems that vascular aging either primes the thoracic aorta for aneurysmal dilatation in response to second insults or it exacerbates dilatation once initiated via other mechanisms, including consequences of particular genetic mutations. The collection of genetic mutations that predispose to TAAs – affecting VSMC contractile proteins, transmembrane proteins, and select ECM proteins – suggests further that the loss of aortic wall integrity that characterizes these lesions may result from dysfunctional mechano-sensing or mechano-regulation of the ECM by the VSMCs[2]. Regardless, the VSMC serves as a central node in establishing, maintaining, or repairing the aortic media, the layer of the aortic wall most affected in TAAs. VSMC vitality is central to aortic health.

NAMPT, Genome Integrity, and Aortopathy

Nicotinamide phosphoribosyltransferase (NAMPT) is a critical rate-limiting enzyme involved in the production of nicotinamide adenine dinucleotide (NAD^+), a key molecule of cellular metabolism as well as gene integrity and gene expression[3,4]. NAD^+ plays a fundamental role in generating adenosine triphosphate (ATP), which is essential to many cellular processes including metabolism, molecular biosynthesis and biotransport, and actomyosin-mediated motions. ATP is also important in signal transduction, as, for example, in the production of the second messenger cAMP and when used by kinases to phosphorylate proteins. NAD^+ is not only central to many downstream gene products, it plays additional roles when consumed by sirtuins (SIRT), poly(ADP-ribose) polymerases (PARP), and CD38 in their functions.

Watson and colleagues[5] report provocative findings in human tissue that correlate the NAMPT-NAD^+ control system with thoracic aortic dilatation. Among other observations, the *NAMPT* promoter was hypermethylated in situ and in vitro in VSMCs from patients with dilated ascending aortopathy, and lower levels of NAMPT correlated with increased DNA breaks. The authors then generated VSMC-specific *Nampt* deficient mice to test the hypothesis that diminished NAMPT leads to aortic dilatation. These mice exhibited an ~40% reduction in aortic NAD^+ and, importantly, presented with mild dilatation, especially in the ascending aorta. When challenged with chronic infusion of angiotensin II (AngII), which increases hemodynamic loads and inflammation, the VSMC-*Nampt* deficient aortas exhibited regions of VSMC loss and intramural hemorrhage, not unlike lesions seen with VSMC-specific postnatal disruption of transforming growth factor-beta receptor II[6]. Both of these studies report localized accumulations of mucoid material, similar to that in human histopathology and a potential initiator of dissection[7]. Watson et al.[5] conclude that aortic integrity depends on an intrinsic NAMPT-NAD^+ system, which when compromised results in unrepaired DNA damage and VSMC senescence.

VSMC Vitality

A striking finding in the pathologic aortas of the AngII-infused VSMC-*Nampt* deficient mice was loss of VSMCs[5]. Although TUNEL staining could not document VSMC apoptosis after 28 days of infusion, the evident senescence and yet dramatic loss of medial cells

suggest that many VSMCs could have undergone apoptosis earlier. Indeed, knockdown of *Nampt* in VSMCs has been shown to increase VSMC apoptosis whereas overexpression of *Nampt* can enhance VSMC survival[8].

Why this focus on the possibility that there was apoptosis? Increased VSMC apoptosis has been observed in the ascending aorta of the most commonly studied animal model of Marfan syndrome, the *Fbn1*$^{C1039G/+}$ mouse, and blocking caspase activity using a pan-caspase inhibitor prevents aortic enlargement[9]. Moreover, these apoptotic VSMCs have increased elastolytic potential when compared with viable cells, and thus can contribute further to the compromised ECM that is characteristic of aneurysmal dilatation. We have also shown that loss of the forkhead transcription factor Foxe3 increases VSMC apoptosis and rupture when the thoracic aorta is stressed mechanically by increasing blood pressure using aortic constriction; these ruptures are prevented by administering a p53 inhibitor (pifithrin-α) or crossing the *Foxe3*$^{-/-}$ mice with *p53*$^{-/-}$ mice[10]. Finally, loss of VSMC attachment to the ECM can drive a form of apoptosis referred to as anoikis (Greek for homeless or wandering). Anoikis-related cell drop-out would be expected following loss of ECM mechano-sensing. Thus, loss of VSMCs can contribute to dilatation and dissection/rupture of the thoracic aorta, suggesting that early VSMC apoptosis could contribute to pathology in AngII infused VSMC-specific *Nampt* deficienct mice.

Inflammation and SIRT1

Inflammation is a hallmark of vascular aging[1] and the associated aortic stiffening that adversely affects the hemodynamics and mechanobiology. Although it is unclear whether inflammation is a consistent initiator of TAAs, inflammatory cells co-localize with medial degeneration in these lesions and appear to contribute to the progressive pathology[11]. Among the many adverse effects of inflammation on the aortic wall, including an excessive proteolytic activity that can compromise structural integrity, increases in oxidative stress can drive cellular damage and dysfunction. Noting that NAD^+ bioavailability decreases with age, augmenting the NAMPT-NAD^+ system via nicotinamide mononucleotide (NMN; see Figure) supplementation reduces oxidative stress within the aorta of aged mice and associated decreases in intramural collagen and aortic stiffness reduce the pulse wave velocity[12]. One possible driver of this beneficial finding is that increased NAD^+ increases SIRT-1, a NAD^+ consuming deacetylase (Figure). Additional findings show that, among other effects, SIRT-1 downregulates AngII type 1 receptors, which reduces oxidative stress in the aorta and its sequelae. Not surprisingly, chronic AngII infusion within VSMC-specific *Sirt1* knock-out mice results in marked increases in oxidative stress and matrix metalloproteinase activity leading to increased aortic stiffness and increased dissection[13].

ECM Integrity and VSMC Contractility

The ECM of a healthy aorta consists of myriad proteins, glycoproteins, and glycosaminoglcans organized within a multi-layered structure – the intima, media, and adventitia. Normal ECM composition and structure endows the aortic wall with considerable compliance and resilience (allowing the media to bear most of the normal pulsatile loading and to store elastic energy during diastole that can be used to augment flow during systole),

but also sufficient strength (primarily within the adventitia, which serves as a protective sheath that prevents acute increases in pressure-induced mechanical stresses from damaging the media). With the exception of the elastic fibers, which are produced early on and typically have a long half-life, most ECM constituents turnover continually during mechanical homeostasis. VSMCs are fundamental to the synthesis, maintenance and, if needed, repair of medial ECM, which requires actomyosin activity both to sense (e.g., assess the stress in or stiffness of) and regulate (i.e., organize newly synthesized) the ECM[2]. For this reason, mutations to genes affecting actomyosin activity, including *ACTA2* (which encodes smooth muscle α-actin) or *MYH11* (which encodes smooth muscle myosin heavy chain), contribute to the propensity to TAAs[15].

VSMC contractility results from ATP-driven interactions between actin and myosin filaments. The NAMPT-NAD^+ control system can affect VSMC contractility in multiple ways, particularly via the production of ATP that fuels actomyosin interactions and the prevention of cell senescence or apoptosis, both of which necessarily reduce overall contractile capacity. Noting that nicotinamide (a key molecule in the salvage pathway for NAD^+ synthesis; Figure) can attenuate VSMC contractility by blocking phosphorylation of myosin light chain, a recent study showed further that an inhibitor (rucaparib) of the NAD^+-consuming DNA-repair enzyme PARP-1 also attenuates VSMC contraction[14], perhaps in a similar manner. A corollary, therefore, is that NAMPT deficiency could increase nicotinamide and thereby decrease VSMC contractility. Hence, despite some controversy[4,5], the NAMPT-NAD^+ control system may influence actomyosin activity directly and indirectly and thereby affect VSMC contraction-mediated ECM integrity.

Conclusion

TAAs are responsible for significant morbidity and mortality. Advances in genetics, molecular and cellular biology, medical imaging, and bioengineering have advanced our understanding of this disease, yet clear pharmacotherapy remains elusive. The paper by Watson and colleagues[5] further highlights the complexity of this disease process. We concur that increased attention should be directed to the NAMPT-NAD^+ control system in studying thoracic aortopathy, but with broad appreciation of its myriad direct and indirect temporal effects on cell vitality and thus matrix integrity.

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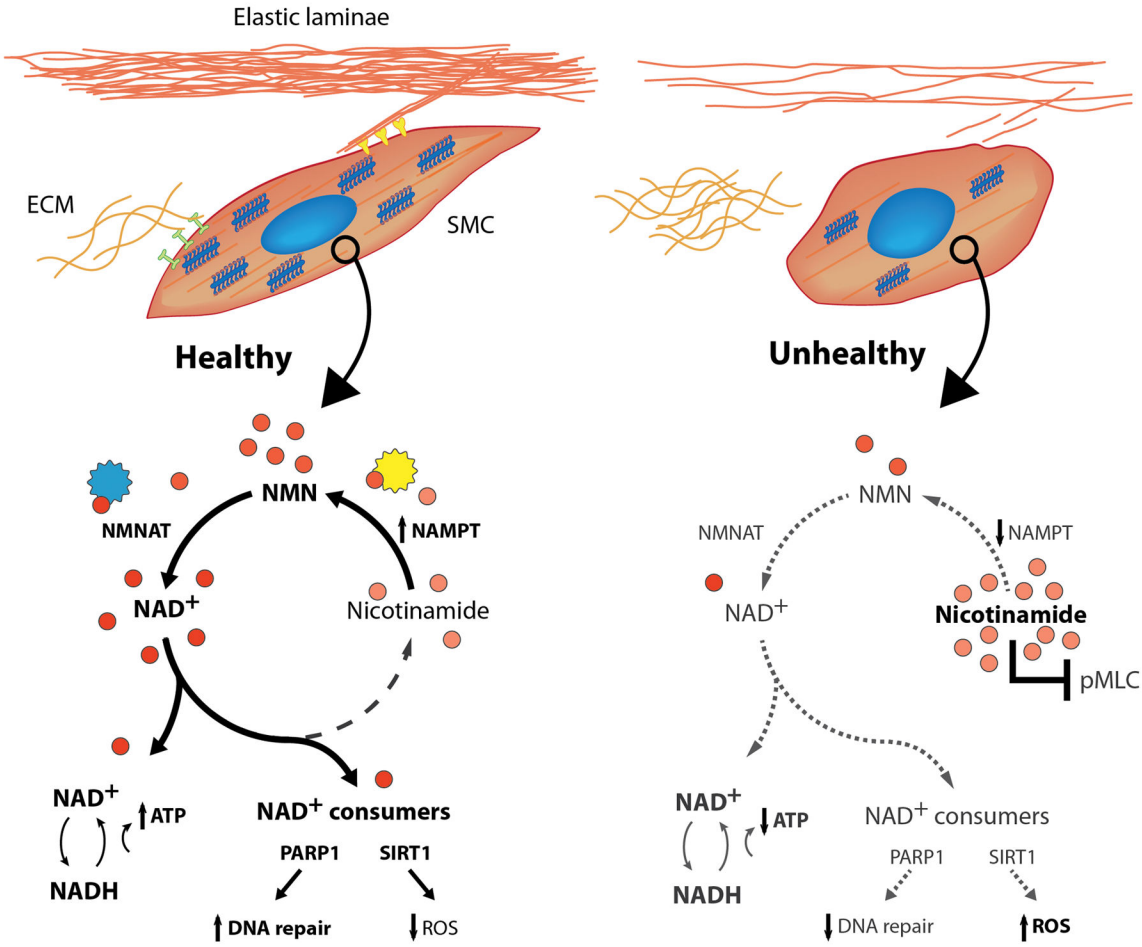

Figure 1.

Healthy (spindle-shaped; left) and unhealthy (rounded; right) vascular SMCs, the latter with few connections to ECM and lower actomyosin activity. Shown too, the salvage biosynthesis pathway with select NAD^+ consuming proteins (SIRT1, PARP1) leading to important biological outcomes. Increased NAMPT-NAD^+ has multiple effects, including suppression of inflammation and direct and indirect influences on contractility. NMNAT = nicotinamide mononucleotide adenylyltransferase 1, NADH = nicotinamide adenine dinucleotide hydrate, ROS = reactive oxygen species, and pMLC (phosphorylated myosin light chain). Courtesy Dr. S-I. Murtada.