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Commentary

Aneurysms are common in the abdominal and thoracic regions of the aorta and can cause death due to dissection or rupture. Traditionally, thoracic aortic aneurysms have been labeled as a degenerative disease, characterized by alterations in extracellular matrix and loss of smooth muscle cells (SMCs) in the medial layer of the aortic wall. In this issue of the *JCI*, Li and colleagues introduce an unconventional concept by demonstrating that mTOR-dependent proliferative SMCs render the aortic wall vulnerable to dilatation and dissection rather than prevent disease progression. These vascular SMCs, termed degradative SMCs, compromise the medial properties and function of the aortic wall by enhanced proteolytic and phagocytic activity; however, the cells do not transdifferentiate into macrophages. The degradative SMC phenotype also worsens atherosclerotic disease and could thus be considered as a therapeutic target for diverse aortic diseases.

Proliferative, degradative smooth muscle cells promote aortic disease

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Aneurysms are common in the abdominal and thoracic regions of the aorta and can cause death due to dissection or rupture. Traditionally, thoracic aortic aneurysms have been labeled as a degenerative disease, characterized by alterations in extracellular matrix and loss of smooth muscle cells (SMCs) in the medial layer of the aortic wall. In this issue of the *JCI*, Li and colleagues introduce an unconventional concept by demonstrating that mTOR-dependent proliferative SMCs render the aortic wall vulnerable to dilatation and dissection rather than prevent disease progression. These vascular SMCs, termed degradative SMCs, compromise the medial properties and function of the aortic wall by enhanced proteolytic and phagocytic activity; however, the cells do not transdifferentiate into macrophages. The degradative SMC phenotype also worsens atherosclerotic disease and could thus be considered as a therapeutic target for diverse aortic diseases.

Aortic wall homeostasis

The aortic wall consists of three layers — intima, media, and adventitia — separated from each other by the internal and external elastic laminae. The intima is thin and composed of endothelial cells and their underlying supporting tissue. The media is the largest portion of the wall and contains smooth muscle cells (SMCs), which are circumferentially aligned and interspaced by thin layers of elastin fibers and extracellular matrix (ECM) proteins including collagens, fibrillins, and glycosaminoglycans. The adventitia is the outermost component of the aortic wall containing connective tissue and a few small blood vessels called the vasa vasorum that support the stromal and parenchymal cells by providing nutrition. Maintaining aortic wall homeostasis is an active, highly dynamic, and tightly regulated mechanism involving all cellular and extracellular components of the aortic wall. Indeed, medial SMCs are not terminally differentiated cells but can alternate between quiescent, contractile, secretory, or proliferative states to ensure adequate function of the aortic wall (1). Dysregulation of one or more of the cellular and extracellular components can pave the way for aortic disease including aortic aneurysms.

Aortic aneurysms or abnormal balloon-like bulging of the vessel can occur anywhere in the aorta and is a common vascular disease increasing the risk of aortic dissection or rupture and accounting for more than 26,000 deaths in the United States annually (2). Thoracic aortic aneurysms (TAAs) are less common than abdominal aortic aneurysms and while they share some physiological characteristics, they are distinct diseases with clear differences in the underlying pathological mechanisms (3). Historically, TAA has been described as a degenerative disease characterized by cystic medial necrosis of the aortic wall (4). However, extensive research in the past decades using genetic studies and different mouse models identified TAA as a multifactorial disease with complex underlying cellular and molecular mechanisms (3, 5). The clinical classification of TAA into syndromic, familial nonsyndromic, and sporadic reflects its heterogeneity with multiple unique mechanisms all leading to a similar clinical manifestation. Syndromic TAAs include Marfan, Loeys-Dietz, and Ehlers-Danlos syndrome, which are caused by mutations in fibrillin-1 (*FBN1*), *TGFBR-1* and *-2*, and type III collagen, respectively (6). TAAs inherited in families without syndromic features are due to mutations in genes encoding proteins involved in SMC contraction (6). Sporadic TAAs do not show any familial transmission and most of these aneurysms are characterized by matrix degradation, elastin fragmentation, and immune cell infiltration (5). Current therapeutic options to prevent rupture are restricted to surgical repair, as there remains a lack of validated pharmaceutical approaches (7). Altogether, TAAs are more than simple, degenerative diseases and are the result of complex changes in the cellular matrix and ECM of the medial layer compromising the aortic wall's structural integrity.

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mTOR-induced SMC hyperplasia aggravates aortic disease

In this issue of the *JCI*, Li et al. investigated the paradigm that SMCs and their phenotypic state play a central role in TAA pathogenesis, either preventing disease by maintaining homeostasis or accelerating disease through inappropriate responses (8). The authors specifically focused on SMC proliferation, which has been thought to limit disease progression — an assumption driven by the observation that SMC death and scarcity associates with end-stage TAA (9). To this end, the authors used a genetic mouse model of conditional *Tsc1* disruption in SMCs (*Myh11*

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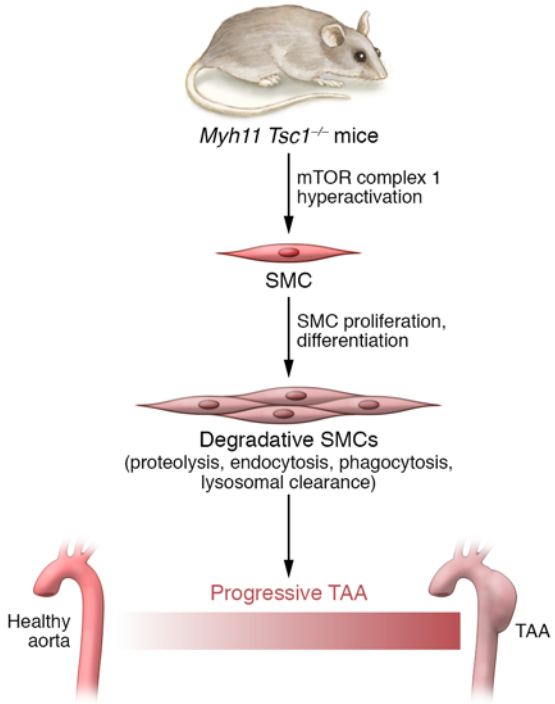

Figure 1. mTOR-induced proliferation of degradative SMCs leads to progressive TAA. Conditional *Tsc1* disruption in SMCs using *Myh11 Tsc1*$^{-/-}$ mice leads to hyperactivation of mTOR complex 1, inducing SMC proliferation and differentiation into degradative SMCs. These modulated SMCs are characterized by increased proteolysis, endocytosis, phagocytosis, and lysosomal clearance compromising aortic medial properties and function and causing progressive TAA.

Tsc1$^{-/-}$ mice) that leads to hyperactivation of mTOR complex 1, a key kinase regulating cell growth and differentiation (10), to investigate whether SMC hyperplasia promoted or prevented TAA and how mTOR modulated SMC phenotype. Postnatal deletion of *Tsc1* in SMCs caused progressive TAA characterized by medial expansion and degradation (Figure 1). Moreover, ascending aortas of knockout mice exhibited SMC proliferation, elastin fragmentation, and decreased elastic energy storage. The initial decrease in elastin synthesis was followed by enhanced susceptibility to elastin proteolysis and in a later phase by increased protease activity, highlighting that both lower production and breakdown contribute to progressive elastin fragmentation. mTOR inhibition by long-term rapamycin therapy reversed the phenotype in *Myh11 Tsc1*$^{-/-}$ mice, which indicates that the observed medial degradation is mTOR dependent. The authors further determined the consequences of mTOR hyperactivation in a clinically relevant model of mild TAA due to *Fbn1* mutation. Chronic mTOR activation in SMCs aggravated disease severity and increased lethality in *Fbn1* mutants. Thus, SMC hyperplasia per se does not prevent TAA, but rather worsens medial degeneration if appropriate cellular function is not maintained.

In addition to aneurysms, the response of vascular SMCs to injury is a major determinant of the development and progression of other vascular diseases, including atherosclerosis (11–13). Therefore, the authors determined the effect of chronic mTOR-mediated SMC hyperproliferation on atherosclerosis by breeding *Myh11 Tsc1*$^{-/-}$ with *ApoE*$^{-/-}$ mice. Conditional deletion of *Tsc1* increased lipid accumulation in intimal cells as well as medial SMCs, further supporting the pathogenic effects of chronic mTOR activation on clinically relevant vascular diseases.

Degradative SMCs drive TAA formation

Given the progressive changes in arterial SMC phenotype in *Myh11 Tsc1*$^{-/-}$ mice, the authors investigated possible differentiation into other cell types. To this end, they combined lineage-tracing experiments with bulk and single-cell RNA sequencing. After conditional mTOR hyperactivation, medial SMCs failed to differentiate to an adipogenic, chondrogenic, or osteogenic phenotype. Instead, they expressed a limited repertoire of fibroblast and macrophage markers. However, they lacked expression of pan-leukocyte and macrophage lineage markers, such as CD45, CD11b, CD68, and F4/80. Transcription analysis further supports the notion that these modulated SMCs fail to transdifferentiate into bona fide macrophages. *Tsc1*-deficient SMCs are characterized by increased lysosomal clearance of ECM and apoptotic cells regulated by mTOR, β-catenin, and melanogenesis-associated transcription factor. These findings support the concept for a distinct SMC phenotype displaying increased proteolysis, endocytosis, phagocytosis, and lysosomal clearance (Figure 1), which does not resemble the canonical contractile-to-synthetic SMC spectrum described in many arterial diseases (13, 14). The authors suggest naming these pathological cells "degradative SMCs" to reflect the main structural and functional changes inflicted by the altered cellular phenotype at the tissue level. These medial SMCs with a degradative phenotype also manifest in human TAA, further supporting their pathological role and opening up new therapeutic strategies focusing on restoring appropriate SMC function. Recent work by Clément et al. also highlights the critical role for autophagy in regulating SMC death and inflammation in angiotensin 2–induced dissecting aortic aneurysms in mice (15).

Are they macrophages or not?

Recent technological advances in biomedical research, such as multicolor lineage-tracing tools, single-cell RNA sequencing, and high-resolution imaging modalities now portray cell identity with greater complexity than previously observed. These advances allow us to abandon oversimplified concepts that do not reflect the cellular phenotypes in a highly dynamic and tightly regulated environment, such as the aortic wall. Previous work reported transdifferentiation of intimal SMCs into macrophages in the setting of atherosclerosis (16–18); however, *Tsc1*-deficient SMCs in hyperlipidemic mice lack expression of conventional macrophage markers except for lysosome-

associated markers (8). In line with the Li et al. findings, Wirka and colleagues recently reported the transformation of SMCs into unique fibroblast-like cells rather than macrophages in mouse and human atherosclerotic lesions (19). Both observations further fuel the ongoing debate whether or not SMCs are able to transdifferentiate into conventional macrophages. Indeed, historically assigned functions, such as phagocytosis, once considered owned by one specific cell type may share the duty with cells originating from different progenitors. Suffice it to say, as opposed to studying pathogenic processes, the claim of SMC-to-macrophage transdifferentiation may even be seen as a matter of semantics. As William Shakespeare wrote more than 400 years ago: "A rose by any other name would smell as sweet."

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