The cellular uncertainty in thoracic aortic dissections: the roles of METTL3 and NOTCH1 on m6A in human aortic smooth muscle cells

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Thoracic aortic dissection (TAD) is a potentially life-threatening pathology with a high mortality rate, which warrants continual development for effective management approaches. TAD is defined by the progressive separation of the thoracic aortic wall layers (1,2). Since the anatomical extent of TAD has different clinical implications and treatment approaches, TAD is classified according to the areas involved into Stanford type A dissections which involves the ascending aorta up to the origin of left subclavian artery, whereas Stanford type B dissections starts from beyond left subclavian artery (3). Stanford type A dissections are more common and dangerous, accounting for approximately two-thirds of TADs (1,4).

Despite extensive investigation pertaining to TAD pathophysiology, the cellular and molecular mechanisms of TAD are inadequately understood. Human aortic smooth muscle cells (HASMCs) have contractile and synthetic phenotypes, both of which are characterised by the expression of different marker proteins. The contractile phenotype is maintained in healthy HASMCs to regulate vascular tone. However, in abnormal proliferation or degeneration of HASMCs, pathological remodelling occurs causing HASMC conversion from contractile to synthetic phenotype.

A pathological process in TAD development is the degeneration of HASMCs; and the association between TAD development and phenotypic HASMC conversion has been reported (5). RNA modifications may therefore play a role in the pathogenesis of TAD. N6-methyladenosine (m6A) is the most common endogenous epigenetic modification on messenger RNA (mRNA) because it can be modified and recognised by enzymes and binding proteins to regulate biological functions (6). Methyltransferase-like-3 (METTL3) is involved in m6A formation; and NOTCH1 signalling is involved in cell proliferation, differentiation, and apoptosis (7-10). METTL3 was reported to regulate the progression of abdominal aortic aneurysms through m6A processing (11). Another study reported that reduced METTL3 expression in urinary tumour cells was associated with upregulation of the NOTCH1 apoptosis pathway (12). However, the role of METTL3 and NOTCH1 on m6A modification in TAD has not been established.

A recent study by Yang *et al.* found that METTL3 was responsible for the m6A modification of NOTCH1 mRNA, which when overexpressed *in vitro* resulted in increased mRNA degradation and subsequent promotion

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of the synthetic HASMC phenotype associated with the progression of TAD (13). This was demonstrated through changes in expression of METTL3, NOTCH1, α-smooth muscle actin (α-SMA) and smooth muscle protein 22-α (SM22α) mRNA via quantitative real-time polymerase chain reaction (qRT-PCR) and relative protein levels via western blot. Given that α-SMA and SM22α are markers of contractile smooth muscle cells (SMCs), their decreased levels of expression with decreased NOTCH1 indicate that the HASMCs had dedifferentiated and subsequently changed phenotype.

To date, studies have identified that the conversion of the contractile SMC to the synthetic phenotype underpins the pathogenesis of most vascular pathologies. Increased METTL3 levels have previously been associated with atherosclerotic plaques, abdominal aortic aneurysm development and the presence of synthetic SMCs (11,14-16). Moreover, given that a study by Qin *et al* identified that METTL3 increased proliferation of PASMCs *in vivo* and *in vitro* via hypermethylation and resultant degradation of phosphatase and tensin homolog (PTEN) mRNA, it should not be surprising that similar results have been concluded regarding NOTCH1 (14).

Nevertheless, these disease processes are multifactorial and must also consider cell-to-cell interactions, endothelial dysfunction and the regulation of gene expression via the application of shear forces induced by blood flow. Consequently, evidence surrounding the relationship between METTL3, NOTCH1 and SMC phenotype is somewhat paradoxical. A number of ApoE(–/–) mouse model studies agree that increased (rather than decreased) NOTCH1 expression induces conversion of the contractile SMC to the synthetic phenotype, thus contributing to the progression of diseases such as atherosclerosis, aortic aneurysm and aortic dissection (17,18). Undoubtedly, the key distinction between these studies is the use of *in vivo* models rather than a two-dimensional *in vitro* model; *in vitro* models are simpler and enable tight control of the physical and chemical environment but often fail to replicate the *in vivo* conditions of that cell type.

Three-dimensional cultures are becoming more popular and offer a compromise between two-dimensional *in vitro* models and mouse models. These enable cell cultures to increase overall cell to cell contact, interact with extracellular matrices, promote differentiation and tissue organisation, as well as enable the development of multi-layered models that could closely replicate the vascular anatomy. HUASMCs in a three-dimensional model have been shown to increase expression of differentiation markers including α-SMA, induce quiescence and amplify transforming growth factor-β (TGF-β) expression, down-regulating downstream expression nuclear factor-kappaB (NF-κB) activity (19). Consequently, provided that NF-κB promotes NOTCH1 signalling, it would be expected that NOTCH1 activity would be low in contractile SMCs and that increased NOTCH1 expression would correlate to an increase in synthetic phenotype.

Moreover, the aorta undergoes approximately 10% stretch under physiological conditions. Less than 10% stretch on SMCs have been shown to inhibit NOTCH signalling and promote the contractile SMC phenotype (20). On the other hand, most aortic dissections occur as a result of high blood pressure, with acute hypertension inducing stretching over 20% resulting in the up-regulation of inflammatory markers such as IL-1β, IL-6, VCAM-1 and ICAM-1, which are controlled by NF-κB. Thus, it could be anticipated that increased stretch in hypertension could also promote the expression of NOTCH1.

Additionally, it was previously noted that SMCs were either of a contractile or synthetic phenotype; yet a recent review by Yap *et al* identified six distinct sub-populations of vascular SMCs via examination of the genotype of SMCs within different vascular pathologies (21). The synthetic SMC phenotype could be divided into mesenchymal-, fibroblast-, macrophage-, osteogenic- and adipocyte-like vascular SMCs. It would therefore be interesting to examine the genotype of the SMCs in aortic dissections to confer whether the subtype(s) correlated to the synthetic SMCs in the study by Yang *et al.* (13). If the SMCs correspond, the results could demonstrate that whilst increased METTL3 expression is associated with promoting the synthetic SMC phenotype, it is not dependant on the augmentation of NOTCH1 and alternative mechanisms may be responsible, such as those involved in mechano-transduction.

Conclusively, whilst the mechanism by which METTL3 contributes to synthetic transformation of SMCs is yet to be confirmed owing to contradictory evidence regarding its relation to NOTCH1 expression, global reduction of METTL3 expression may prevent the progression of several critical vascular diseases and have a widespread impact on mortality rates and healthcare costs attributed to cardiovascular disease. Despite this, given that METTL3 is responsible for mRNA processing, translations efficiency, editing and stability in fundamental biological processes, it may be difficult to specifically augment METTL3 expression in SMCs to reduce progression of TAD without

inducing some off-target effects (22). The severity of these implications would be difficult to determine prior to *in vivo* testing but evidence suggests that further analysis would be warranted to perceive probability of success.

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