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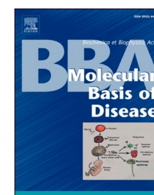

Identifying and targeting the molecular signature of smooth muscle cells undergoing early vascular ageing

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ABSTRACT

Early vascular ageing (EVA) is a pathological phenomenon whereby the vascular system ages more quickly than chronological age. This underpins many cardiovascular diseases including the complications of type 2 diabetes, aneurysm formation and hypertension. Smooth muscle cells (SMC) are the principal cell type in the vascular wall and maintain vascular tone. EVA-related phenotypic switching of these cells contributes towards disease progression. EVA is distinct from chronological ageing, and research is ongoing to identify a definitive molecular signature of EVA. This will facilitate the discovery of new clinical tests for early detection of EVA and identify therapeutic targets to halt (or prevent) EVA in SMC, thus reducing macrovascular morbidity and mortality.

1. Introduction

Our population is becoming increasingly aged. Since the turn of the millennium, the global average life expectancy has increased from 66.8 to 73.4 years [1]. In developed countries such as the UK, average life expectancy is even higher at 81.3 years [2]. Unfortunately, healthy life expectancy has not followed suit meaning that our aged population is suffering more and more from age-related morbidity, including increased risk and severity of cardiovascular disease.

Smooth muscle cells (SMC)[1] are the principal cell type within the vascular wall and are critical for both normal vascular function (*e.g.* maintaining vascular tone, responding to vasodilators/vasoconstrictors) and in the vascular response to injury. This is facilitated by their phenotypic plasticity; SMC can exist in either a contractile, differentiated state or synthetic, dedifferentiated state according to the needs of the blood vessel at any particular time. In young healthy people, SMC are contractile with a low turnover rate and are characterised by an abundance of contractile proteins such as alpha smooth muscle actin (α-SMA), smooth muscle myosin heavy chain (SM-MHC), smoothelin and calponin, amongst others. During disease or in response to injury, SMC can transiently and reversibly transform into a synthetic or inflammatory phenotype with a loss of contractile proteins and an increase in proliferation, migration and clonality (reviewed in [3]). This phenotype is also present in SMC from older individuals and is associated with increased prevalence of atherosclerosis, hypertension and peripheral artery disease.

SMC phenotypic plasticity is key to the correct functioning and responsiveness of blood vessels. In addition to the two classical SMC phenotypes mentioned above (contractile and synthetic), SMC can also adopt a number of other phenotypes in either a transient and permanent manner. These include macrophage-like SMCs, stem cell-like SMCs, myofibroblast-like SMCs and osteogenic SMCs (Fig. 1). Whilst this initially may be part of an adaptive response to changes in blood flow, injury or disease, if the contractile phenotype is not restored it can

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[1] α-SMA, alpha smooth muscle actin; AAA, abdominal aortic aneurysm; AGE, advanced glycation end products; AngII, angiotensin II; ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and Rad3-related; C3, complement factor 3; DAMP, damage associated molecular patterns; ECM, extracellular matrix; ERK1/2, extracellular signal-regulated kinase 1/2; EVA, early vascular ageing; HGPS, Hutchinson-Gilford progeria syndrome; KLF4, Kruppel-like factor 4; KV, voltage-gated potassium channel; L3MBTL4, lethal(3) malignant brain tumor-like protein 4; LMNB1, lamin B1; IL, interleukin; JNK, c-Jun N-terminal kinase; MCP1, monocyte chemoattractant protein 1; MFG-E8, milk fat globule-epidermal growth factor 8; miR, microRNA; MMP, matrix metalloproteinase; NAMPT1, nicotinamide phosphoribosyl-transferase 1; NFκB, nuclear factor kappa B; Nlr, nod-like receptor; Nrf2, nuclear factor erythroid 2-related factor 2; OPN, osteopontin; OSX, osterix; PDGF, platelet-derived growth factor; RCN2, reticulocalbin 2; ROS, reactive oxygen species; Runx2, runt-related transcription factor 2; SASP, senescence-associated secretory phenotype; SIRT-1, sirtuin-1; SM-22α, smooth muscle cell protein 22 alpha; SM-MHC, smooth muscle myosin heavy chain; SMC, smooth muscle cell; SOC, store-operated channel; sRAGE, soluble receptor for advanced glycation end products; SV, saphenous vein; T2DM, type 2 diabetes mellitus; TGF, transforming growth factor; TRPC, transient receptor potential channel; YVC, Youth Vascular Consortium.

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quickly transform into a maladaptive response that contributes to atherosclerotic plaque progression and the acquisition of functional defects common to both physiological and premature vascular ageing [4,5]. Thus, retention of the ability to switch phenotypes is critical for maintaining vascular health.

2. Chronological (physiological) vascular ageing

The vascular system evolves throughout an individual's lifetime and accumulates functional defects such as increased stiffness, vessel wall thickness and SMC proliferation. These can all be identified at the molecular level and lead to age-related decline in cardiovascular health characterised by increased atherosclerosis and neointimal hyperplasia, ultimately leading to increased risk of myocardial infarction and stroke. It is impossible to study SMC from the same individual across their lifetime, and so instead *in vitro* assays have been developed to mimic the ageing process, which could be extrapolated to *ex vivo* studies of old *versus* young tissue.

One of the hallmarks of ageing in any tissue is senescence and the development of a senescence-associated secretory phenotype (SASP). The senescent fingerprint of human coronary artery SMC using both *in vitro* replicative senescence and DNA-damage-induced senescence methods has recently identified the acquisition of a rhomboid shape, enlarged ovoid nuclei, reduced lamin B1 (LMNB1) expression and increased expression of interleukins IL-1β and IL-6 as markers for physiological ageing [6]. Whilst this is not an ideal study – it uses *in vitro* methods on SMC isolated from one individual to induce the aged phenotype – it does at least use human cells and two different models to validate its findings on senescence. In support of this, expression of sirtuin-1 (SIRT-1; a longevity protein) is reduced in senescent cells and in SMC from both aged humans and mice [7,8]. In contrast, the effect of age on SMC proliferation is inconsistent with reports of both enhanced [9,10] and impaired proliferative capacity [11]. It may be that SMC undergo an initial period of hyperproliferation early on in the ageing process which then switches to a replicative senescence model, further inducing senescence in surrounding SMC and leading to functional decline.

Chronic low-grade inflammation, such as that caused by persistent SASP, is a feature of 'inflammaging'. Very recently, cytoplasmic Nod-like receptor (Nlr) inflammasomes have been implicated in promoting SMC senescence and age-related phenotypic switching through induction of IL-1β and IL-6 [12], providing an alternative route for production of these cytokines in addition to SASP [6]. Furthermore, monocyte chemoattractant protein 1 (MCP1) is elevated in aged SMC and can induce dedifferentiation in naïve younger cells [13], propagating the inflammatory response.

Advanced glycation end-products (AGEs) accumulate in the body over time and contribute to vascular stiffness. Cross-linking of extracellular matrix (ECM) components *via* AGEs causes stiffening and translates into SMC dysfunction through outside-in signalling [14]. Indeed, a recent study has highlighted that individuals who have elevated levels of soluble receptor for AGEs (sRAGE) are protected somewhat from age-related vascular stiffening [15]. AGEs induce SMC inflammation through triggering the nuclear factor kappa B (NFκB) signalling pathway, contributing to impaired vasodilation and vascular calcification [16,17]. Activation of RAGE also downregulates the expression of SMC differentiation marker SM-22α [18]. Such a decline in contractile SMC markers has long been recognised as a response to

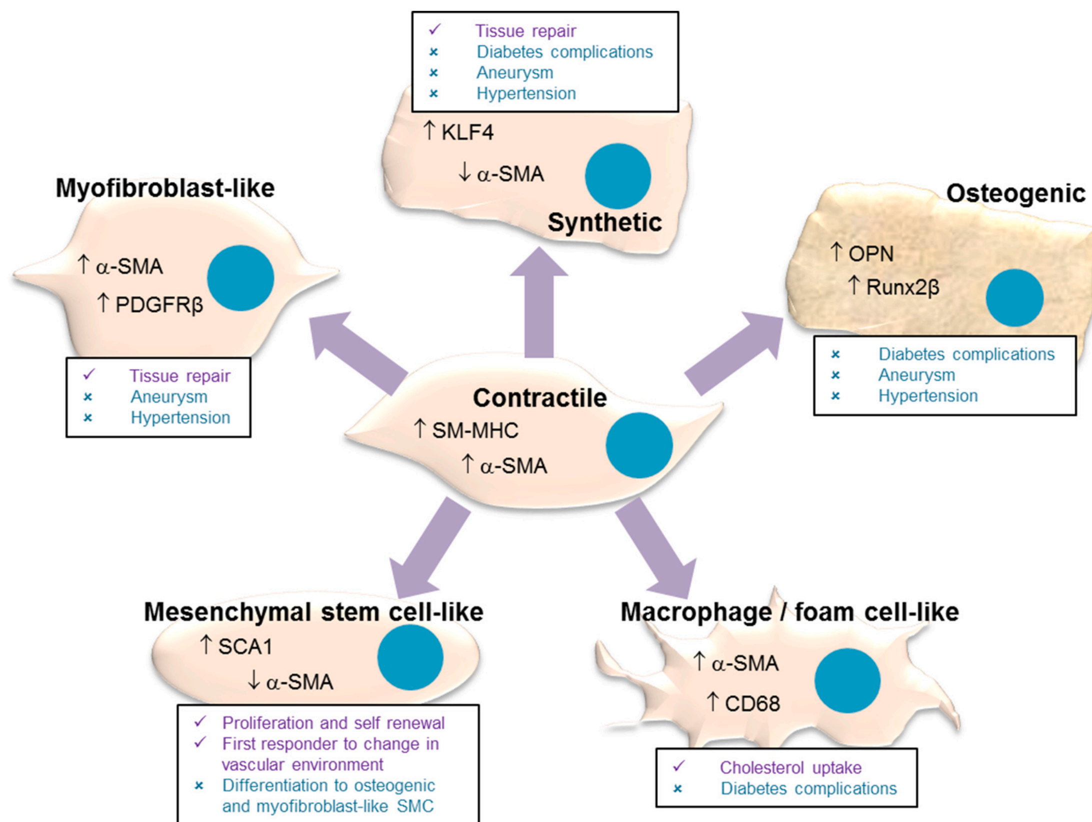

Fig. 1. Diversity of vascular smooth muscle cell phenotypes. Under healthy conditions, SMC exist in a contractile phenotype that contracts and relaxes to maintain vascular tone. This is characterised by expression of key contractile markers such as SM-MHC and α-SMA. In cases of injury or disease, SMC can differentiate into a number of different phenotypes that are initially adaptive but can ultimately contribute to vascular complications of both physiological and premature ageing. Positive roles are indicated by ✓ and negative roles are indicated by ×. Abbreviations: α-SMA; alpha smooth muscle actin; CD68, cluster of differentiation 68; KLF4, Kruppel-like factor 4; OPN, osteopontin; PDGFRβ, platelet-derived growth factor receptor beta; Sca1, stem cells antigen-1; Runx2β, Runt-related transcription factor 2 beta.

ageing [19], although a recent study has challenged that assertion [20].

Matrix metalloproteinases MMP-2 and -9 are important for SMC migration into the intima and are positively associated with atherosclerosis development. Expression of both is enhanced in ageing-associated arterial stiffening in mice, as is expression of transforming growth factors TGFβ1 and β3 [7]. MMP-2 can further activate TGFβ1 in a positive feedback loop [21], perpetuating matrix remodelling. Milk fat globule-epidermal growth factor 8 (MFG-E8) has recently been highlighted as a key modulator of vascular age. Expression is upregulated in aged SMC and vascular tissue, and it signals *via* MCP-1 to stimulate SMC invasion (reviewed in [22]). Given that MCP-1 can induce MMP-9 expression [23], this could be a likely mechanism for the functional effect.

Defects in autophagy have recently been postulated as a mechanism for premature ageing in the cardiac system ([24,25] and references therein) and thus could also be important in ageing of the vasculature. A thorough overview of the different theories underpinning physiological vascular ageing (including senescence, epigenetic changes and autophagy), is given in a recent review by Wang et al. [26]. For the purposes of focussing in specifically on SMC, a summary of the recent developments and molecular changes during physiological SMC ageing is depicted in Fig. 2.

3. Lessons from premature ageing syndromes

To examine the influence of age on SMC, it can be useful to look to the extremes. Premature ageing syndromes such as Hutchinson-Gilford progeria syndrome (HGPS) affect multiple organ systems including the cardiovascular and skeletal systems, and skin [27]. Whilst this is a very rare condition, data from HGPS SMC have provided insight into molecular changes associated with age. HGPS is caused by mutations in *LMNA* or *Zmpste24* which causes the accumulation of a mutated form of lamin A. This induces DNA damage and nuclear morphology defects; these features are also evident in peripheral artery SMC from aged individuals [28]. In a recent murine and human HGPS-induced pluripotent stem cell-derived SMC study, SMC were more susceptible to mechanical damage from shear stress due to increased expression of the collagenase MMP-13 [29]. Elevated MMP-13 has been identified in premature skin (photo)ageing [30] but as yet has not been assessed fully in premature or physiologically aged vasculature. An overview of the multi-system phenotypes seen in mouse models of accelerated ageing reveals a wealth of data on skeletal, dermatological and ocular defects in premature ageing but there is comparatively less known specifically about SMC [31].

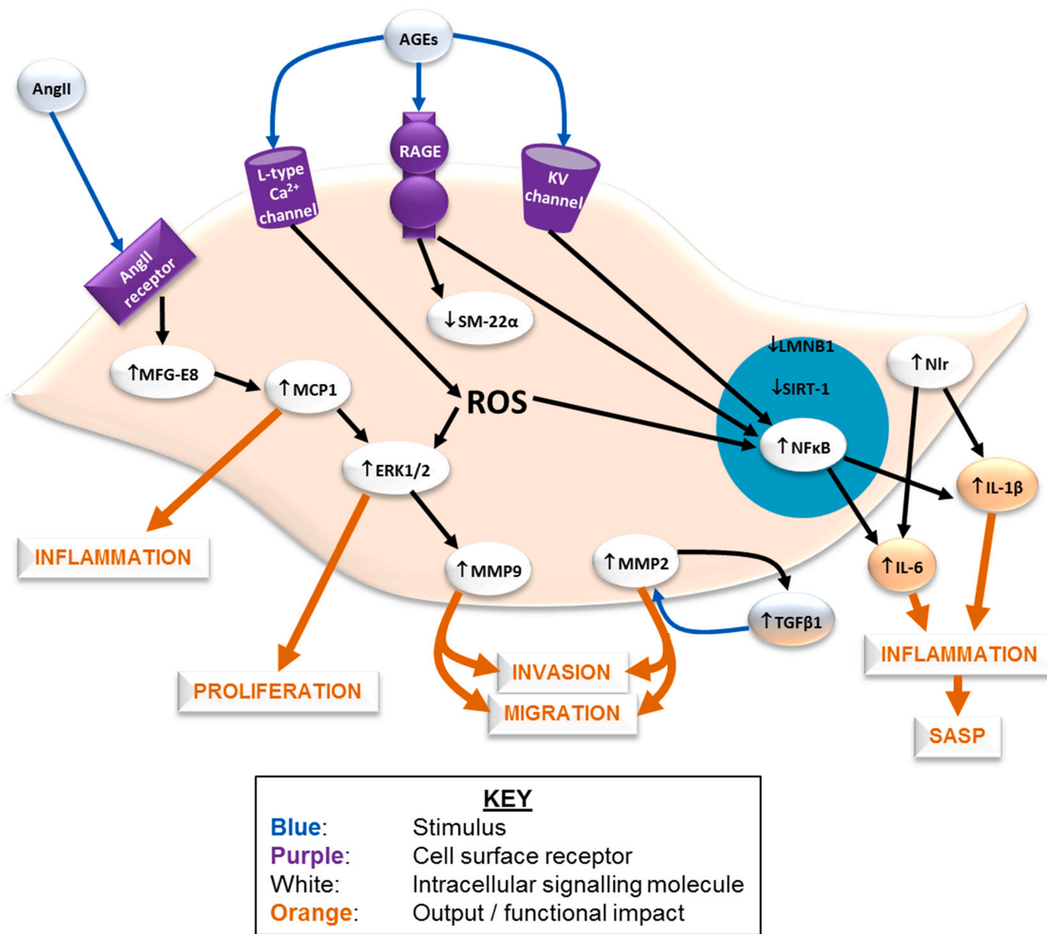

Fig. 2. Chronologically aged smooth muscle cells. During ageing, circulating factors such as AngII, AGEs and TGFβ1 increase and stimulate multiple signalling pathways leading to functional changes in proliferation, senescence, migration, invasion and inflammation. Abbreviations: AGEs, advanced glycation end products; AngII, angiotensin 2; ERK1/2, extracellular signal regulated kinase 1/2; IL, interleukin; KV, voltage-gated potassium channel; LMNB1, lamin B1; MCP1, monocyte chemoattractant protein 1; MFG-E8, milk fat globule epidermal growth factor 8; MMP, matrix metalloproteinase; NFκB, nuclear factor kappa B; Nlr, Nod-like receptor; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; SIRT-1, sirtuin-1; SM-22α, smooth muscle cell protein 22-alpha; TGFβ1, transforming growth factor beta 1.

4. Early vascular ageing (EVA)

EVA is defined as a disconnect between the relative age of an individual's vascular system and the actual chronological age of their body. Blood vessels develop structural changes that mimic ageing phenotypes resulting in premature development of atherosclerosis, neointimal thickening and vasoconstriction, and EVA is clinically identified as elevated carotid-femoral pulse wave velocity and arterial stiffness [32–34]. Dysfunction of the cells lining the blood vessels – the endothelial cells – has been a central tenet since the identification of EVA [34], underpinned by traditional ageing features of oxidative stress and inflammation [35]. Reduced endothelial nitric oxide production causes dysfunction in neighbouring SMC which manifests as vasoconstriction (reviewed in [36]). Similarly, increased arterial stiffness has been long-recognised as a feature of EVA and causes changes in the mechanical signals that vascular cells – in particular SMC – are exposed to. Ageing is associated with changes in the ECM including degradation of elastin. This causes hyperproliferation of SMC [37] and, given elastin is only produced early in life, could indicate that individuals can be genetically primed to undergo EVA if they produce less elastin. This transition to a classically dedifferentiated, synthetic SMC phenotype results in increased production of collagen and consequently arterial stiffness; a feature also seen in prematurely aged HGPS individuals [38]. Whilst it is clear that cross-talk throughout the three layers within the vascular wall is complex, this mini-review will focus solely on the impact of EVA on SMC.

Whilst EVA was first hypothesised and discussed in 2008 [34], research into this area has gained traction in recent times. Search terms for EVA in PubMed yields 102 returns in 2008 when the term first came into common use, doubling ten years later. In 2021 this figure is projected to rise to ~300 (data collected 13th August 2021) highlighting the growing recognition of EVA and its contribution to multiple pathologies including type 2 diabetes mellitus (T2DM) [39], abdominal aortic aneurysm (AAA) [40] and hypertension [41]. Given the inherent plasticity of SMC and their acquisition of multiple alternative phenotypes (see Fig. 1), it is inevitable that these will have an impact on the initiation, development and propagation of EVA.

To fully understand EVA, a robust and reliable set of benchmarking characteristics of vascular function, behaviour and metabolism needs to be identified not only in EVA, but also in physiological ageing. The recently-announced Youth Vascular Consortium (YVC) Protocol aims to establish references for vascular ageing in the younger population [42]. As atherosclerosis development begins in childhood and clinical EVA can be observed in adolescents with T2DM [39], this study may provide the scientific community with a wealth of data that can differentiate and identify EVA at early time points when interventions may be at their most efficacious.

4.1. SMC phenotype in T2DM EVA

T2DM is a chronic condition that is caused by a loss of insulin sensitivity and subsequent failure of insulin secretion. The leading cause of mortality in T2DM patients is cardiovascular disease which begins with endothelial dysfunction and culminates in premature development of atherosclerosis, thrombotic disease and/or cardiomyopathy. The vascular systems of patients with T2DM are reportedly 15 years older than the patients' chronological age [43]. Thus, SMC from T2DM patients are an ideal model in which to study EVA. Complications arise when considering the source vessel of SMC – the behaviour of SMC from arteries and veins can differ in both non-diabetic [44] and T2DM individuals [45] so care must be taken in evaluating the relative contribution of different pathways to T2DM SMC dysfunction.

Saphenous vein (SV) SMC from T2DM individuals share some similarities with the chronological ageing phenotype described in Stojanovic et al. [6], namely increased senescence, enlarged cellular and nuclear morphology, reduced LMNB1 expression and increased levels of IL-1β and IL-6 [45–48]. However, T2DM SMC also had an increased incidence of γH2AX-positive nuclear foci, and elevated ataxia-telangiectasia mutated (ATM), ataxia-telangiectasia and Rad3-related (ATR) and p21 expression levels suggesting persistent activation of the DNA damage signalling pathway [46]. This could conceivably explain the increased SMC senescence found in this population. T2DM SMC also tended towards increased expression of contractile SMC marker protein such as α-SMA [48], however as contractile markers also increase with chronological age in SV grafts [20], this is unlikely to be useful as a marker specific for EVA. The influence of microRNAs (miRs) on SMC function has received much attention. One of these, miR-145, is a master regulator of SMC phenotype [49]. It was elevated in T2DM SV SMC [48] and was responsible for many of the changes in DNA damage signalling [46]. However, expression of miR-145 was unchanged in arterial SMC from T2DM patients, with their phenotype reportedly being more influenced by increased levels of miR-126 [50]. Whether miR-126 expression is more consistent across different source vessels in T2DM remains to be seen.

T2DM blood vessels become stiffer over time, which again is accelerated from their chronological age. Stiffening can be caused by a multitude of factors; changes in the proportions of extracellular matrix components such as elastin and collagen, increased contractility, or SMC differentiation into an osteogenic, calcified phenotype. Ion channel expression and function is a critical modulator of SMC phenotype and tone. For example, L-type Ca^{2+} channel activity is enhanced in ageing SMC [17], yet in rodent T2DM SMC L-type Ca^{2+} channel activity is reduced and replaced with increased store-operated Ca^{2+} (SOC) channels *via* induction of Orai1. This caused increased osteopontin (OPN) expression, acquisition of an osteogenic phenotype and a subsequent increase in vascular stiffness [51]. Indeed, enhanced T2DM SMC calcification appears consistent across human [52] and rodent [53] vascular sources, controlled by increased expression of Runx2.

4.2. SMC phenotype in AAA EVA

During AAA the lumen of the abdominal aorta becomes enlarged and the aortic wall dilates, making it prone to catastrophic rupture. EVA is a classical hallmark of patients with AAA. Patients with overt AAA are offered open repair when the risk of rupture outweighs the risk of surgery. There is an AAA screening programme in place in the UK to identify patients who may benefit from intervention as AAA rupture is associated with 90% mortality. During open repair, end-stage AAA tissue can be removed and the phenotype of SMC examined.

Studies on AAA SMC from end-stage human tissue have revealed a loss of contractile function [54] and shared several features in common with T2DM SMC, namely increased spread cell area, disorganised cytoskeleton, reduced proliferative capacity, increased proportion of senescent cells, elevated markers of DNA damage (γH2AX-positive nuclei with an aberrant shape, increased p21 and p53 expression) and increased expression of miR-145 [40,55,56]. Importantly, the expression levels of miR-145 in AAA SMC were not correlated with the patients age [40] and, given the commonality with T2DM SMC phenotype, is more likely related to premature EVA. It is interesting to note that this observed induction of miR-145 was only present in human AAA SMC, and was not evident in an *ex vivo* porcine model of AAA development either early or late in the disease process [40].

Together with medial SMC loss, inflammation is a hallmark of AAA. Recent murine and human studies have demonstrated that aneurysm size is inversely proportional to nicotinamide phosphoribosyltransferase 1 (NAMPT1) expression. Restoration of NAMPT1 ameliorates AAA-associated inflammation and SMC apoptosis [57]. Given that NAMPT is also reduced in diabetes (though not specifically in SMC) and is tightly woven within the reactive oxygen species (ROS) and SIRT signalling cascades [58], this could be a potential marker for EVA.

AAA SMC had a reduced secretion of MMP-2 compared to non-aneurysmal SMC [55] which is in contrast to that described in

chronological ageing in Section 2. Nonetheless, there are some features of AAA SMC that match with chronological ageing. During the early stages of aneurysm development, it is well-recognised that SMC adopt a hyper-proliferative synthetic phenotype which contributes to dilatation of the vessel wall, before becoming more senescent and lowly-proliferative as the disease progresses. This is reminiscent of the serial passaging method of studying chronological vascular SMC age [6] and has been captured by Tao et al. in human *ex vivo* AAA tissue [56]. It is therefore unsurprising that both chronological ageing and AAA cause reduced SIRT-1 expression [40,56]. Very recently, this has been discovered to be due to enhanced expression of miR-199a-5p in AAA patients [56], however the correlation of miR-199a-5p with chronological ageing remains to be seen.

4.3. SMC phenotype in essential hypertensive EVA

Hypertension is one of the most common age-associated cardiovascular disorders, but hypertension itself can also accelerate the ageing process and cause EVA [41]. It is characterised by dysfunction in the renin-angiotensin-aldosterone system and increased sympathetic nervous system activity, leading to elevated peripheral resistance and high blood pressure. The regulation of SMC phenotype in essential hypertension is complex and has recently been thoroughly reviewed [59]. Comparably fewer studies have been carried out on human tissue or primary cells in essential hypertension, when compared to T2DM or AAA. The impact of hypertension on SMC phenotype can also seem counter-intuitive – whilst one might expect hypertensive SMC to possess an exclusively hyper-contractile phenotype, there is a large body of evidence demonstrating that in fact hypertensive SMC are hyper-synthetic; causing fibrosis and deposition of ECM that causes stiffening of the vascular wall. The increased proliferation inherent to hyper-synthetic SMC will conceivably lead to EVA.

In direct contrast to findings in patient-derived T2DM and AAA SMC, miR-145 expression is reduced in SMC isolated from spontaneously hypertensive Wistar rats [60]. This reduction leads to a cascade of cellular effects that promote the synthetic SMC phenotype including activation of the renin-angiotensin system (which can increase basal Ca^{2+} levels through activation of transient receptor potential canonical 3 (TRPC3) channels), increased expression of mitogenic platelet-derived growth factor (PDGF) to enhance proliferation, and concomitant increases in pro-fibrotic TGFβ [60,61]. However, similarly to T2DM and AAA SMC, hypertension induced by chronic angiotensin II administration is associated with increased expression of p53, p21 and consequent senescence [62,63]. Recent studies identified increased levels of Lethal (3) malignant brain tumor-like protein 4 (L3MBTL4; a protein involved in epigenetically-regulated gene transcription in which polymorphisms predispose to hypertension) as causing persistent activation of the p38 and c-Jun N-terminal kinase (JNK) stress pathways leading to SMC proliferation, migration and acquisition of the synthetic phenotype [64].

It is clear that there is no classical EVA SMC phenotype that can encompass dysfunction caused by multiple pathologies and so a more nuanced approach to defining one is needed. A summary of the molecular signatures in all three examples of EVA is given in Fig. 3.

5. Moving towards detecting, halting or reversing vascular age

EVA is readily detected in the clinic using pulse wave velocity, so one could question why we need to find a molecular signature. The answer is simple – timing. Reversing well-established pathological processes is harder and more complex than halting them at an earlier stage to prevent their progression. Identifying molecular signatures of EVA that precede overt clinical presentation opens up the possibility of screening patients and intervening before these individuals continue down a path that will escalate to chronic disease.

5.1. Identifying the molecular signature of EVA

The characteristics of EVA-specific therapeutic targets need to satisfy a number of criteria:

1. Targets are altered in EVA but not affected by chronological ageing (or are affected in a different way)
2. Targets are present in more than one example of EVA
3. Targets are altered in human vascular tissue (regardless of whether these changes are mirrored in animal models of vascular conditions)

Given these criteria, to date only a few molecular markers can be identified. Interestingly these are all related to the DNA damage pathway and senescence including elevations in p21 and p53 in addition to a higher frequency of aberrant, non-ovoid nuclear morphology. The presence of these, in addition to traditional chronological age markers (for example, reduced LMNB1 and SIRT-1), are likely to be indicative of EVA. Much more work needs to be done to determine a comprehensive, unique molecular signature that defines EVA which is distinct from senescent or chronologically aged cells.

5.2. Detecting the molecular signature of EVA in the clinic

One of the major challenges in identifying a molecular signature of EVA in SMC is accessing SMC for testing. Whilst many of the papers cited in this report have examined human cells, these are invariably isolated from end stage tissue collected during surgical intervention, cadaveric donations, or derived from iPSCs (Table 1). There would have to be a compelling reason to biopsy any major blood vessel, and the potential identification of EVA would likely not reach that threshold. Minimally invasive procedures must be developed instead.

One such method could be using blood samples, as these are often taken during routine health checks and could easily be applied to EVA screening. However, whilst this may provide circulating biomarkers or profiles that could be indicative of EVA, it would be impossible to tell if they were representative of what was happening specifically in SMC. Any number of vascular cells – endothelial, smooth muscle, white blood cells *etc.* – can contribute to circulating biomarkers and any related to SMC function may be drowned out in the noise of other vascular contributors.

An alternative would be to identify blood vessels that can be accessed superficially, and that retain the molecular signature of EVA. Skin is a highly vascularised organ [65] which can be sampled through a minimally invasive punch biopsy. If an EVA signature can be defined in dermal blood vessels, then it could be tested by either immunohistochemistry or explant of resident SMC before it was clinically visible by having functional effects on pulse wave velocity.

5.3. Avenues for future therapeutic investigation

Clinical 'reprogramming' of SMC to revert to a younger, more differentiated phenotype has been suggested as a potential therapeutic strategy. This would presumably be easier to conduct early on in the ageing (or EVA) process which further highlights the importance of identifying an EVA-specific molecular signature. Recent studies have demonstrated that reprogramming SMC to adopt a mesenchymal phenotype can recapitulate all the features of AAA development [66]. Theoretically then it would be possible to do the reverse and reprogramme EVA SMC into a differentiated contractile phenotype but the practicalities of doing this, particularly in humans, are extensive. Furthermore, reprogramming would have to drive stem cell populations into SMC as reprogrammed somatic cells from HGPS models retain age-related phenotypes [67]. Altogether, this raises questions about the clinical potential of reprogramming. Likewise, recent discoveries in autophagy-related ageing (and, potentially, EVA) have led to the suggestion of autophagy induction as a therapeutic strategy but the non-

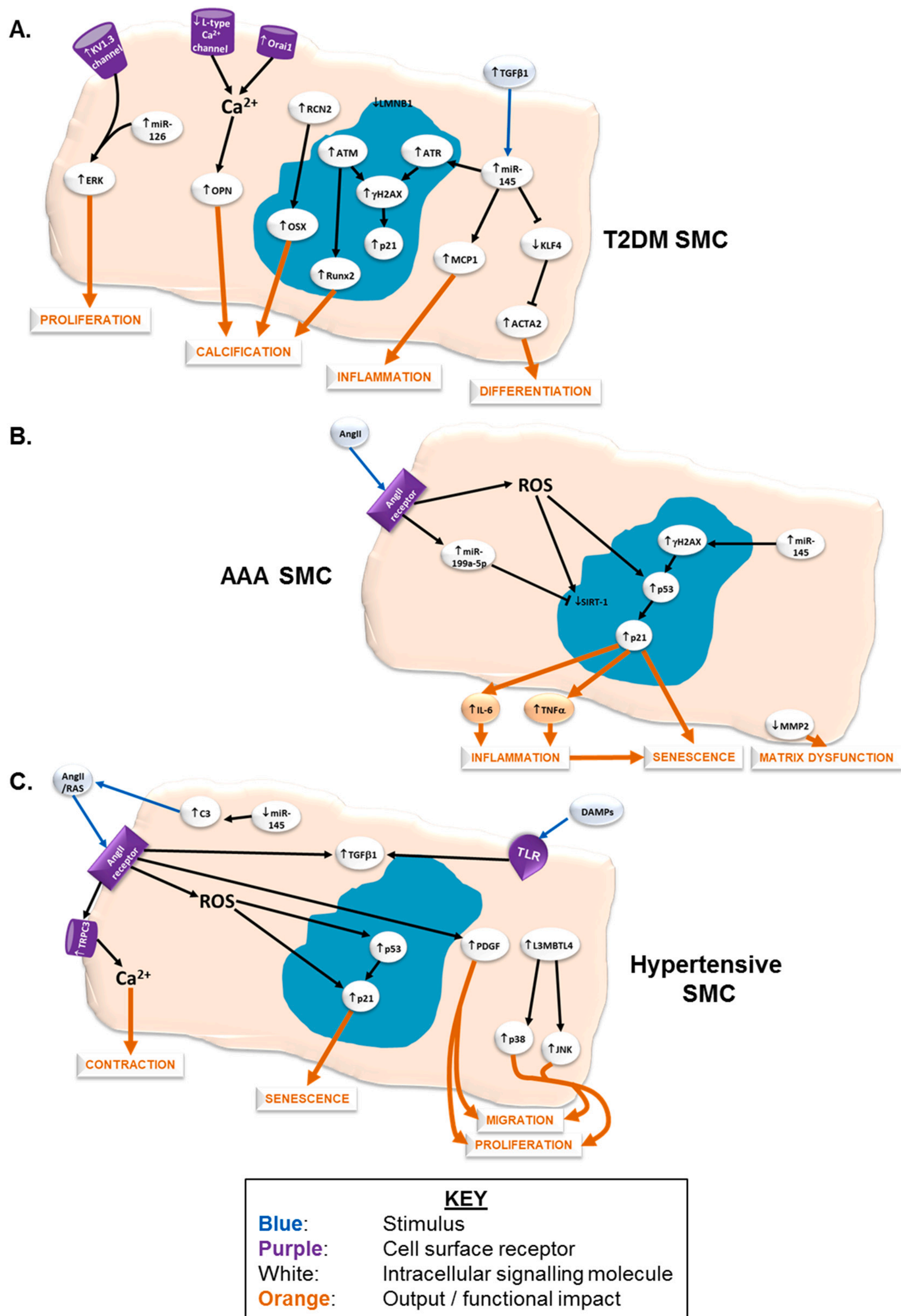

Fig. 3. Smooth muscle cells undergoing EVA. Many cardiovascular pathologies can be characterised with EVA, including (**A**) the vascular complications of T2DM, (**B**) abdominal aortic aneurysms and (**C**) essential hypertension. Circulating factors including AngII, DAMPs and TGFβ1 promote the acquisition of dedifferentiated, calcified and hypercontractile phenotypes that contribute to disease progression. Abbreviations: AAA, abdominal aortic aneurysm; ACTA2, alpha smooth muscle actin; AngII, angiotensin 2; ATM, ataxia-telangiectasia mutated; ATR, ataxia-telangiectasia and Rad3-related; C3, complement 3; DAMP, damage associated molecular patterns; ERK, extracellular signal regulated kinase; IL, interleukin; JNK, c-Jun N-terminal kinase; KLF4, Kruppel-like factor 4; KV, voltage-gated potassium channel; L3MBTL4, Lethal(3) malignant brain tumor-like protein 4; LMNB1, lamin B1; MCP1, monocyte chemoattractant protein 1; miR, microRNA; MMP, matrix metalloproteinase; NAMPT, nicotinamide phosphoribosyl-transferase 1; Nrf2, Nuclear factor erythroid 2-related factor 2; OPN, osteopontin; OSX, osterix; PDGF, platelet-derived growth factor; RAS, renin-angiotensin system; RCN2, reticulocalbin 2; ROS, reactive oxygen species; Runx2, runt-related transcription factor 2; SIRT-1, sirtuin-1; SMC, smooth muscle cell; T2DM, type 2 diabetes mellitus; TGFβ1, transforming growth factor beta 1; TLR, toll-like receptor; TNF-α, tumor necrosis factor alpha; TRPC, transient receptor potential channel.

Table 1
SMC models for studying disorders presenting with EVA.

Model	SMC characteristics	References
AngII-stimulated murine SMC	↑ DNA damage ↑ Senescence	[62]
AngII-stimulated rodent SMC	Enlarged morphology ↑ DNA damage ↑ ROS ↑ Senescence	[63,71]
High glucose-stimulated primary human aortic SMC	↑ Calcification ↓ Contractile markers	[52]
iPSC-derived human HGPS SMC	↓ Adhesion ↑ Apoptosis ↑ Calcification ↓ Contractility ↑ DNA damage ↑ MMP-13 ↓ Proliferative capacity ↑ Senescence	[29,67,76,77]
Primary human AAA SMC	Enlarged morphology Nuclear defects ↓ Contractility ↑ DNA damage ↓ MMP-2 secretion ↑ ROS ↑ Senescence	[40,54–56]
Primary human HGPS SMC	Nuclear defects ↑ DNA damage ↑ Senescence	[28]
Primary human mammary artery T2DM SMC	↑ Migration ↑ Proliferation	[50]
Primary human saphenous vein T2DM SMC	Enlarged morphology Nuclear defects ↑ DNA damage ↓ Proliferative capacity	[45–48]
Primary insulin resistant ob/ob murine SMC	↑ Calcification ↑ Osteogenesis	[53]
Primary spontaneously hypertensive rat SMC	↑ Osteogenesis ↑ Proliferation	[60]
Primary Zucker diabetic fatty rat SMC	Ca^{2+} handling defects ↓ Contractility ↑ Osteogenesis	[51]

Studies of EVA specifically on SMC biology are restricted by the lack of dedicated human or animal models. Consequently, many studies utilise *in vitro* methods from human or animal donors with a particular EVA-related pathology, or examine tissue-level changes in transgenic animals with mutations in *LMNA* to mimic HGPS [78].

specificity of clinical agents and potential side effects may limit their development [68].

Given the growing body of literature on the topic, it is inevitable that epigenetic changes in SMC across the life-course must contribute to EVA. The disorders discussed in this manuscript (T2DM, AAA, essential hypertension) are all associated with increasing age as well as EVA, with the two acting cumulatively to significantly enhance patient risks of mortality and morbidity. The deregulation of epigenetic signalling molecules (*e.g.* SIRTs) has recently been reviewed [69], and the discovery of EVA-associated microRNAs holds therapeutic potential. For example, miR-145 is up-regulated in T2DM and AAA human tissue. This could be a compensatory mechanism – antioxidant Nuclear factor erythroid 2-related factor 2 (Nrf2) is reduced in EVA [70,71] but can be rescued by miR-145 expression in cardiomyocytes [72]. This microRNA could be targeted therapeutically, however one concern is the fine balance between what is a 'normal' expression range, and what flips into the putative 'EVA' range. If the scientific and medical community can come to a consensus on what a reliable cut-off for expression was (either for miR-145 or any other microRNAs that appear to be differentially regulated in EVA), then therapies could be designed based on anti-miR or pre-miRs respectively. Importantly, this technology already exists and so could be progressed to clinical trials in an expedient manner [73].

Whilst SMC are the principal cell type in the vascular wall, they are not in constant contact with the circulation. Treatments to alter SMC epigenetic marks can traverse the endothelial barrier *in vivo* [74] but this may not be the case for all therapeutic options. Given the intimate relationship between endothelial cells and SMC, an alternative approach could be to administer drugs that act on endothelial cells to produce anti-EVA effects in SMC through, for example, microvesicles [75]. Research into this area is scant but it may be a viable avenue for treating EVA systemically throughout the body, rather than individual isolated vessels during surgical interventions.

6. Conclusion

Is all EVA the same? It is highly unlikely. One of the major challenges in identifying markers of a condition that exists on a spectrum across different disorders is the difficulty in recognising subtle alterations in expression profiles in patients individually, rather than as part of a cohort. Furthermore, differing disease endotypes where the same disorder can present differently between patients may cause a challenge, particularly given the plasticity of SMC. This is why it is important that the EVA signature encompasses a range of different markers rather than simply relying on one. It is probable that we need even greater, more subtle subdivisions of EVA. For example if a patient has increased x, decreased y and no z then they are likely to have a type of EVA related to T2DM (or hypertension *etc.*). Whilst much more research is needed to conclusively identify these molecular signatures, the inception of the YVC to create benchmarking characteristics and the continued progress in miR-based therapies mean that targeting EVA SMC to improve vascular health is an exciting and realistic prospect.

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Declaration of competing interest

The authors declare that they have no known competing financial interests of personal relationships that could have appeared to influence the work reported in this paper.

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