

REVIEW

TRAIL signals, extracellular matrix and vessel remodelling

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Abstract

The extracellular matrix (ECM) is an essential part of the vasculature, not only providing structural support to the blood vessel wall, but also in its ability to interact with cells to regulate cell phenotype and function including proliferation, migration, differentiation and death – processes important in vascular remodelling. Increasing evidence implicates TNF-related apoptosis-inducing ligand (TRAIL) signalling in the modulation of vascular cell function and remodelling under normal and pathological conditions such as in atherosclerosis. TRAIL can also stimulate synthesis of multiple ECM components within blood vessels. This review explores the relationship between TRAIL signals, the ECM, and its implications in vessel remodelling in cardiovascular disease.

Key Words

- ▶ vascular remodelling
- ▶ extracellular matrix
- ▶ TNF-related apoptosis-inducing ligand

Introduction

Blood vessels, consisting of arteries, arterioles, capillaries, venules and veins, are channels with intricate networks that supply blood around the body and remove waste. Most blood vessels are composed of three layers (Fig. 1). The interior layer, called the tunica intima, consists of a monolayer of endothelial cells supported by the sub-endothelial space of connective tissue. This layer is always exposed to the blood and forms the first layer of defence. The tunica media is the second layer, partitioned from the intima by the internal elastic lamina, and consists predominantly of vascular smooth muscle cells (VSMCs), elastic and connective tissues, arranged in a circular fashion. The VSMCs can constrict and dilate, playing a key role in blood pressure control. The third (outer) layer of the blood vessel, the tunica adventitia, is comprised of fibroblasts and connective tissue fibres surrounded by the external elastic lamina. On the other hand, the smallest of blood vessels, the capillaries, consist of a thin endothelial cell tube, surrounded by pericytes, a mural cell, similar to VSMCs.

A major component of the blood vessel wall is the extracellular matrix (ECM). In most vessels, it is synthesised by endothelial cells, VSMCs and fibroblasts and found within the space between the tunica layers and cells, contributing to more than half of the vessel wall mass (1). In capillaries, a thin layer of ECM (or basement membrane) is located between the endothelial tubes and pericytes. ECM composition varies in each vessel type, as it is essential not only for the structural support of blood vessels, but also for the regulation of cell processes including proliferation, migration, differentiation, survival, adhesion, contractility, polarity and phenotype, as well as matrix protein synthesis, assembly and degradation (2). These processes are essential in vascular remodelling where structural changes to the blood vessel wall are necessary during normal physiology, aging, injury, and disease. Cells act in response to the ECM and remodel, but this process becomes impaired in vascular diseases such as atherosclerosis (3, 4).

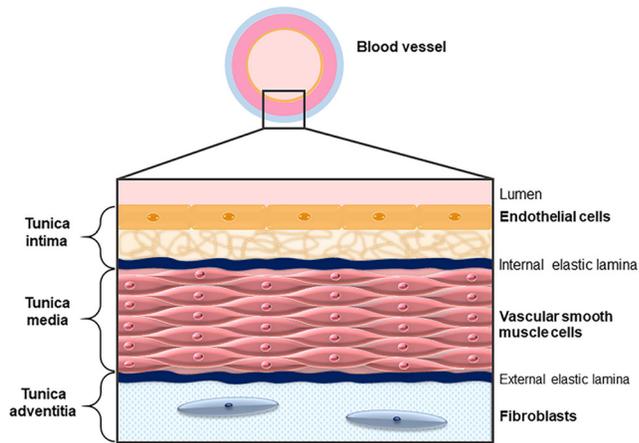


Figure 1
Structure of the blood vessel wall. The vessel wall is comprised of three layers: (1) the tunica intima, consisting of endothelial cells within the subendothelial space, (2) the tunica media, primarily composed of vascular smooth muscle cells with extracellular matrix proteins, and (3) the tunica adventitia, where fibroblasts and connective tissues reside.

TNF-related apoptosis-inducing ligand (TRAIL) was discovered for its unique ability to selectively kill cancer cells (5, 6). In the vasculature, TRAIL has a multifunctional role. In addition to cell death, TRAIL can contribute to cell survival, proliferation, migration and differentiation (7, 8, 9) and in doing so modulate vascular remodelling in cardiovascular diseases (CVDs). Furthermore, in many vascular cells, TRAIL has been linked to regulating components of the ECM. This review summarises our current understanding of the contribution of TRAIL signals to the ECM and vascular remodelling in disease. A better understanding of ECM synthesis, composition, function and alterations to blood vessel remodelling in CVD could offer new strategies for treatment.

ECM components, structure and function

The ECM is a highly organised support network primed to provide cell anchorage and define tissue architecture. Each matrix protein possesses specific properties that define structural, mechanical and chemical characteristics of the ECM important for elasticity, organisation, blood vessel function and vascular cell activity (2). The major ECM proteins involved in these processes are glycosaminoglycans (GAGs), collagens, elastin, fibronectins, integrins, growth factors and matrix metalloproteases (MMPs) (Fig. 2).

GAGs are the major component of the ECM ground substance, a gel-like substance in the extracellular space, arranged as unbranched chains of repeating units of disaccharides (10). GAG chains covalently link to protein

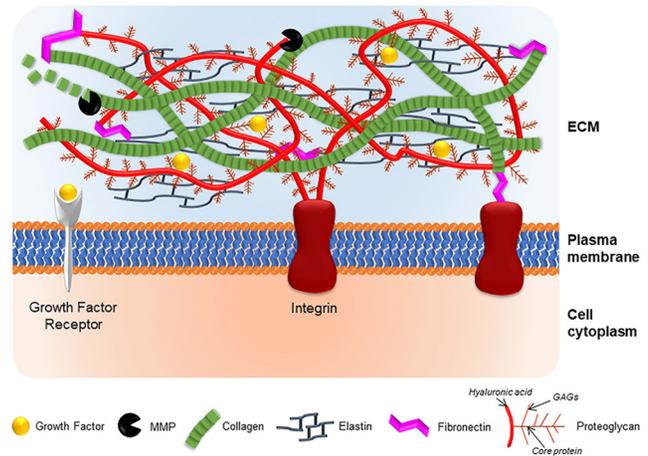


Figure 2
The ECM structure and its components. The ECM is a complex environment comprised of multiple proteins including collagens, fibronectin, proteoglycans, integrins, growth factors and metalloproteinases (MMPs). The ECM regulates a plethora of functions to maintain vessel structure and homeostasis.

core molecules to produce proteoglycans found in all layers of the vessel wall. Among many molecules, hyaluronic acid polymers form complexes with proteoglycans. Because these polymers can be large, they can displace large volumes of water, thus one function of proteoglycans is to maintain tissue hydration (11). Examples of GAGs found within the vasculature include hyaluronan and heparan sulphate; proteoglycans include syndecan, versican and aggrecan (12, 13, 14). Proteoglycans regulate movement of molecules in and out of the matrix, as well as signal transduction (15). As such, they have a unique ability to interact with other ECM proteins including integrins, growth factors, chemokines and cytokines to modulate cellular processes (reviewed in 16).

Collagen is the most abundant matrix protein with 28 family members identified to date (17); 13 of these are found in the vascular wall, synthesised by endothelial cells, VSMCs and fibroblasts (18). They have a triple stranded helical structure arranged into fibrils which provides tensile strength (19). For example, type 1 and 3 (the most abundant collagens in the vasculature) provide strength in the media and adventitia (20), while type 7 is important for the maintenance of vessel structure (21). Collagens also interact with vascular cells to influence cellular processes and ECM regulation. For example, VSMCs interact with collagen 1, and this interaction stimulates VSMC growth and migration, as well as the production of matrix degrading enzymes, MMP2 and 9 (22). Additionally, collagen maintains the ECM by binding to other ECM proteins such as fibronectin to regulate

collagen assembly, which in turn stabilises fibronectin structure during mechanical stretching (23).

Elastin is another ECM protein synthesised by vascular cells. Its precursor, tropoelastin, is secreted as a highly soluble monomer, but following post-translational modification, tropoelastin subunits are crosslinked into elastin fibres, becoming insoluble and giving elastin its durable property (24). As such, elastin is essential for the maintenance of elasticity and structure of the vessel wall. Elastin can also regulate cell behaviour. For example, elastin-derived peptides can activate the mitogen-activated protein kinase (MAPK) pathway in VSMCs to stimulate proliferation (25). Further, endothelial nitric oxide synthase (eNOS) produced by endothelial cells can be activated by tropoelastin, leading to the subsequent release of nitric oxide (NO), a molecule important for its vascular protective functions (26).

In order to interact within their microenvironment and modulate behaviour, differentiation, metabolism and survival, cells are required to interact with the ECM. They do this primarily via integrins, which are the largest family of adhesion receptors on cells, binding collagens, laminin and fibronectin in the vessel wall. By binding ECM proteins, integrins orchestrate bi-directional signals, outside-in and inside-out, to regulate endothelial cell and VSMC behaviour during vessel remodelling such as in angiogenesis (27). For example, endothelial cells interact with collagen type 1 via integrins to form new capillaries (28), and integrins on VSMCs interact with collagen in the media promoting survival and growth signals (27) as well as adhesion to the basement membrane during vessel maturation (29).

Fibronectin is a large secreted dimeric protein with multiple isoforms. In the vasculature, it is expressed on the surface of cells and becomes part of the ECM as fibronectin fibrils, a process which requires activation via binding of integrin receptors. Together with other fibres, matrix proteins and integrins, fibronectin forms a meshwork important for signalling (1). It can also be released into plasma upon induction of injury to support thrombus formation (30). The significance of fibronectin in the vessel wall was demonstrated when a fibronectin inhibitor reduced cellular proliferation, VSMC ECM secretion and differentiation, vascular remodelling, inflammatory responses and ECM accumulation following injury (31). One example of this is fibronectin's ability to regulate the production of collagen 1 fibres (32).

The ECM also consists of matrix proteases known as MMPs which are enzymes that degrade matrix proteins to

release growth factors and cytokines and generate bioactive fragments capable of remodelling the vessel (2). MMP1, 2 and 9 are the most common MMPs in the vessel wall and predominantly synthesised by endothelial cells, VSMCs and fibroblasts (33). MMP production is controlled by cells via ECM-cell interactions and counterbalanced by inhibitors of MMPs, called tissue inhibitor of metalloproteinases (TIMPs), an essential mechanism required to maintain homeostasis. The ECM is also a storage reservoir for many growth factors, derived from all cells within the vasculature, including vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF2), platelet-derived growth factor (PDGF), insulin-like growth factor receptor-1 (IGF1R) and transforming-growth factor- β 1 (TGF β 1). Many of these growth factors are necessary for physiological remodelling of blood vessels, for example, in angiogenesis and blood pressure control; however, they can also act as potent mitogens and chemoattractants, contributing to abnormal cell processes (proliferation and migration) in states of pathological vascular remodelling, such as intimal thickening and atherogenesis.

TRAIL signals and the ECM

TRAIL

TRAIL is expressed on most cells as a transmembrane-bound protein which can be cleaved by proteases to release a soluble form. Both forms are bioactive (5). In humans, TRAIL signalling is the most complex of all TNF members because it can bind five different receptors. Although multimers have been identified (5, 34, 35), TRAIL's most common form is a homotrimer that binds and activates its signalling receptors, death receptor-4 and -5 (DR4 and DR5) (36, 37). Upon binding these, TRAIL can stimulate apoptosis by activating the caspase cascade or activating signalling pathways such as phosphoinositide 3-kinase (PI3K), MAPKs including extracellular signal-regulated kinase (ERK), Jun N-terminal kinase (JNK) and p38, as well as the transcription factor NF κ B, for pro-survival signals (37, 38, 39, 40, 41). Additional receptors include decoy receptors-1, -2 (DcR1, DcR2), and osteoprotegerin (OPG), the only known secreted receptor for TRAIL, which regulates osteoclastogenesis by binding receptor activator of NF κ B ligand (RANKL). It is suggested that these decoy receptors can compete with DR4 and DR5 for ligand binding and protect cells from apoptosis. However, the function(s) of TRAIL receptors in the vasculature are not fully defined.

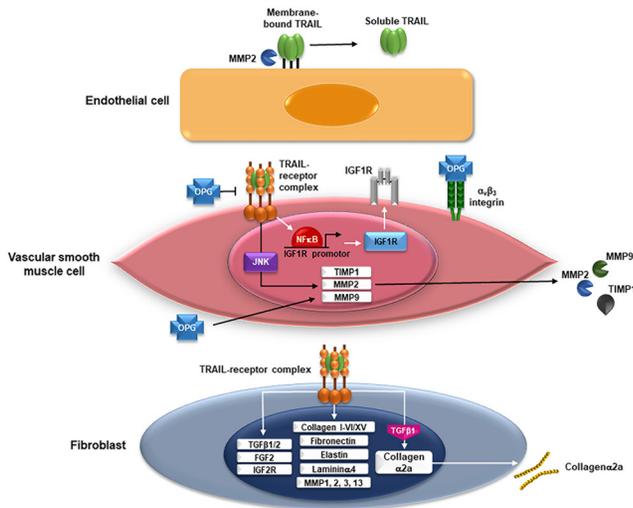


Figure 3 TRAIL signals and ECM components in vascular cells. *Top*, in endothelial cells, MMP2 can cleave membrane-bound TRAIL to produce a soluble form. *Middle*, exogenous TRAIL exposure to vascular smooth muscle cells stimulates IGF1R mRNA and protein expression via NFκB. TRAIL can stimulate MMP2, MMP9 and TIMP1 expression and secretion via JNK, whereas OPG inhibits TRAIL-induced MMP expression. In contrast, OPG exposure alone increases MMP protein expression in VSMCs. In response to hypoxia, OPG and $\alpha_v\beta_3$ integrin associate. *Bottom*, exogenous TRAIL exposure to fibroblasts can increase mRNA expression of ECM proteins: MMP1, 3, 11 and 13; growth factors including TGFβ1, FGF2 and IGF2R; structural proteins including collagens 1, 6 and 15, as well as fibronectin, elastin and lamininα4. TRAIL-inducible expression and secretion of collagenα2a occurs via TGFβ1.

TRAIL and ECM components

Although the effect of TRAIL on ECM components and function is not established, a link between TRAIL and collagen, MMP and growth factor synthesis has been described (Fig. 3). Yurovsky *et al.* showed that low concentrations of human recombinant TRAIL at ~1 ng/mL for 24 h significantly increased collagenα2a mRNA and protein secretion from human fibroblasts, without affecting cell viability (42). Using DNA microarray hybridisation, the same group identified multiple genes important in tissue remodelling to be altered by TRAIL including collagens 1-6, collagen 15, fibronectin, elastin, lamininα4, growth factors (TGFβ1/2, connective tissue growth factor) and glycoproteins (thrombospondin 1/2), whereas collagen 11 and the proteoglycan decorin levels were reduced (42). To demonstrate functionality of their findings, the authors focussed on TGFβ1 signalling. They found that treatment of fibroblasts with a neutralising antibody to TGFβ1 inhibited TRAIL-mediated collagenα2a gene expression and total collagen secretion, implying a TRAIL-TGFβ1-collagen axis (42). While fibroblasts express all TRAIL receptors (43, 44), it is unclear whether the TRAIL-TGFβ1-collagen

axis involves a specific receptor. These suggest that TRAIL may contribute to the regulation of tensile strength in the blood vessel wall, in part, by regulating synthesis of collagen.

MMP2 can cleave membrane-bound TRAIL to a soluble form in endothelial cells *in vitro* (45); however, the extent of this occurring in other vascular cells or *in vivo* is unclear. It is also unclear as to whether MMPs can modulate soluble and matrix-bound TRAIL in cells or tissues to influence remodelling events. Interestingly, in fibroblasts, TRAIL can stimulate the expression of MMP1-3 and 13 (42), in cancer cells by increasing MMP7 and 9 (46, 47) and in VSMCs by increasing the expression of *Mmp2*, *Mmp9* and *Timp1* via JNK signals (48). In the latter, TRAIL-induced *Mmp9* and *Timp1* expression was inhibited by treatment with recombinant human OPG, suggesting that these effects may require TRAIL-OPG signalling. Indeed, treatment of RAW264.1 cells or VSMCs isolated from *ApoE*^{-/-} mice with OPG at 4 nM increased MMP9 protein expression, as well as MMP9 activity in *ApoE*^{-/-} bone marrow-derived macrophages (49). Importantly, *Opg*^{-/-}*ApoE*^{-/-} mice had increased collagen, proteoglycan and laminated elastin content in their atherosclerotic lesions (49). Interestingly, OPG can bind GAGs and proteoglycans in non-vascular cells (50); however, the extent and functional consequences of these in the vasculature is unclear. In contrast, OPGs ability to bind $\alpha_v\beta_3$ integrin in VSMCs can stimulate proliferation of these cells and contribute to disease (51). Collectively, these findings suggest that TRAIL signals may regulate ECM to control cell processes necessary for vascular remodelling.

Another ECM component that TRAIL is known to regulate is the IGF1R, a potent anti-apoptotic growth factor receptor. In VSMCs, exogenous TRAIL treatment stimulated *IGF1R* gene expression, whereas serum-inducible IGF1R protein expression was blocked with a neutralising antibody to TRAIL (52). A novel NFκB site (-325/-315) was identified on the human IGF1R promoter responsible for TRAIL-induced IGF1R expression, since a seven-fold induction of IGF1R promoter activity was abolished when this element was mutated and NFκB binding ability inhibited (52). Interestingly, TRAIL's ability to regulate growth factor expression may be a common theme, since it can also increase *IGF2R*, *TGFβ1/2* and *FGF2* mRNA and/or protein expression in vascular cells (42, 53). Collectively, these demonstrate that TRAIL can regulate the expression of several ECM proteins and may contribute to ECM function in the blood vessel wall. Further studies are needed to delineate whether

TRAIL signals can modulate the expression, synthesis or function of other ECM molecules such as GAGs, elastin, fibronectins and integrins.

TRAIL signals and vascular remodelling

Vascular remodelling occurs under normal physiology and during pathological disease. TRAIL-dependent processes in vascular remodelling under these conditions are summarised in Fig. 4A and B and described in greater detail subsequently.

Angiogenesis

Under normal physiology, endothelial cells contribute to vascular remodelling by releasing or activating factors to stimulate cell proliferation, migration and death,

contributing to the synthesis and composition of the ECM. The endothelium plays a crucial role in maintaining barrier homeostasis, controlling exchanges between the blood and the vessel wall, controlling adherence of immune cells, modulating vessel tone, reducing VSMC proliferation and controlling thrombotic activity. Endothelial cells are also essential in angiogenesis. These processes, however, are impaired or dysregulated when the endothelium becomes dysfunctional as observed in CVD. Multiple lines of evidence indicate that TRAIL signalling can stimulate endothelial cell apoptosis, proliferation, migration, and differentiation, as well as regulate cell-cell junctions and influence cell adhesion.

Although Li *et al.* (2003) showed that recombinant human TRAIL used at concentrations up to 80 ng/mL killed human umbilical vein endothelial cells (HUVEC), ~70% of the cultured cells survived TRAIL's cytotoxic actions, suggesting TRAIL's killing effects in these cells may require additional factors. Indeed, a marked increase in

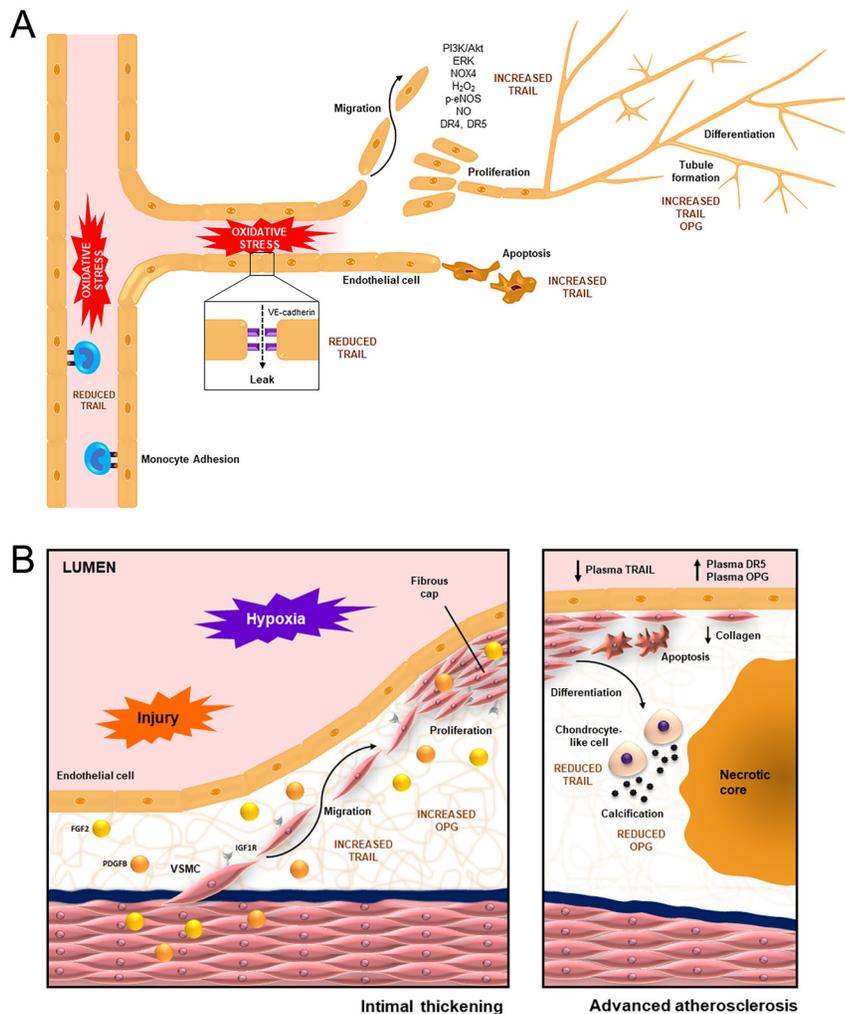


Figure 4

(A) TRAIL-dependent effects on endothelial cell processes during vascular remodelling. TRAIL stimulates endothelial cell proliferation, migration and tubule formation via activating PI3K, ERK and NOX4- and H₂O₂-inducible eNOS and nitric oxide release (NO). TRAIL can stimulate expression of its receptors, DR4 and DR5. Under certain conditions of heightened stress, TRAIL promotes apoptosis of endothelial cells. TRAIL protects endothelial cells from oxidative stress, by improving vascular leak, in part, by controlling VE-cadherin reorganisation on the cell membrane and by reducing monocyte adhesion to the vessel wall. The soluble TRAIL receptor OPG can also stimulate tubule formation in endothelial cells. (B) TRAIL-dependent effects during intimal thickening and atherosclerosis. In response to injury, FGF2 or PDGFB, TRAIL stimulates VSMC migration and proliferation, contributing to atherogenesis and fibrous cap formation in atherosclerotic plaque. TRAIL also stimulates cell migration and proliferation via upregulating IGF1R. Under hypoxic conditions, TRAIL and OPG expression is augmented to modulate VSMC processes in intimal thickening. In advanced atherosclerosis, TRAIL-expressing T cells stimulate VSMC apoptosis reducing collagen content. Deletion of TRAIL accelerates atherosclerosis and stimulates differentiation of VSMCs to chondrocyte-like cells. Deletion of TRAIL accelerates calcification in advanced lesions; a finding also observed with OPG deletion. A common feature in CVD is reduced circulating TRAIL levels while plasma DR5 and OPG are increased in atherosclerosis; the latter associating with disease severity.

apoptosis, up to 80%, was only observed in the presence of TRAIL and the protein synthesis inhibitor cycloheximide (54). Inducible cell death was also observed in tumour endothelial cells *in vitro*; supported by disruption of tumour endothelium in mice treated with recombinant soluble Flag-tagged human TRAIL (55). Interestingly, this group crosslinked their soluble Flag-tagged human TRAIL with an anti-Flag antibody producing higher order oligomers, which enabled stimulation of apoptosis in multiple cancer cells (55). These suggest that, under certain circumstances, TRAIL may promote endothelial cell death in the vessel wall.

We and others have shown TRAIL to stimulate proliferation, migration and differentiation of endothelial cells into tube-like structures (39, 56, 57) – *in vitro* processes of angiogenesis. Human recombinant TRAIL can activate kinases important for cell survival such as Akt and ERK (39, 56), and although endothelial cells express DR4, DR5, Dcr1 and Dcr2 at similar levels (39), their role in activation of these kinases is unclear. Interestingly, 18 h of trophic withdrawal resulted in ~45% apoptosis of HUVEC, which was rescued to ~30% by TRAIL exposure, suggesting that TRAIL may act as a survival factor (39). When the system was overloaded with TNF- α and/or a PI3K inhibitor, TRAIL's protective effects were no longer evident, implying that, in a toxic environment (such as in advanced atherosclerosis), TRAIL's protective effects may become void. TRAIL at 10 and 100 ng/mL can also stimulate phosphorylation and trafficking of eNOS, NOS activity and generation of NO (57, 58, 59) – key factors regulating vessel patency. Importantly, exposure of human endothelial cells to TRAIL receptor-Fc fusion proteins for DR4 and DR5 inhibited apoptosis due to serum deprivation, implicating both DR4 and DR5 in TRAIL's ability to promote survival in these cells (60).

Using a model of peripheral artery disease in mice, we identified a role for TRAIL in ischaemia-induced angiogenesis. In response to hindlimb ischaemia, *Trail*^{-/-} mice had impaired recovery of limb movement and increased limb necrosis, with markedly reduced (~70%) capillary density in ischaemic hindlimbs. Viral TRAIL gene therapy dramatically improved limb perfusion mediated by NOX4- and H₂O₂-inducible eNOS phosphorylation and generation of NO. These effects may be mediated by DR4 or DR5, since ischaemia and viral TRAIL gene therapy increased *Dr5* mRNA expression in WT, but not in *Trail*^{-/-} hindlimbs, and TRAIL was shown to stimulate *Dr4* mRNA expression in human endothelial cells *in vitro* (57). Interestingly, OPG has also been implicated in the

regulation of angiogenesis (61). Using the aortic ring model, the authors showed that human recombinant OPG stimulated angiogenic sprouting, in part, by activating Akt and ERK, which was inhibited by 5 times more TRAIL (61). These findings demonstrate our lack of understanding of complex TRAIL-receptor interactions.

More recently, we showed *Trail*^{-/-} mice had increased vascular leak, and *in vitro*, TRAIL (10 ng/mL) reduced angiotensin II-induced oxidative stress, leukocyte adhesion, and permeability by preventing redistribution of VE-cadherin from the cell membrane, an adherens junction important for endothelial cell integrity (62). Importantly, deletion of TRAIL in atherosclerotic mice resulted in endothelial dysfunction associating with increased oxidative stress and inflammation in the vessel wall (62). TRAIL regulates the production of prostanoids in HUVEC, key molecules controlling vascular tone, inflammation and leakiness, and this supports a role for TRAIL in maintaining endothelial cell function (59). However, some studies report otherwise; TRAIL (10 and 100 ng/mL) had no effect on proliferation, migration or tubule formation using the human brain endothelial cell line, hCMEC/D3 (63). Instead, in these cells, TRAIL stimulated caspase-1 activity and cell death (60, 63). These discrepancies may reflect receptor expression, cell type, origin, or differences in the *in vitro* angiogenic assay methods used. Nevertheless, these findings suggest that TRAIL signals regulate multiple cellular functions in endothelial cells involving the ECM necessary for vascular remodelling.

Intimal thickening

Under normal conditions, VSMCs are quiescent and contractile in phenotype, exhibiting minimal proliferation and migration. However, VSMCs are remarkably plastic in nature, and in response to environmental and mechanical cues, they can change and become 'synthetic', adopting a proliferative form, capable of synthesising ECM (64). Proliferation of VSMCs can contribute to the remodelling of the vessel wall early in lesion development, leading to intimal thickening. We and others have shown that low physiological levels of TRAIL stimulate VSMC proliferation, migration and survival (52, 65, 66, 67, 68). Although all receptors are expressed in human VSMCs, albeit at different levels, we showed that TRAIL-induced human VSMC proliferation was inhibited with neutralising antibodies to DR4 and Dcr1, but not to neutralising antibodies targeting Dcr1, OPG or using Fc-DR5 (52). These suggest that TRAIL's proliferative effects

are mediated by signalling and decoy TRAIL receptors. Interestingly, TRAIL-inducible aortic VSMC migration was blocked by recombinant OPG (48); OPG acting as a true decoy receptor in this setting.

Because growth factors reside in the ECM and modulate cell processes by actively interacting and signalling with other ECM components, there is some evidence linking TRAIL to growth factor signalling to regulate cell proliferation and migration in disease. For example, in people, TRAIL expression was evident in the intima of failed saphenous vein bypass grafts, co-localising with proliferating VSMCs and the IGF1R, but not active caspase-3 (52); pharmacologically blocking IGF1R's actions can inhibit VSMC proliferation *in vitro* and *in vivo* (69). Importantly, TRAIL-induced VSMC proliferation was inhibited with IGF1R antisense oligonucleotides, but not with control oligonucleotides, confirming that TRAIL-induced VSMC proliferation, in part, involved the IGF1R (52) and that this process may contribute to vessel remodelling during intimal thickening and atherogenesis.

PDGFB, is a mitogen and potent chemoattractant involved in the promotion of atherogenesis; inhibition of PDGFB or its receptor PDGFR β reduced intimal thickening and VSMC proliferation following carotid injury compared to control rat (70), whereas increased PDGFR β signalling augmented atherosclerosis in *ApoE*^{-/-} and *Ldlr*^{-/-} mice (71). In addition to the TRAIL-IGF1R axis described previously, PDGFB can regulate TRAIL gene expression and stimulate VSMC proliferation and migration. We showed evidence of PDGFB in modulating chromatin and implicated Sp1-acetylated histone-3-p300 interactions to stimulate TRAIL transcriptional activity and gene expression. Importantly, PDGFB-induced VSMC proliferation and migration was inhibited using siRNA targeting TRAIL, and in support, *Trail*^{-/-} VSMCs displayed impaired PDGFB inducible responses to cell proliferation and migration, revealing a novel role for TRAIL in PDGFB-stimulated processes that modulate vessel remodelling (67).

FGF2 is a potent growth factor, released from VSMCs within minutes in response to injury to the vessel wall (53). Intimal thickening is an early stage of atherosclerosis development and can be induced experimentally using multiple methods including placement of a non-occlusive cuff around the femoral artery. We showed that *Trail*^{-/-} mice were protected from intimal thickening, whereas WT mice developed neointimal hyperplasia 15 days after cuff placement (53). VSMC proliferation and neointimal thickening was dependent on injury- and FGF2-inducible *Trail* expression, mediated by the transcription factor

complex containing Sp1 and NF κ B (53). Interestingly, FGF2 expression was down-regulated in injured femoral arteries of *Trail*^{-/-} mice with reduced neointimal thickening. Collectively, these findings suggest an interplay between FGF2 and TRAIL in the ECM necessary for remodelling following injury to the vessel wall.

Pulmonary hypertension

Like intimal thickening, pulmonary hypertension (PAH) is caused by aberrant proliferation and migration of VSMCs resulting in medial thickening of pulmonary vessels, namely the arterioles. Clinically, PAH is characterised by an elevation in pulmonary artery pressure and right ventricular hypertrophy, leading to right ventricular failure. In 2012, Hameed *et al.* identified TRAIL as an important mediator of this disease (68). The authors showed that TRAIL expression was increased in vascular cells isolated from PAH patients and in the pulmonary vasculature of multiple models of experimental PAH, implying that increased TRAIL expression in pulmonary vessels may cause PAH features. Indeed, anti-TRAIL antibody treatment returned the elevated right ventricular systolic pressure, elevated right ventricular end-diastolic pressure, and reduced pulmonary artery acceleration time, back to normal compared to IgG-treated rats. Notably, the anti-TRAIL antibody prevented pathological remodelling of VSMCs observed in the pulmonary vasculature; findings which were confirmed genetically since *Trail*^{-/-} mice were protected from chronic hypoxia-induced PAH (68). Similarly, in a more severe murine PAH model, sugen-hypoxia, *Trail*^{-/-} mice displayed no echocardiographic or haemodynamic signs with reduced proliferative pulmonary vascular remodelling in the media compared to WT mice (72).

OPG has also been implicated to play a role in PAH since soluble levels are increased in serum of patients compared to control and its expression is elevated in human PAH lung samples, correlating with severity of disease (51, 73). Furthermore, OPG expression was increased in VSMCs *in vivo*, in hypoxia- and sugen-induced PAH (51). Consistent with these, PAH was attenuated in *Opg*^{-/-} mice, whereas OPG reconstitution exacerbated disease, suggesting that OPG may regulate processes that promote PAH pathology. Indeed, OPG stimulated VSMC proliferation and migration, and these processes were mediated via $\alpha_v\beta_3$ integrin, since immunoprecipitation studies revealed the physical association of OPG with integrin $\alpha_v\beta_3$, but not with α_1 and α_8 in hypoxia (51). Furthermore, the authors

identified that OPG-mediated proliferation of VSMCs occurred via activation of $\alpha_v\beta_3$ integrin/FAK/Akt signals, since blocking $\alpha_v\beta_3$ integrin using siRNA technology in the presence of OPG prevented phosphorylation of FAK and Akt, and VSMC proliferation (51). More recently, OPG's pro-proliferative and pro-migratory effects in VSMCs was found to be mediated via another TNF receptor, Fas (74). Here, the authors established that the physical interaction of OPG with Fas was necessary to activate cell proliferation, migration and survival of pig aortic VSMCs, since neutralising Fas inhibited these processes (74). Most significantly, the therapeutic potential of OPG in attenuating established PAH was shown – treatment of mice with a human antibody targeting OPG in multiple models of PAH inhibited pulmonary vascular remodelling and features of PAH (74). Collectively, these findings confirm a role for TRAIL and TRAIL receptors in remodelling of the vasculature during disease.

Atherosclerosis

VSMCs are critical for maintaining the physiological functioning of the vasculature. Not only can they contract and relax, contributing to blood pressure control, but like fibroblasts, they are a major source of collagen synthesis. Studies by our group showed that *Trail*^{-/-}*Apoe*^{-/-} mice had accelerated atherosclerosis, displaying vulnerable plaque with large necrotic cores, thin fibrous caps, and significantly increased macrophage accumulation and apoptosis observed in the cap and shoulder regions of the plaque compared to *Apoe*^{-/-} mice (75). Importantly, lesions of mice lacking TRAIL had significantly reduced VSMC and collagen content, suggesting that in advanced lesions the presence of TRAIL may be necessary for VSMC survival, collagen synthesis, stability, and tensile strength of the vessel wall. Indeed, recombinant TRAIL administration to diabetic *Apoe*^{-/-} mice increased VSMC-positive cell numbers within the plaque cap with a tendency for increased collagen (76). These are significant since plasma TRAIL levels are suppressed in patients with atherosclerosis (77, 78, 79, 80).

TRAIL receptors have also been implicated in atherosclerotic disease. For example, plasma OPG and DR5 levels are increased in stroke patients with large artery atherosclerosis (80) and a significant positive correlation was identified between plasma OPG and severity of coronary artery disease (81). OPG and DR5 are also expressed in human atherosclerotic tissues (82, 83) and predominantly by VSMCs (83, 84). However, in contrast to increased plasma levels and expression in human disease, *Opg*^{-/-}*Apoe*^{-/-} mice

had accelerated atherosclerosis compared to *Apoe*^{-/-} mice at 40 and 60 weeks of age, and unlike *Trail*^{-/-}*Apoe*^{-/-} lesions, atherosclerotic lesions from *Opg*^{-/-}*Apoe*^{-/-} had significantly more collagen deposition, although VSMC content was not measured (49). The role of DR5 in atherosclerotic disease is yet to be established, but adoptively transferred TRAIL-expressing CD4 T cells induced VSMC apoptosis via DR5 in atherosclerotic plaque engrafted into immunodeficient mice (83). How TRAIL-dependent VSMC apoptosis effected plaque stability, collagen content or other ECM components was not examined.

Calcification

Within the ECM, multiple ECM proteins (e.g. collagens and proteoglycans) provide charged regions where calcium phosphate can accumulate (85). Abnormal deposition of calcium phosphate salts or hydroxyapatite in the vascular wall (vascular calcification) not only contributes to vascular stiffness but is a major risk factor for the morbidity and mortality associated with CVDs including atherosclerosis (85). As mentioned earlier, VSMCs are incredibly plastic, and in addition to the synthetic phenotype, they are able maintain a spectrum of phenotypes in the vasculature including becoming adipocytes, foam cells, or osteochondrogenic (bone-forming) cells (86). Previous work by our group showed that atherosclerotic lesions from *Apoe*^{-/-} mice lacking TRAIL had significantly more chondrocyte-like cells, which secrete cartilage matrix, as well as increased calcification in plaque compared to atherosclerotic lesions from *Apoe*^{-/-} mice alone (87). An alizarin red assay was performed to assess calcification *in vitro*; VSMCs isolated from *Trail*^{-/-} or *Trail*^{+/+} mice were grown in osteogenic media, and *Trail*^{-/-} VSMCs developed more calcification compared to *Trail*^{+/+} VSMCs, suggesting that TRAIL inhibits VSMC osteochondrogenic transdifferentiation (87). Indeed, exogenous recombinant TRAIL attenuated calcium-induced calcification of human VSMCs, in part, by inhibiting RANKL (87), an initiator of osteoclastogenesis. In support of these findings, Zauli *et al.* demonstrated that recombinant TRAIL inhibits osteoclastogenesis (88); however, others report the opposite; recombinant TRAIL promoted calcification of human VSMCs (89), and TRAIL was detected in tissues from patients with calcific aortic valvular disease associating with cell death (90). This group did not examine the effect of TRAIL on VSMCs *in vitro*, but they found that exogenous TRAIL treatment of valvular interstitial cells isolated from patients with calcific disease had enhanced calcification

in osteochondrogenic media vs non-calcific cells (90). Furthermore, calcific interstitial cells expressed more DR4, DR5, DcR1 and DcR2 compared to non-calcific cells, whereas only DR4 expression was enhanced in response to osteogenic media (90), suggesting that TRAIL's effects dependent on DR4 may be cell-type specific and reliant on the local micro-environment.

Like DR4, DR5, DcR1, and DcR2 expression in calcified interstitial cells described previously, OPG is also expressed in arteries of aged *ApoE*^{-/-} mice with significantly more chondrocyte-like cells (91), suggesting that OPG expression may associate with calcification. However, OPG deletion in *ApoE*^{-/-} mice accelerated calcification in the vessel wall. In this study, calcium content was measured by Von Kossa staining from brachiocephalic arteries of *Opg*^{-/-}*ApoE*^{-/-} mice and compared to littermate control arteries, showing increased calcification within the media and intima which was age dependent (49). Aortic calcium content was also increased ~12-fold in 60-week-old *Opg*^{-/-}*ApoE*^{-/-} vs *Opg*^{+/+}*ApoE*^{-/-} mice, and interestingly, mice lacking OPG had elevated levels of plasma RANKL, ~4-fold, suggesting that mechanisms controlling bone turnover may also be relevant in vascular calcification (49). These studies show that TRAIL signals play important role(s) in the blood vessel wall; however, further work is warranted to understand the TRAIL-receptor contribution.

Conclusions and future perspectives

The remodelling of the vasculature is a key response in physiology and in pathology. Although there have been significant advances in our understanding of remodelling events in the blood vessel wall, these processes are highly complex, involving multiple cells, the expression of genes, their secreted factors, the ECM and the local microenvironment. TRAIL was identified because of its ability to selectively kill cancer cells and leave normal cells resistant to its cytotoxic actions. The emerging concept that TRAIL through its multiple receptors also regulates vascular cell survival, proliferation, migration, differentiation, permeability, adhesion, ECM expression and synthesis suggests that it plays a key role in the profound changes to the architecture of the blood vessel wall during vascular remodelling. Understanding how TRAIL signals control these responses and interact with the ECM under normal and in disease states would increase knowledge that could offer new and selective therapeutic strategies for adjusting undesirable processes during vascular remodelling.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contributions statement

M P, S C and M K contributed to writing this review.

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