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Expression, regulation and function of trail in atherosclerosis

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ABSTRACT

Atherosclerosis is a condition where vascular smooth muscle cells (VSMCs), inflammatory cells, lipids, cholesterol and cellular waste accumulate in the inner lining of an artery, producing a fibro-fatty plaque and resulting in the thickening of the arterial wall. The tumor necrosis factor (TNF) family of cytokines plays a major role in the progression of atherosclerosis. Recently, TNF-related apoptosis-inducing ligand (TRAIL), a member of the TNF superfamily, has been implicated in the development of atherosclerosis since it has been detected in normal and diseased atherosclerotic tissue. Not only is TRAIL involved in apoptosis and immune regulation, recent studies have provided a new function of TRAIL on vascular cells, such that TRAIL can promote endothelial cell (EC) and VSMCs migration and proliferation. In addition, TRAIL is implicated in regulating vascular tone. This review discusses our current understanding of TRAIL expression, regulation and function, and summarises the recent data implicating a role for TRAIL in atherosclerosis.

1. Atherosclerosis

No longer considered just a degenerative disorder of ageing, atherosclerosis is a highly dynamic chronic arterial disease that leads to the pathogenesis of myocardial infarction, stroke and gangrene. It is the most common cause of mortality in the western world and subsequently remains a burden on health care costs (reviewed in Ref. [1]). Atherosclerotic lesions can occur as early as adolescence and progression of disease relies on genetic and environmental factors [1]. The progression of an atherosclerotic lesion is depicted in Fig. 1. Atherosclerotic lesions usually develop in the arterial intima where vascular smooth muscle cells (VSMCs), inflammatory cells, lipids and connective tissue accumulate. Initiation of lesion progression occurs due to dysfunction of the endothelium and accumulation of low-density lipoprotein (LDL) in the subendothelial matrix. Monocytes and T lymphocytes attach to these lipoproteins and migrate between endothelial cells. This interaction is mediated by adhesion molecules and chemotactic factors released by altered endothelium, adhered leukocytes and VSMCs.

Beneath the arterial surface, foam cells are generated from macrophages through the accumulation of highly oxidised LDL. Together with leukocytes, foam cells produce the fatty streak. Fatty streaks are not clinically significant but can ultimately progress to form more advanced lesions. 'Fibrous' lesions are described by the accumulation of VSMCs and lipid-rich necrotic debris. Proliferation of cells under normal physiological conditions is regulated by growth factors and cytokines, and continued VSMCs proliferation, accumulation of extracellular lipid and VSMCs derived extracellular matrix, give rise to a fibrous plaque. The most important clinical complication of advanced atherosclerotic plaques is plaque rupture, followed by formation of a thrombotic occlusion and either myocardial infarction or sudden death. Atherosclerosis therefore, represents a unique case of inflammatory and immune responses evoked by complex interactions between vascular cells, immune cells and lipids. Due to the highly

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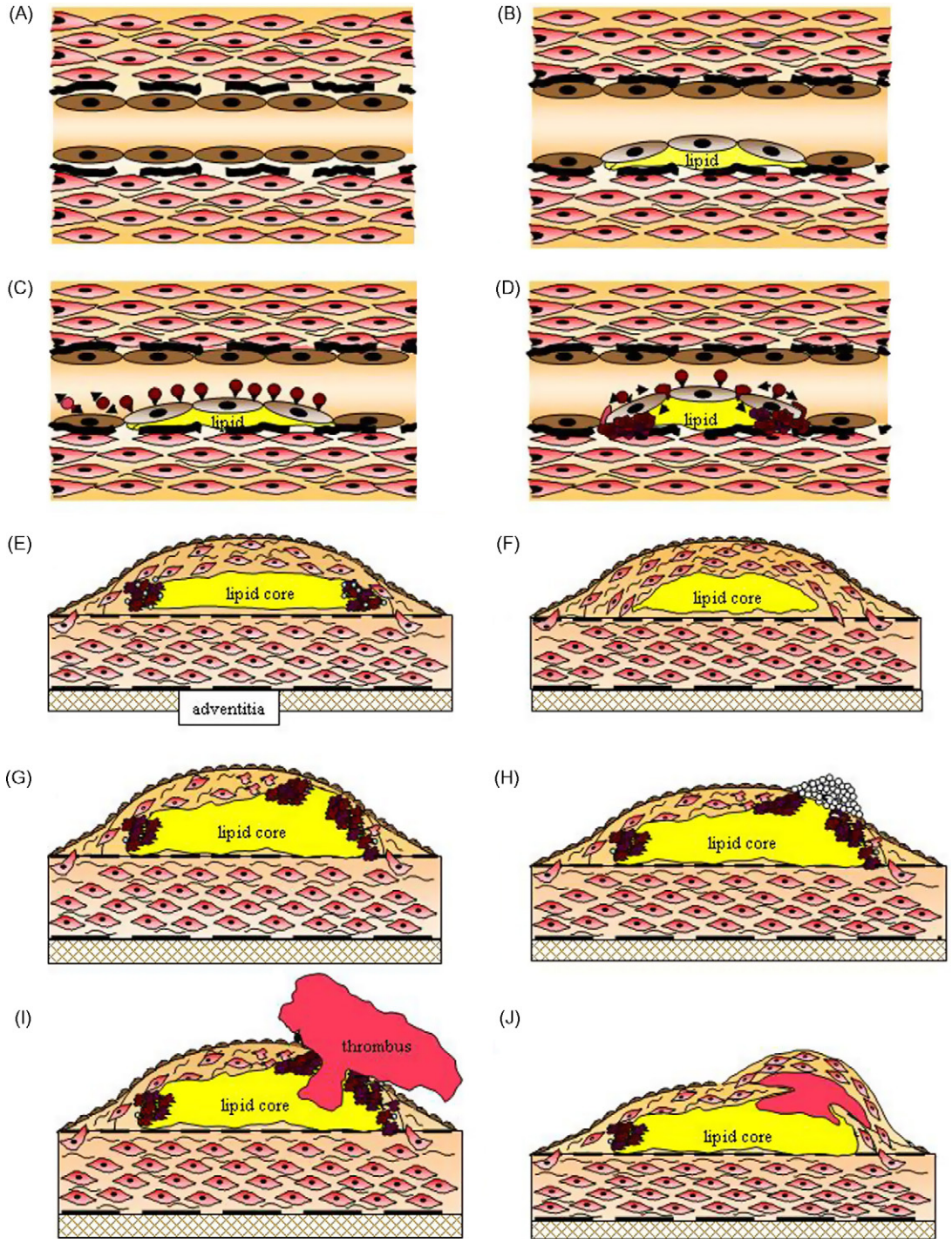

Fig. 1 – Schematic diagram of atherogenesis and plaque progression. (A) The normal artery intima consists of a single layer of endothelial cells resting on basement membrane. In humans, there may also be vascular smooth muscle cells (VSMCs) present. (B) Atherogenesis starts with lipid insudation into the vessel wall, where it is trapped in the intima. The endothelium above the collection becomes dysfunctional, resulting in monocytes rolling and then adhering to the endothelium, prior to migrating into the intima (C), resulting in a macrophage collection. Macrophages ingest lipid to become resident foam cells (D). (E) VSMCs migrate and proliferate over the lipid and macrophage accumulation to create the fibrous cap, a structure separating the lipid core from the lumen. (F) A stable plaque is created with a thick fibrous cap, a low proportion of macrophages and a relatively small lipid core. In contrast, macrophages release matrix metalloproteinases that degrade extracellular matrix, and VSMCs undergo apoptosis and become senescent, thinning the fibrous cap (G). If the cap ruptures, platelets accumulate at the site (H), followed by thrombus formation (I). If the event is not fatal, the thrombus is reorganised by VSMCs migrating into it, and depositing new matrix and cells, creating a new fibrous cap. In this way, the plaque grows by repeated rounds of rupture and repair (J).

complex nature of atherosclerosis, the molecular and cellular interactions mediating disease progression are not completely understood.

2. TRAIL

Tumour necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL/Apo2L) is a member of the TNF family of ligands. It was originally identified and characterised by high sequence homology to CD95L/FasL and TNF [2]. TRAIL is a type II transmembrane protein and like other TNF ligands, TRAIL occurs as a trimer, which can be proteolytically cleaved at its C-terminus to form a soluble ligand. Both the membrane-bound and soluble forms of TRAIL rapidly induce apoptosis in multiple transformed cell lines and tumour cells, although normal cells appear to be resistant [2–4]. This is not surprising, given that TRAIL expression is detected in a variety of human tissues and cells [2]. Based on these observations, and on *in vivo* studies demonstrating little toxicity [5–7], TRAIL is considered to be a promising therapeutic agent for anti-tumour therapy.

2.1. *TRAIL receptors—human*

TRAIL signalling is the most complex of all TNF family members since it is known to bind five different receptors (reviewed in Ref. [8]). TRAIL induces an apoptosis signal following engagement with its specific death-domain containing receptors, DR4 (TRAIL-R1) and DR5 (TRAIL-R2/APO2/Killer/TRICK2) [9,10]. Studies have identified additional membrane-bound receptors of TRAIL including, DcR1 (TRAIL-R3/TRID/LIT) which lacks the intracellular death domain and is anchored to the membrane via a glycophosphatidyl inositol (GPI) tail, and DcR2 (TRAIL-R4/TRUNDD), which lacks a functional death domain by virtue of a truncation [9–13]. TRAIL also binds osteoprotegerin (OPG, TR1), the only known secreted receptor for TRAIL, which regulates osteoclastogenesis by binding receptor activator of nuclear-factor κB ligand (RANKL) [14–16] (Fig. 2). It is suggested that these decoy receptors can compete with DR4 and DR5 for ligand binding and thus protect cells from the induction of apoptosis [9,10]; however, the biological significance of all decoy receptors *in vivo* needs further elucidation.

2.2. *TRAIL receptors—murine*

TRAIL signalling in mice appears to be similar to the human system although there is only one known death-inducing TRAIL receptor, namely mDR5 (TRAIL-R/MK) [17]. This receptor has high sequence homology to the death domains of DR4 and DR5 (76% and 79%), with an overall sequence similarity of 43% and 49%, respectively [17]. Recently, two additional receptors have been identified in the mouse TRAIL-signalling system and resemble the human decoy receptors, since they do not induce apoptosis [18]. mDcR1 and mDcR2 were identified by sequence homology through genome mining of classic cysteine-rich repeats found in TNF receptor family members [18]. Interestingly, two alternatively spliced variants of mDcR2 exist—the long (mDcR2L) membrane-bound receptor, and the short (mDcR2S) secreted form [18]. Murine OPG is also present and highly conserved to the human form [14] (Fig. 2).

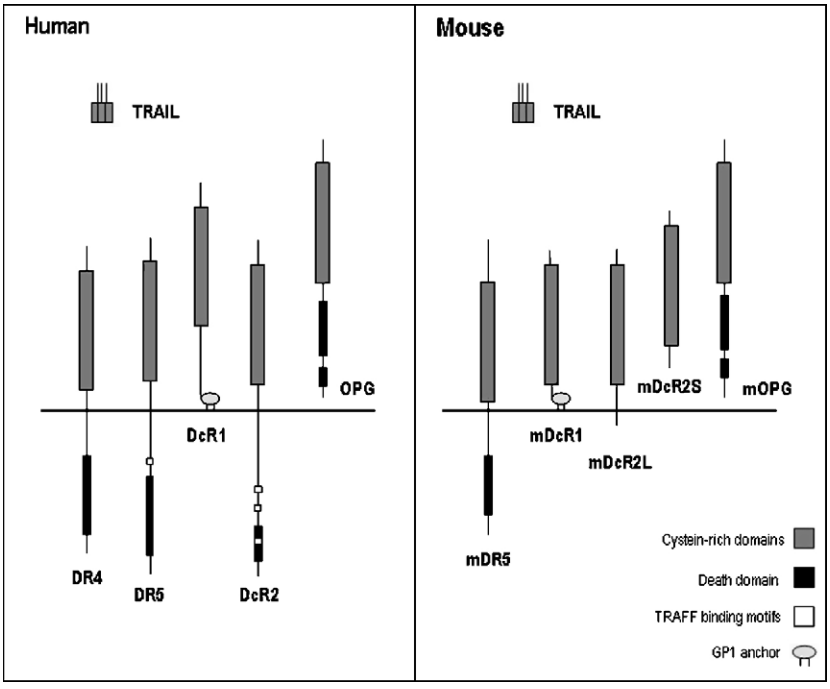

Fig. 2 – Summary of human and mouse TRAIL receptor–ligand interactions. DR4, DR5 and mDR5 contain intracellular death domains and upon ligand binding can signal to initiate apoptosis. DcR2 contains a truncated death domain. DcR1 and mDcR1 are Gp1 anchored receptors. mDcR2 has two spliced variants resulting in membrane-bound (long, L) or secreted forms (short, S).

3. TRAIL signalling

3.1. *Apoptotic role of TRAIL*

Upon ligation of TRAIL, DR4 or DR5 undergo trimerisation and recruit the formation of the death-inducing signalling complex (DISC). TRAIL DISC components include Fas-associated death domain (FADD) and the apoptosis initiator, caspase-8 [19]. Caspase-10 is also recruited and activated and can transmit an apoptosis signal in the absence of caspase-8 [20]. Like FasL, the response to TRAIL is cell type-specific and characterised by two distinct cell type pathways (reviewed in Ref. [21]) (Fig. 3). In the type 1 pathway, extrinsic signals result in DISC formation with release of active caspase-8 to the cytosol. Caspase-8 activation leads to cleavage of effector caspases (e.g. caspase-3 and -7). In the type II pathway, intrinsic signals (e.g. by DNA damage) direct the translocation of Bax to the mitochondria, resulting in loss of transmembrane potential, which induces release of cytochrome c and Smac/DIABLO. Cytochrome c and Apaf-1 can then activate caspase-9 with subsequent activation of effector caspases. The complexity of the apoptotic pathway is further demonstrated by additional regulation involving Bcl-2 family members. For example, while Bcl-2 and Bcl-X_L can inhibit apoptosis, the pro-apoptotic members Bax and Bak, can induce cytochrome c release. Both the intrinsic and extrinsic pathways can direct the apoptotic signalling cascade, and also involve cross-talk. For example, when low concentrations of caspase-8 or -10 are evident with insufficient activation of caspase-3, the cleavage of Bid allows for translocation to the mitochondria where it can activate Bax and Bak to stimulate apoptosis (Fig. 3).

3.2. *Anti-apoptotic role of TRAIL*

Recent studies have illustrated anti-apoptotic functions of TRAIL (reviewed in Refs. [22,23]). For example, studies using a neutralising TRAIL monoclonal antibody defined a role for TRAIL in immune surveillance against tumour development in mice [24]. Administration of this antibody promoted outgrowth of TRAIL-sensitive tumours, but not of TRAIL-resistant tumours [25]. Interestingly, TRAIL expression in ovarian cancer and B chronic lymphocytic leukaemia is also associated with longer cancer survival [26,27]. In corroboration of these findings, TRAIL and its receptors (DR4 and DR5) have been implicated in the survival and proliferation of multiple cells including leukaemia cells, EC, VSMCs, eosinophils, and fibroblasts [28–32].

It is now widely accepted that TRAIL, DR4 and DR5 can activate the transcription factor NF-κB, via release from the inhibitory IκB proteins [28,33,34]. In response to stimuli, IκB kinase (IKK) becomes activated and phosphorylates IκB, which in turn results in the ubiquination and degradation of IκB, thus

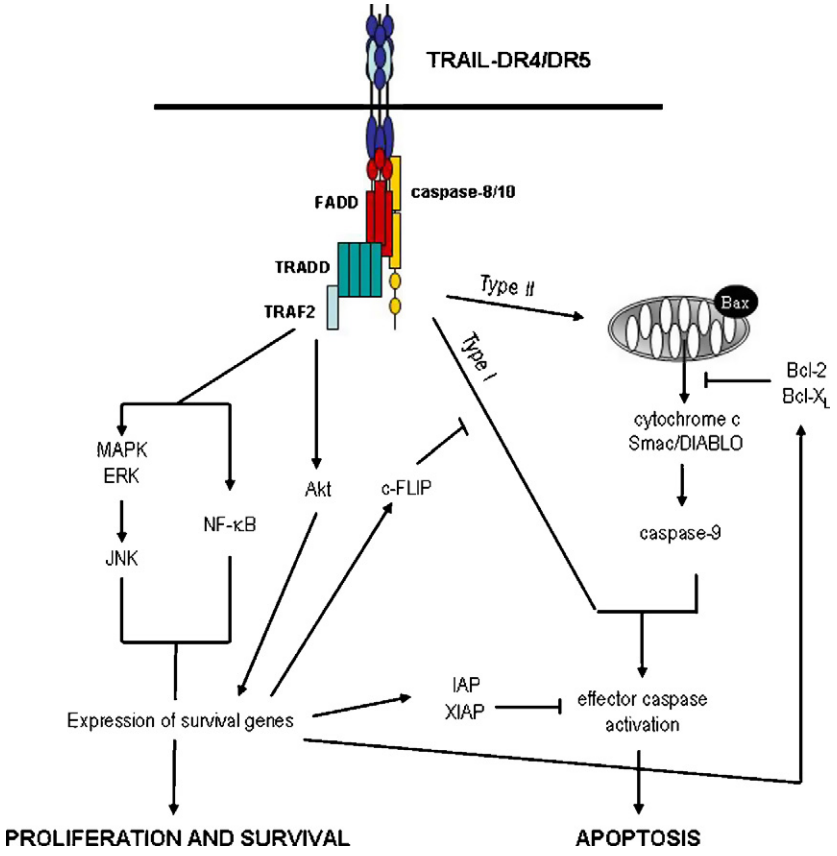

Fig. 3 – Schematic representation of TRAIL-receptor signalling. Ligation of TRAIL to DR4 or DR5 can lead to both the apoptotic and survival pathways. TRAIL-DR4/5 can initiate apoptosis by recruiting FADD and caspase-8 or 10, followed by activation of the caspase cascade. TRAIL-DR4/DR5 can also initiate survival signals via NF-kB and JNK activation and subsequently lead to upregulation of survival genes.

releasing NF-κB/Rel proteins from the inhibitory complex and allowing them to translocate to the nucleus. Once in the nucleus, NF-κB can activate anti-apoptotic genes including c-IAP1 and c-IAP2 (cellular inhibitor of apoptosis proteins 1 and 2) [35], XIAP (X-linked mammalian inhibitor of apoptosis protein) [36], TRAF1 and TRAF2 (TNF-receptor associated factor 1 and 2) [35], c-FLIP (cellular FLICE-like inhibitory protein) [37] and Bcl-X_L [38]. TRAIL, DR4 and DR5 can also activate ERK1/2 [29], mitogen-activated protein kinase (MAPK) [29], c-Jun N-terminal kinase (JNK) [39] and Akt [32], processes involved in survival. Interestingly, NF-κB and JNK activation is mediated by recruitment of TRADD (TNF-R1-associated death domain protein) [40], RIP (receptor-interacting protein) [41] and TRAF2 (TNF-receptor associated factor 2) (Fig. 3) [41] and can occur independently of caspase-8 or -10 [33,39]. The mechanisms for the anti-apoptotic role of TRAIL at present are largely undefined.

4. Regulation of TRAIL

The regulation of TRAIL gene expression is limited (Table 1). The human TRAIL promoter has been described and several putative consensus transcription factor sites have been identified, e.g. GATA, C/EPB, Sp1, Ap1, Ap3, CF-1 and ISRE [42,43]. The interferon (IFN) family of cytokines, plays an important role in the development of atherosclerosis [44] and has been shown to regulate TRAIL expression [42,45–48]. IFNγ stimulated Stat1 activation and increased secretion of soluble TRAIL [47]. The role of Stat1-dependent TRAIL expression has also been described and involves stimulation by IFNβ [45]. Using a RNAse protection assay and U3A cells deficient in Stat1, Choi et al. no longer observed the induction of TRAIL mRNA by IFNβ [45]. Interestingly, IFNα induced rapid activation of Stat1 and accumulation of IRF-1 [48]. Furthermore IFNα stimulated Stat1 and IRF-1 DNA binding to the TRAIL promoter, and SiRNA targeting both transcription factors were able to inhibit IFNα-induced expression of TRAIL, implying that both transcription factors are required for inducible expression of TRAIL [48]. IRF-3 [49] and IRF-7 [50] have also been implicated in regulating TRAIL expression. Sendai virus infection (SeV) induced TRAIL mRNA in an IRF-3-dependent manner in human foreskin keratinocytes [49] and constitutively active IRF-3 (but not mutant IRF-3) activated the TRAIL promoter [49]. IRF-3 was also demonstrated to bind the interferon-stimulated response element on the TRAIL promoter following SeV infection [49]. In contrast, adenovirus-mediated transduction of IRF-7 was more potent than IRF-3 in up-regulating TRAIL mRNA in primary macrophages [50].

The role of NF-κB-signalling and TRAIL regulation has been implied, given that NF-κB-dependent induction of TRAIL has been observed [51,52]. Virus-induced TRAIL RNA expression and TRAIL surface expression was completely inhibited by the IKK/IKβ mutants in kidney cells [52]. Tax, an oncoprotein encoded by human T-cell leukaemia virus induced apoptosis (via TRAIL) which was dependent on NF-κB-signalling [51]. Interestingly, Tax can mediate the activation of adhesion molecules and may regulate the interaction of T cells with EC [53]. Tax-inducible TRAIL expression was completely abrogated in IKKγ-deficient Jurkat cells compared to parental cells [51]. These findings suggest that the NFκB pathway mediates inducible expression of TRAIL by Tax [51]. Since TRAIL also activates NF-κB-signalling, this may serve as a feedback mechanism in the regulation of TRAIL.

Egr transcription factors have been implicated in the development of atherosclerosis [54] and are known to regulate TNF family members FasL, TRAIL and TNF [55]. In particular, Egr2 and Egr3 transcription factors induced endogenous TRAIL mRNA expression in intestinal epithelial cells which was blocked by overexpression of Nab1, a co-repressor of Egr transcription factors [55]. In contrast Egr1 significantly repressed TRAIL mRNA expression in EC [56]. Interestingly, overexpression of NAB2, upregulated TRAIL and abrogated the early inhibitory effect of TNFα on TRAIL expression, implying that Egr1 may play a role in TNFα-induced repression of TRAIL [56].

Finally, insulin also plays a role in TRAIL mRNA expression. A significant decrease in TRAIL mRNA and protein was observed in the aortas of diabetic mice that received insulin compared with control [57]. A similar finding was also observed in vitro [57]. VSMCs release bioactive nitric oxide (NO) in response to recombinant TRAIL [58,59], and this suggests that TRAIL may regulate vascular tone under physiological conditions. Thus, down-regulation of TRAIL by insulin may be recognised as a likely pathway of diabetic vasculopathy [57].

5. Expression and function of TRAIL in atherosclerosis

TRAIL and its receptors are expressed in normal arteries, in lesions of atherosclerosis and in Monckeberg's sclerosis, a

Table 1 – Regulation of TRAIL by transcription factors and cytokines

Agonist	Transcription factors involved	Effect	Reference
IFNγ	Stat1	Increased protein	[47]
IFNβ	Stat1	Increased mRNA	[45]
IFNα	Stat1 and IRF-1	Increased protein, promoter activity	[48]
Viral infection	IRF-3	Increased mRNA	[49]
	IRF-7	Increased mRNA	[50]
NF-κB	Tax	Increased protein, mRNA	[51,52]
Viral infection	Egr2 and Egr3	Increased mRNA	[55]
TNFα	Egr1	Decreased mRNA	[56]
Insulin	?	Decreased protein	[57]

References are outlined in the text.

form of arteriosclerosis typically occurring in diabetes [30,60–65]. TRAIL expression can be found in VSMCs, EC and inflammatory cells within the vessel wall [29,61,63,65–67]. TRAIL is also expressed in smooth muscle cells of the airways, spinal cord and uterine spiral arteries [31,60]. Interestingly, serum levels of soluble TRAIL are reduced in patients predisposed to coronary artery disease [64,68] a trend also observed in patients with type II diabetes [57]. These findings suggest, that TRAIL may be an important factor regulating cardiovascular disease.

5.1. *Endothelial cells*

Vascular EC act as barriers that limit thrombosis, and are the principle targets of pro-inflammatory actions by T cells and macrophages. The endothelium can also regulate inflammation, vascular tone and vascular remodelling in the vessel wall [1] and apoptosis of EC can therefore increase the likelihood of atherosclerotic plaque formation. TNF ligands have been implicated in apoptosis of EC [69]. Furthermore, human aortic EC (HAEC) and human umbilical vein EC (HUVEC) express TRAIL, DR4, DR5, DcR1 and DcR2 [29,67] and in support of these findings, TRAIL-induced apoptosis of EC in culture has been reported [70]. Although only 30% of EC were subject to death by TRAIL, the remaining 70% of EC did not die, and demonstrated increased NF-κB activity, and increased expression of E-selectin, ICAM-1 and IL-8, molecules involved in promoting leukocyte adhesion to EC [70]. The proadhesive activity of TRAIL in EC was also observed by Secchiero at el., although in contrast, no increase in NF-κB activity and no upregulation of E-selectin, ICAM-1 and VCAM-1 was observed [71,72]. TNFα and IL-1β strongly induce leukocyte adhesion to EC. Interestingly, pre-treatment of EC with TRAIL significantly reduced the pro-adhesive activity of these two cytokines [71]. Furthermore, TNFα-induced mRNA expression of multiple cytokines involved in leukocyte adhesion (e.g. CCL8, CXCL10, CCL20, and CXCL5) was reduced with TRAIL pre-treatment [71]. These studies imply that TRAIL may also play a role in leukocyte/EC adhesion.

Endothelial nitric oxide synthase (eNOS), an enzyme which stimulates the production of NO, plays an important role in the regulation of cardiovascular function including vascular tone, endothelial cell survival/migration, anti-thrombotic and anti-inflammatory activity [73]. Recently, eNOS and NO have been implicated in TRAIL-mediated EC biology. TRAIL stimulated NO production and migration of HUVEC via the phosphorylation and subsequent activation of eNOS at ser^{1177} in an Akt-dependent manner [74]. Reorganisation of the cytoskeleton can also lead to alteration of NO production [75]. Interestingly, TRAIL-inducible NO synthesis occurs by regulating the redistribution of eNOS from the plasma membrane to the cytoplasm (Golgi) without inducing apoptosis [76]. Given that eNOS is an important regulator of cardiovascular homeostasis and vascular tone, these findings have significant implications for TRAIL-mediated EC function.

Not only is TRAIL involved in migration of EC, TRAIL also promoted proliferation and inhibited apoptosis by activating Akt and ERK pathways [29]. Interestingly, TRAIL did not activate p38, JNK or NF-κB [29]. Furthermore, Alladina et al (2005) [67] showed that TRAIL at 100 ng/ml and 300 ng/ml significantly increased EC numbers in low serum. When the cells were co-treated with the PI3K inhibitor LY294002, increased cell numbers were no longer observed. Instead, PI3K inhibition reduced phosphorylation of Akt, c-$FLIP_S$ and Bcl-2 expression and sensitised HUVEC to TRAIL-induced apoptosis [67]. A summary of TRAIL signalling in EC is outlined in Fig. 4A.

5.2. *VSMCs*

VSMCs are the principle cellular components in atherosclerotic plaques that produce collagen types I and III and extracellular matrix, required to maintain tensile strength. Excessive repair by VSMCs can lead to post event stenosis, for example in restenosis after angioplasty or stenting. On the contrary, reduction of VSMCs by apoptosis in advanced lesions can contribute to plaque instability and rupture [77]. Although not fully established, TRAIL, DR4, DR5 and elements of the apoptotic pathway have been implicated in VSMCs apoptosis [61,62,64]. Apoptosis of VSMCs can lead to features of atherosclerotic plaque rupture [77] and recently Michowitz et al. [64] demonstrated increased TRAIL protein expression from cells of plaques vulnerable to rupture, compared to cells from stable plaques [64]. In addition, TRAIL is expressed in atherosclerotic coronary artery lesions in regions of apoptosis [62]. VSMCs express DR4 and DR5 and are susceptible to apoptosis induced by TRAIL-expressing CD4+ plaque cells [63,78]. In the study by Sato et al. [63], immunodeficient mice were implanted with carotid plaque tissue with adoptive transfer of CD4 T cells. VSMCs accounted for the majority of apoptotic death in the plaque, and VSMCs apoptosis could be blocked using an anti-TRAIL antibody. Furthermore, recombinant TRAIL-induced apoptosis of VSMCs in culture was inhibited by anti-DR5 antibodies [63], implicating the TRAIL-death receptor mediated pathway in VSMCs cytotoxicity, a finding also supported by others [61,78].

Although apoptosis of VSMCs may control cell turnover in the vessel wall and promote atherosclerotic plaque rupture, recent studies have identified an opposing role for TRAIL in VSMCs. Recombinant human TRAIL administration attenuated development of plaques in diabetic ApoE-null animals, and affected cellular composition of plaque lesions by inducing apoptosis of macrophages and not VSMCs [65]. Instead, VSMCs content in the plaque was increased [65]. TRAIL also promoted the migration of human VSMCs (but not of monocytes or macrophages) [65]. In support of this finding the same group demonstrated that TRAIL protected VSMCs from treatment with TNF-α, IL-1β, IFN-γ, or serum withdrawal, and promoted migration of VSMCs by activation of ERK1/2 and Akt but not p38 [30].

Until recently NO generation in the vessel wall was believed to be restricted to the endothelium [79]. We now know that VSMCs from carotid and renal arteries, as well as aortas have been shown to generate physiologically significant amounts of NO [58,59]. VSMCs may also modulate vascular contractility in the vessel wall. In light of this, TRAIL has been shown to induce the release of bioactive NO by not only EC, but also in VSMCs in culture [57]. This increase in NO synthesis was inhibited by L-NAME, a pharmacological inhibitor of the eNOS pathway [57]. Thus, implicating TRAIL as an endogenous

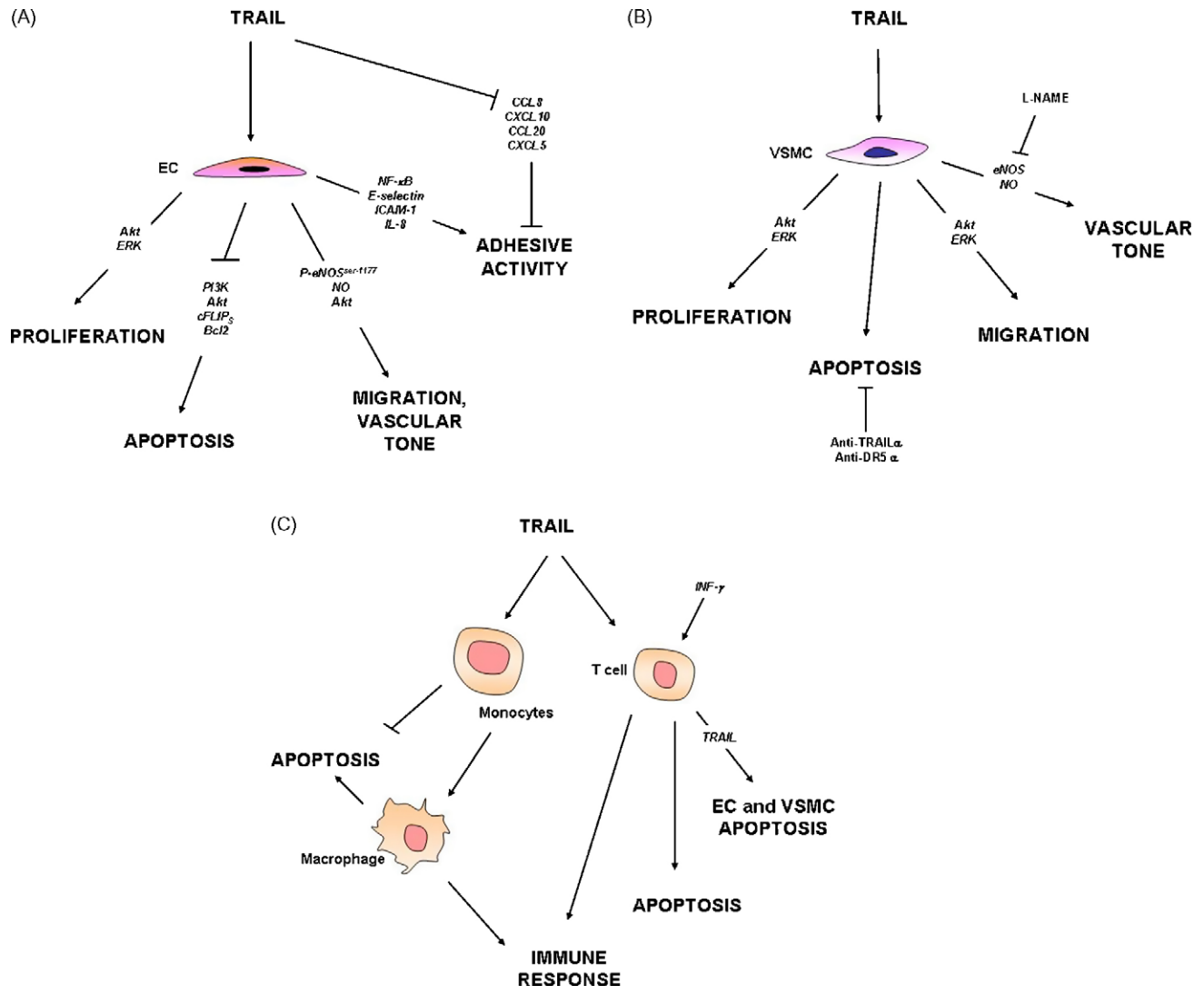

Fig. 4 – TRAIL function in atherosclerosis. (A) In endothelial cells (EC), TRAIL can regulate multiple functions including apoptosis, proliferation, leukocyte adhesion, migration and vascular tone. (B) In vascular smooth muscle cells (VSMCs), TRAIL can promote apoptosis, proliferation, migration and vascular tone. (C) TRAIL can also regulate apoptosis and evoke immune responses in inflammatory cells within the vessel wall.

regulator of myo-relaxation via a VSMCs-specific and endothelium-independent NOS pathway [57]. The effects of TRAIL on VSMCs is outlined in Fig. 4B.

5.3. *Inflammatory cells*

Atherosclerosis is characterised by the recruitment of monocytes and lymphocytes into the vessel wall and this process may act as a critical step from the transition of a stable plaque to an unstable plaque [80]. T cells and macrophages generate cytokines that regulate the biochemical processes during all stages of atherosclerosis. TRAIL plays an important role in the immune response, and has been shown to promote apoptosis of macrophages and lymphocytes [81,82]. Removal of atherogenic and inflammatory-prone macrophages by apoptosis may be beneficial to the development of atherosclerosis. In contrast, apoptosis of macrophages in advanced atherosclerosis may promote plaque instability [83], although our recent data suggest that it does not [84].

Systemic administration of recombinant TRAIL in diabetic ApoE knockout mice resulted in apoptosis of infiltrating macrophages and attenuated the development of the plaque [65]. Interestingly, TRAIL did not induce apoptosis of circulating CD14+ monocytes [65]. Plaque-infiltrating CD4 T cells are also cytotoxic to EC and VSMCs [63,85], and TRAIL-mediated cytotoxicity of VSMCs has been observed from plaque-derived CD4 positive T cells [63]. IFNα stimulation of CD4 T cells from patients with acute coronary syndromes also kills VSMCs in a TRAIL-dependent manner [66]. TRAIL is expressed in plaque-infiltrating CD3 and co-localises to regions in atherosclerotic plaques expressing ox-LDL, a proinflammatory and proatherogenic protein that promotes T cells/monocyte chemotaxis, adhesion and induction of inflammatory genes [86]. These studies are summarised in Fig. 4C and suggest that TRAIL has a

key role in the inflammatory process by inducing apoptosis of vascular cells and promoting the initiation, progression and stability of atherosclerotic plaques.

6. Conclusions

The studies reviewed above provide greater insight into the role of TRAIL in cells of the vasculature. However, the pathogenesis of atherosclerosis is a multifactorial process involving multiple cell types, and interactions between TRAIL and its receptors could have multiple sequelae, that differ at different stages of the disease, or that are conflicting. For example, although TRAIL-induced apoptosis of EC could lead to atherosclerosis, EC proliferation and migration induced by TRAIL could help reduce monocyte/leucocyte infiltration into the vessel wall. In VSMCs, TRAIL-induced apoptosis might be beneficial during early stage atherogenesis. On the contrary, TRAIL-induced VSMCs proliferation in early atherosclerosis or in in-stent restenosis after percutaneous coronary interventions may lead to renarrowing of the lumen. In late stage atherosclerosis however, VSMCs proliferation may stabilise the atheromatous plaque and prevent it from rupture. In inflammatory cells, TRAIL predominantly functions to regulate an immune response or to induce apoptosis, but it is not clear whether macrophage apoptosis has different effects at different stages of the disease.

TRAIL function therefore demonstrates greater complexity than originally envisaged, such that multiple pathways and outcomes other than apoptosis, including differential regulation of different cell types, are beginning to be described. Further studies on TRAIL expression, regulation and function need to be performed to fully understand the complex signalling pathways mediated by TRAIL in atherosclerosis. In particular, the current studies infer a role for TRAIL signalling in vascular disease from the association of TRAIL/TRAIL-R expression in the vessel wall with a particular cellular phenomenon. We need to study what happens to atherosclerosis and other vascular pathologies when we manipulate TRAIL signalling, both via administration of recombinant TRAIL, and also more importantly by inhibiting TRAIL or TRAIL receptors in vivo to determine the role of endogenous expression of these proteins.

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