[Frontiers in Bioscience 13, 135-147, January 1, 2008]

TRAIL and osteoprotegerin: a role in endothelial physiopathology?

Federica Corallini [1], Erika Rimondi [1], Paola Secchiero [1]

[1] *Department of Morphology and Embryology, University of Ferrara, Ferrara, Italy*

TABLES OF CONTESTS

1. ABSTRACT

Increasing experimental evidence suggests that both tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and its soluble decoy receptor osteoprotegerin (OPG) are involved in vascular biology. In particular, emerging data indicate that recombinant soluble TRAIL may act as a molecule with potential anti-inflammatory activity in vascular physiopathology. Conversely, the presence of leukocytes expressing membrane-bound TRAIL in atherosclerotic lesions might be involved in the destabilization of atherosclerotic plaques by inducing apoptotic cell death of vascular smooth muscle cells in an inflammatory milieu. Also OPG seems to be involved in vascular homeostasis, by acting in a paracrine or autocrine manner as a survival factor for endothelial cells. However, an increased production of OPG may have a role in the development of vascular dysfunction likely by multiple potential mechanisms, not only related to its ability to neutralize TRAIL-activity but also mediated by its heparin-binding domain. In this review we have summarized and discussed both *in vitro* and *in vivo* data that suggest potential roles of TRAIL and OPG in vascular physiopathology. Further studies are needed to address how the TRAIL/OPG interaction, their reciprocal balance and/or interplay affect vascular biology in order to design innovative therapeutic strategies in vascular diseases.

2. INTRODUCTION

The vascular endothelium is a thin monocellular layer that covers all the inner surface of the blood vessels, separating the circulating blood from the tissues. It is not an inactive organ, but plays a key role in the maintenance of vascular homeostasis. Under normal conditions, the endothelium secretes a variety of vasoactive substances, including nitric oxide (NO) and prostacyclins, which protect the vascular wall against vasoconstriction, inflammatory and proliferative changes, and thrombus formation (1-2). Among the plethora of intracellular signaling pathways involved in endothelial cell biology, the mitogen-activated protein kinase (MAPK)/ extracellular signal-regulated kinase (ERK) pathway is a central element in transducing mitogenic signals in endothelial cells, while phosphatidylinositol 3-kinase (PI3K/Akt) pathway is of central importance in conferring survival to endothelial cells in response to angiogenic cytokine stimulation, fluid shear stress, and matrix attachment signals (3).

Angiogenesis, the formation of capillaries from preexistent blood vessels, is an essential process in development, reproduction, and tissue repair, which however also occurs in the adult under pathological conditions such as ischemic disease, arthritis and tumor growth. Angiogenesis is stimulated by a number of well-

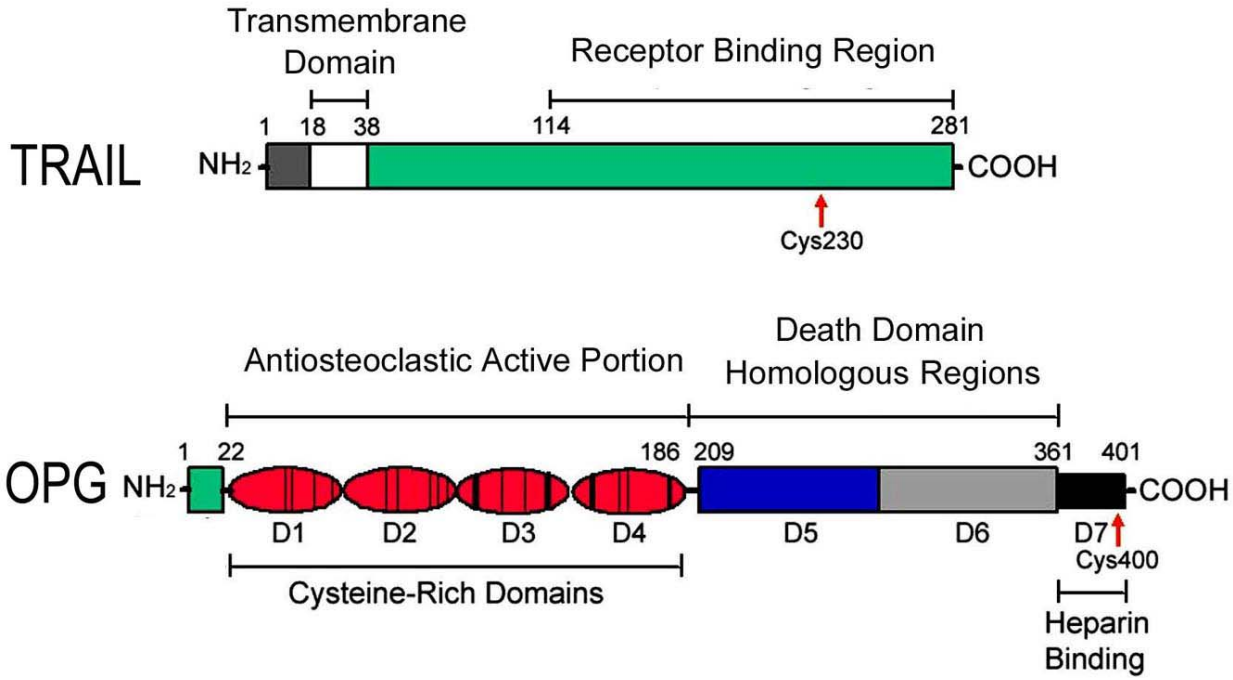

Figure 1. Schematic representation of the structure of TRAIL and OPG. Main domains and their biochemical and/or functional properties are indicated. NH2 indicates amino-terminus; COOH, carboxy-terminus.

characterized pro-angiogenic cytokines, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), as well as by pleiotropic cytokines, such as transforming growth factor-beta (TGF-beta) or chemokines, such as macrophage inflammatory protein (MIP) (3-7). It is increasingly clear that angiogenesis is a tightly regulated process, in which endogenous inhibitors of revascularization, such as thrombospondin (TSP-1), interferon-gamma (IFN-gamma), interleukin-10 (IL-10), and tissue inhibitors of metalloproteinases (TIMPs), play a role as important as that of pro-angiogenic cytokines (4). In addition, two recently recognized inhibitors of angiogenesis, angiostatin and endostatin, have been identified (3,7,8). A well-characterized effect of pro-angiogenic cytokines is the promotion of endothelial cell survival by inhibiting apoptosis (9-12). In this respect, it should be noticed that, upon endothelial injury, loss of the anti-thrombotic properties of the vessel wall occurs, and the concomitant enhancement of the number of damaged circulating endothelial cells acts as a critical initial step in the development of atherosclerotic plaques (13-14). Fast and complete regeneration of injured endothelium is of central importance in the biology of vascular system. The endothelial repair may occur by migration and proliferation of surrounding mature endothelial cells, processes involved also in adult angiogenesis (15). Another important process in endothelial physiopathology is inflammation, which is mediated by endothelial activation. Long-term exposure of endothelial cells to pro-inflammatory cytokines accelerates oxidative stress and apoptosis, and promotes leukocyte extravasation and thrombosis. Interestingly, anti-inflammatory cytokines exerting inhibitory effects on vascular cells include TGF-beta and IL-10 (16), which exert opposite effects on angiogenesis, clearly demonstrating that the effects on angiogenesis are uncoupled from those on inflammation. However, endothelial cells should not be considered passive targets of exogenous cytokines, since contribute themselves to cytokine secretion (17-19). In fact, it has been clearly established that human endothelium is capable of expressing a broad spectrum of pro- and anti-inflammatory cytokines, including interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), monocyte chemotactic protein-1 (MCP-1), granulocyte/macrophage colony stimulating factor (GM-CSF), granulocyte CSF (G-CSF), macrophage CSF (M-CSF), PDGF, VEGF and FGF (19). Moreover, exposure of endothelium to the prototypical pro-inflammatory cytokine TNF-alpha, results in the rapid activation of nuclear factor-kB (NF-kB), which in turn modulates the endothelial synthesis of proinflammatory cytokines and chemokines (6). In the context of the physiopathology of the endothelium, recent findings imply a role for TRAIL and OPG, two members of the TNF superfamily of cytokines and receptors, respectively.

3. TRAIL AND THE VASCULAR SYSTEM

3.1. TRAIL structure and expression

TRAIL was originally identified by two independent groups and characterized as a member of the TNF family of death-inducing ligands (20-21). TRAIL is a type II membrane protein of about 33-35 kD, which can be cleaved from the cell surface to form a soluble ligand that retains biological activity (Figure 1) (22). The extra-cellular domain of TRAIL forms a bell shaped homo-trimer, much

like other ligands of the TNF family. However, there is a unique insertion loop of about 16-20 amino acids in soluble TRAIL near its amino-terminal end (Figure 1) (23-25). Unlike other members of the TNF ligand family, TRAIL carries a zinc ion at the trimer interface, coordinated by the single unpaired cysteine residue (Cys 230) of each monomer (Figure 1); this zinc ion is essential for structural integrity of TRAIL and to maintain its capacity to induce apoptosis (23-25). In this respect, in the last years TRAIL received particular attention because both full-length membrane expressed TRAIL and the soluble ligand can rapidly induce apoptosis in a wide variety of human cancer cell lines and primary tumors, showing minimal or absent toxicity on normal cells (20); thus TRAIL was identified as a potential tumor-specific cancer therapeutic.

TRAIL is expressed on the surface of activated immune cells, such as natural killer (NK) cells, T cells, macrophages and dendritic cells, where it apparently functions as an immune effector molecule, mediating antitumor cytotoxicity and immune surveillance (26-30). Importantly, this biological role of TRAIL is consistent with its tumor selective properties, since it implies that normal tissues are constitutively protected from circulating immune cells bearing TRAIL. Besides, a significant level of TRAIL transcript has been detected in many human tissues and is expressed constitutively in some cell lines (30). Such widespread distribution of TRAIL transcripts differs from that of other members of TNF family, which suggests that TRAIL may not only be an anti-tumoral mediator *in vivo*.

3.2. TRAIL receptors

TRAIL can bind to five different receptors found on a variety of cell types: four membrane-bound and one soluble receptor (31-32). Two of these membrane receptors, TRAIL-R1/death receptor 4 (DR4) and TRAIL-R2/death receptor 5 (DR5), act as agonistic receptors, containing a cytoplasmic death domain through which TRAIL can transmit an apoptotic signal. The other two membrane receptors, TRAIL-R3/decoy receptor 1 (DcR1) and TRAIL-R4/decoy receptor 2 (DcR2), can also bind TRAIL, but may act as antagonistic receptors, lacking the death domain. In addition to these four transmembrane receptors, a fifth soluble antagonistic receptor, osteoprotegerin (OPG), has been identified.

Among the various receptor-ligand interactions described within the TNF-superfamily, the TRAIL/TRAIL-receptors system is considered to be the most complex (30). Like most TNF family members, TRAIL forms a homotrimer that binds to the high affinity TRAIL-R1/DR4 and TRAIL-R2/DR5, inducing the trimerisation of these receptors. This leads to the assembly of a death-inducing signaling complex (DISC) and the subsequent recruitment of the adaptor protein Fas associated death domain (FADD) that acts as a bridge between the death receptor complex and the pro-domain of the initiator caspase 8. Dimerisation of caspase 8 molecules at the DISC leads to the formation of mature caspase 8 that is capable of activating downstream effector caspases, such as caspases 3, 6 and 7, which execute apoptosis (33). This apoptosis pathway is referred to as the extrinsic apoptosis pathway. However, in certain cell types the activation of initiator caspases must be amplified by involvement of the so-called intrinsic mitochondrial pathway of apoptosis (34-35). In both cases, activated executioner caspases induce apoptosis of TRAIL-sensitive cells. Concerning the two decoy receptors, TRAIL-R3/DcR1, attached to the cell membrane *via* a GPI linker (36-40), lacks the cytoplasmatic region and TRAIL-R4/DcR2 possesses a truncated death domain (38,41). Thus both are unable to confer a pro-apoptotic signal. These antagonistic decoy receptors are able to compete with the agonistic TRAIL-receptors for binding of the ligand, and might thus protect normal cells from apoptotic cell death. Of note, following stimulation of TRAIL-R1/DR4 or TRAIL-R2/DR5, TRAIL is also able to up-regulate the transcription factor NF-kB and c-Jun-N-terminal kinase (JNK) (42-45). The delineation of the actual role of NF-kB in modulating TRAIL-mediated signaling is complicated by the pleiotropic effects induced by NF-kB (46). However, a recent paper has shed light on the interplay between TRAIL and NF-kB demonstrating that the ability of TRAIL to activate NF-kB is ineffective in protecting TRAIL-sensitive cells to TRAIL-mediated apoptosis. On the other hand, pre-activation of NF-kB confers resistance to TRAIL-mediated apoptosis (47). Similarly, it is incompletely understood to what extent activation of the JNK pathway contributes to the pro-apoptotic activity of TRAIL (45,48,49).

Although the expression pattern of the various pro- and anti-apoptotic receptors may influence the balance between sensitivity and resistance towards TRAIL, the assessment of the relative levels of the transmembrane receptors alone does not reliably predict the response of the respective cell (50-51). In fact, our and other groups of investigators have clearly shown that TRAIL is able to activate simultaneously pro-apoptotic pathways as well as anti-apoptotic (Akt/eNOS) and proliferative (MAPK/ERK) pathways (51-57). Of note, the activation of pro-apoptotic and anti-apoptotic intracellular pathways by TRAIL seems to be cell-type specific.

In addition to the TRAIL-membrane receptors already discussed, a fifth receptor for TRAIL, the soluble decoy OPG, has been described (58). This soluble member of the TNF receptor family, initially characterized for its ability to block RANKL-stimulated osteoclast formation, also interacts with TRAIL (58). Although one study has claimed that binding of TRAIL to OPG is weak at physiological temperature (37°C) as compared to the binding of TRAIL to trans-membrane TRAIL receptors (59), more recent studies have underlined the biological relevance of OPG/TRAIL interactions in different *in vitro* cell models (60-61).

3.3. Effects of soluble TRAIL on endothelial cells

In considering the potential physiopathological role of TRAIL on vascular biology, it is noteworthy that TRAIL protein is expressed in the medial smooth cell layer of aorta and pulmonary artery (62). Whereas cleavage of other member of the TNF super-family from the cell surface usually requires the action of zinc-dependent metalloproteases, generation of soluble TRAIL involves the

Table 1. Biological effects and expression of TRAIL in the vascular system

Biological effects on endothelial cells	**References**
• No induction of apoptosis	52, 54, 68-71
• Induction of apoptosis in serum- and/or extracellular matrix-deprivation	52, 71-73
• Induction of prostanoid and nitric oxide release	54
• Induction of endothelial cell survival/proliferation and promotion of endothelial tube-like organization	52, 69, 75
• Down-modulation of TNF-alpha-induced leukocyte adhesion	78
Expression/effects in vascular diseases	
• Expressed in atherosclerotic lesions	83-86
• Reduction of atherosclerotic lesion in diabetic ApoE-null mice treated with human recombinant TRAIL	88
• Reduced serum levels in patients with acute coronary sindrome and neagative correlation with the levels of C-reactive protein	84, 89

action of cysteine proteases (63). Notably, the vessel wall is a rich source of cysteine proteases (64). TRAIL is also detectable in the plasma and/or serum of normal individuals (65-67). The possibility that circulating TRAIL may be involved in regulation of endothelial cell function is underscored by the observation that human endothelial cells obtained from different vascular districts express detectable amounts of all transmembrane TRAIL receptors (TRAIL-R1-R4).

In spite of the presence of death receptors, most of the studies performed on endothelial cells reported no apoptosis induction by the addition in culture of recombinant soluble TRAIL (Table 1) (52,54,68-71). Although a couple of studies have reported that endothelial cells may be sensitized to TRAIL-induced apoptosis (72-73), it should be pointed out that particular conditions, however, were required to disclose the ability of TRAIL to induce apoptosis, such as serum- and extracellular matrix-deprivation, or inhibition of pro-survival pathways (i.e., Akt/PI3K) (Table 1) (52,71-73). On the other hand, we have demonstrated that TRAIL induces a moderate but significant upregulation of prostanoid production/release (PGE2, PGI2, TXA2) in endothelial cell cultures and upregulates eNOS activity and NO synthesis/release (54). Of note, the NOS pathway is essential also for endothelial cell differentiation and migration (3). Among the members of the MAPK family, TRAIL rapidly activates the ERK1/2 pathway (52), which is a central element in transducing mitogenic signals in endothelial cells (74).

The ability of TRAIL to induce prostanoid production and nitric oxide release (54), as well as to activate the Akt and ERK1/2 pathways (52) lead us to hypothesize potential vasoprotective functions of TRAIL, since PGE2 and PGI2, together with NO, regulate the vascular tone and permeability, calm down activated platelets and leukocytes, prevent the occurrence of parietal thrombotic events, promote thrombolysis, maintain tissue perfusion and protect vascular wall against acute damage and chronic remodeling. These effects together with the ability of TRAIL to promote the survival/proliferation, actin reorganization and migration of endothelial cells (Table 1) (52,54,75) strongly suggest that the TRAIL/TRAIL-R system may play important roles in endothelial cell biology. In this contest however, the role of TRAIL on angiogenesis is subtler than expected and likely depends on the basal endothelial cell conditions. In fact, Cantarella et al. (76) has recently demonstrated that in different human glioblastoma cell lines recombinant TRAIL inhibited the orchestra of factors contributing to glioblastoma biological aggressiveness, by down-modulating mRNA expression of VEGF, along with those of matrix metalloproteinase-2 (MMP-2), and of the tissue inhibitor of matrix metalloproteinases-2 (TIMP-2). In line with a potential role of TRAIL in counteracting tumor angiogenesis, Carlo-Stella et al. (77) have recently demonstrated that membrane-bound TRAIL, over-expressed by CD34+ cells, is very effective in inducing apoptosis and necrosis of tumor endothelial cells associated to hematopoietic malignancies.

Another important aspect of TRAIL in endothelial cell biology is its ability to counteract leukocyte adhesion induced by inflammatory cytokines (Table 1) (78). At variance to TNF-alpha, TRAIL doesn't activate the NF-kB pathway in primary endothelial cells and consequently it doesn't upregulate the surface level of adhesion molecules, like ICAM-1, VCAM-1, and E-selectin (52,78), key molecules for the endothelium-leukocyte interaction in inflammation. Of note, an abnormal increase of the leukocyte adhesion is considered an early step in endothelial cell dysfunction (2,79,80). It is particularly interesting that TRAIL, by selectively counteracting the up-regulation of CCL8/MCP-2 and CXCL10/IP-10 chemokines, significantly reduces the potent pro-adhesive activity of canonical inflammatory cytokines, such as TNF-alpha or IL-1beta (78). Thus, in analogy to TGF-beta1 (81), also TRAIL, by promoting endothelial cell survival and proliferation, might have a role in the later phases of inflammation when repair and tissue regeneration start to occur (52,82).

3.4. TRAIL and vascular diseases

The presence of TRAIL-expressing leukocytes has been documented in both mouse and human atherosclerotic plaques (Table 1) (83-86) and a potential pathogenetic role of these TRAIL-expressing leukocytes in inducing the destabilization of atherosclerotic plaques has been proposed mainly through the induction of apoptosis in vascular smooth muscle cells (85-86). However, it should be noticed that soluble TRAIL promotes the survival and growth of vascular smooth muscle cells of both human and rat origin (55). Whether this reflects a differential ability of membrane-bound versus soluble TRAIL in inducing apoptosis of vascular smooth muscle cells it is not known. In this respect, it is noteworthy, however, that membrane-bound TRAIL is able to trigger apoptosis via both TRAIL-

R1 and TRAIL-R2, while soluble TRAIL is much less effective in inducing apoptosis through TRAIL-R2 (87), whose expression is widespread in normal tissues.

Recently, the potential role of soluble recombinant TRAIL in the pathogenesis and/or treatment of atherosclerosis has been investigated *in vivo*, in an animal model represented by apolipoprotein E (apoE)-null mice, in which diabetes mellitus was induced by destruction of islet cells with streptozotocin (88). Diabetes mellitus in apoE-null mice was associated with a significant increase in atherosclerotic plaque area and complexity in the aorta, as assessed by a marked increase in interstitial collagen, cellular proliferation, macrophage-infiltration and by a focal loss of endothelial coverage. Repeated intraperitoneal injections of recombinant human TRAIL significantly attenuated the total extension of the plaques development, and contributed to stabilize atherosclerotic plaques by selectively decreasing the number of infiltrating macrophages and increasing the vascular smooth muscle cell in the atherosclerotic lesions (Table 1) (88).

The potential clinical relevance of the results obtained in the apoE-null mice is corroborated by two studies carried out in patients with acute coronary syndrome, that revealed significantly lower soluble TRAIL serum levels compared to patients with stable angina or normal coronary arteries (Table 1) (84,89). In particular, it has been shown that TRAIL serum levels negatively correlated with the level of C-reactive protein (84), a nonspecific "acute-phase" protein, which represents a known marker of acute vascular events (90). Since C-reactive protein serum levels are closely associated with plaque instability and oxidative stress (91), these data further support a protective role of TRAIL against atherosclerosis development and plaque instability and indicate TRAIL as a promising therapeutic agent, not only for anti-tumor therapy but also for its anti-atherosclerotic activity.

4. OPG AND THE VASCULAR SYSTEM

4.1. OPG structure and expression

OPG is a member of the TNF-receptor superfamily, which was isolated independently by two laboratories (92-93). Unlike all other receptors of the family, OPG lacks a transmembrane and cytoplasmic domains and is secreted as a soluble protein. It is a secretory basic glycoprotein that exists in a 60-kd monomeric form and a disulfide-linked homodimeric form of 120 kd (94). It has also been detected in a cell surface–associated form with some cell types (95), although sequence analysis failed to detect a classical hydrophobic transmembrane domain, which is typical for all other members of the TNFR superfamily (94). OPG consists of 7 structural domains, of which the amino-terminal cysteine-rich domains 1 to 4 share some features with the extracellular domains of other members of the TNFR family (Figure 1) (96). The carboxy-terminal portion of the protein with domains 5 and 6 contains two putative death domain homologous regions, motifs that are found in the cytoplasmic region of transmembrane receptors mediating apoptotic signals, such as TNFR1, CD95/Fas, or TRAIL-R1 and TRAIL-R2 (Figure 1) (97-99). Finally, domain 7 harbors a heparin-binding region, a common feature of peptide growth factors and signal molecules (100-102), as well as an unpaired cysteine residue required for disulfide bond formation and dimerization (Figure 1) (94,103).

OPG is produced by a variety of tissues including the cardiovascular system (heart, arteries, veins), lung, kidney, intestine, stomach, and bone, as well as hematopoietic and immune cells (B cells and dendritic cells) (92,95,104). The major biologic action of OPG described to date is the inhibition of osteoclast differentiation and activity (92,105), but the potential role of OPG in the other tissues is still under investigation and remains to be established.

OPG has two known TNF family ligands: receptor activator of NF-kB ligand (RANKL) (106) and TRAIL (58). RANKL normally binds to its membrane receptor RANK inducing differentiation, activation, and survival of osteoclasts. By binding to RANKL, OPG acts as a soluble inhibitor that prevents RANKL/RANK interaction and subsequent osteoclastogenesis (106). However, it has been reported that also OPG binding to TRAIL inhibits TRAIL/TRAIL-receptors interaction, as revealed by the inhibition of TRAIL-induced apoptosis (58). Vice-versa, TRAIL can block the inhibitory activity of OPG on osteoclastogenesis (58). Therefore, potential cross-regulatory mechanisms involving the balance among RANKL, OPG and TRAIL has to be considered. Initially, the physiological roles of OPG have been revealed by studies in OPG deficient mice, produced by targeted disruption of the gene (107-108). OPG (-/-) mice were viable and fertile, but they exhibited severe osteoporosis caused by enhanced osteoclast formation and function. These results have indicated that OPG is a physiological regulator of osteoclast-mediated bone resorption during postnatal bone growth. *In vivo* administration of OPG resulted in an increase in bone mineral density and bone volume associated with a decrease of active osteoclast number in normal and ovariectomized rats (92). OPG has also been shown to regulate B-cell development and function and dendritic cell function (95,109), making OPG a paracrine mediator of both bone metabolism and immune functions. Consistently, OPG has been clearly implicated in various skeletal and immune disorders and diseases at the interface between bone metabolism and the immune system, such as rheumatoid arthritis (110).

4.2. Endothelium as cellular source and target of vascular OPG production

Beside the expected localization of OPG in bone tissues, OPG expression has been documented at high levels also in human arterial walls (111-113), and it is easily detectable in the serum and/or plasma of healthy subjects (67,84,89,114-116). Compared with the concentration of OPG in plasma, it has been reported that the content in aorta is approximately 500 times higher (116), assuming a water content of arterial tissue of 80% (117), and is at a level similar to that reported for bone (118). It has been found that the most efficient extraction

Table 2. Biological effects and expression of OPG in the vascular system.

Biological effects on endothelial cells	**References**
• Released in responsed to inflammatory cytokines by endothelial cells	67, 123
• Induction of endothelial cell survival	73, 129, 130
• Induction of endothelial cell growth and promotion of endothelial cord-like structures	113
Expression/effects in vascular diseases	
• Elevated expression in endothelial cells in malignant tumors	113
• Elevated plasma levels in subjects with vascular damage	67, 84, 89
• Elevated plasma levels in patients affected by coronary artery disease	114, 115
• Elevated plasma levels in diabetic patients with vascular complications	116

procedure was seen with solutions containing GnHCl; this could indicate that OPG is associated with anionic low-solubility components of the vascular wall, for example proteoglycans, which is consistent with the fact that OPG contains a heparin-binding domain (94). In this respect, heparan sulfate proteoglycans are important participants in cell-surface signaling and have been involved in actin cytoskeleton regulation, cell adhesion and migration, and modulation of specific receptor interactions (119-120). Of note, the affinity of OPG for cell surface heparan sulfates is strong enough to block infection of various continuous cell lines by the human T lymphotropic virus-type 1 (HTLV-1), which uses heparan sulfates to enter cells (121). An increased concentration of OPG in tunica media has observed in samples from diabetic individuals compared with non-diabetic individuals. This was the case under both normal-appearing intima and plaques; on the other hand, no differences between diabetic and non-diabetic subjects were observed when intimal tissue was compared (112). Altered arterial OPG content may be a consequent modification of the effects of hormones and cytokines, like insulin and TNF-alpha. Thus, it can be hypothesized that increased levels of serum OPG documented in diabetes (67,114,116) may reflect increments of arterial expression of the molecule.

In vitro, both vascular smooth muscle cells (122) and endothelial cells (73) have been shown to produce OPG, with vascular aortic smooth muscle cells producing 20-30 times more OPG than human endothelial cells. Moreover, it has been reported that exposure of both micro- and macro-vascular endothelial cells to the inflammatory cytokines TNF-alpha and IL-1beta elevates OPG expression and release by 5 to 40-fold (Table 2) (67,123). Recent studies on the intracellular localization of OPG (124) have indicated that OPG protein is found in the Weibel-Palade Bodies (WPB) of these cells, in physical association with von Willebrand Factor. Upon thrombogenic and inflammatory challenge, the contents of WPB are rapidly translocated to the plasma membrane or extracellular space, where they serve to facilitate the emigration of leukocytes and platelets into sites of inflammation and thrombus formation (125,126). These observations strongly support a modulatory role of OPG in hemostasis, vascular injury and inflammation. Consistently, in pathological conditions with prolonged exposure to inflammatory molecules (i.e., TNF-alpha and IL-1beta) such as rheumatoid arthritis, multiple myeloma, diabetes, or hyperlipidemia, OPG synthesis and storage in endothelial cells has been shown to be low, or completely absent (114,127,128), may be due to the chronic release of OPG, which may result in the exhaustion of OPG production over extended periods of time.

Based on the aforementioned evidences, it has been suggested an involvement of OPG in the inflammatory functions of endothelial cells, with endothelium acting as both cellular source and target of vascular OPG production (73). In this respect, there are accumulating data indicating a role for OPG in the regulation of endothelial cell survival *in vitro* (129-130). In particular, recombinant OPG promotes the survival of endothelial cells under conditions of serum deprivation (Table 2). Although it has been proposed that the pro-survival activity of OPG on endothelial cells may be due to inhibition of TRAIL-induced apoptosis, as also underlined in the previous sections, several reports have shown that endothelial cells are resistant to TRAIL-induced apoptosis and only under certain conditions endothelial cells may be sensitized to death induced by TRAIL (52,130). Consistently, it has been reported that endothelial cells plated on the extracellular matrix protein osteopontin, which is able to promote endothelial cell survival, had increased OPG RNA and protein secretion into the media (73). Moreover, Asou et al. (131), by using a bone disc implantation model, observed that in an osteopontin null background, angiogenesis was stimulated and resorption was inhibited. These results, in concert with the data on the possible role of OPG in endothelial cell survival, have suggested that molecules known to regulate bone cells might also regulate angiogenesis in the bone environment. More recently, Cross et al. (113) have shown that OPG stimulates endothelial cell growth, as well as the formation of cord-like structures on a matrigel substrate, providing the evidence that OPG may modulate also endothelial cell differentiation (Table 2). In addition, they reported that although endothelial cells in various normal tissues or benign tumors do not express high levels of OPG, it is highly expressed by endothelial cells in the majority of malignant tumors examined (Table 2). In particular, in breast cancers endothelial expression of OPG seems to be associated with increasing tumor grade (113). Taken together, these results have suggested that the increased levels of OPG expression may be associated with tumor development and/or progression.

4.3. OPG and vascular diseases

OPG knockout mice do not display a phenotype implicating disrupted vascular function, suggesting that OPG is not essential for the development and maintenance of the normal vasculature. However, OPG-deficient mice, behind a decrease in total bone density with a high

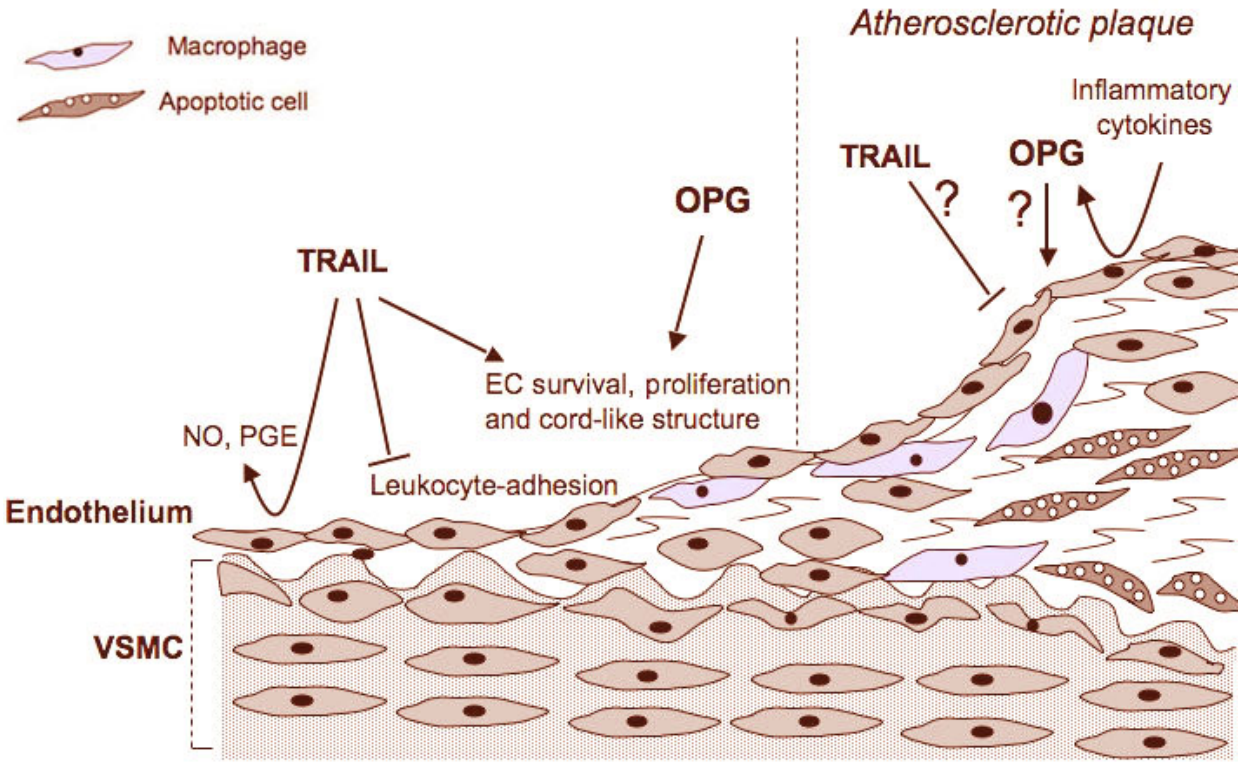

Figure 2. Schematic diagram of potential functions of TRAIL and OPG in the vascular contest. EC: endothelial cells; VSMC: vascular smooth muscle cells.

incidence of bone fractures (107-108), did show calcification of the aorta and renal arteries (111), suggesting that OPG might play a role in preserving from vascular calcification. In this respect, the study by Min and coworkers (132) reported multinucleated osteoclast-like cells in the calcified vascular lesions of OPG-deficient mice. The hypothesis that the RANKL/OPG system could link osteoporosis and arterial calcification is further underlined by the high clinical prevalence and coincidence of arterial calcification and cardiovascular disease in postmenopausal women and elderly people with osteoporosis (133-135). Interestingly, a study in elderly women found a significant correlation of elevated OPG serum levels and cardiovascular mortality and similarly, an earlier study detected increased serum concentrations of OPG in osteoporotic and postmenopausal women as compared with age-matched women without osteoporosis, and OPG levels were highest in those with the highest bone turnover and the most severe osteoporosis (136-137). The potential links between OPG and vascular disease in humans have been further suggested by the detection of a single nucleotide polymorphism in the promoter region of the human gene for OPG related to vascular morphology and function (138) and several independent studies have reported elevated serum and/or plasma OPG levels in subjects with vascular damage (Table 2) (67,84,89,114-116). In particular, a strong association between plasma levels of OPG and the presence and severity of coronary artery disease was observed in non-diabetic subjects (Table 2) (114-115). In a prospective study of almost 500 women, high OPG values were associated with an increased cardiovascular mortality (114) and in another investigation, the authors found an association between OPG levels and the presence and severity of coronary artery disease in subjects undergoing coronary arteriography (115). Moreover, in a large observational study, serum concentrations of OPG were higher in diabetic than in non-diabetic subjects (114), in particular in diabetic patients with vascular complications (116), suggesting that elevated plasma levels of OPG may reflect vascular damage among patients with diabetes rather than the diabetic state per se. Although Hofbauer and Schoppet (139) have proposed that increased OPG levels may represent a defense mechanism against other factors that promote vascular pathologies, the increased levels of OPG in plasma from patients with vascular complications supports the growing concept that OPG acts as an important regulatory molecule in the vasculature and, particularly, that an abnormal and prolonged elevation of OPG levels might be involved in the development of vascular dysfunction.

5. PERSPECTIVE

In this review, we have delineated possible vascular physiopathological functions of the TRAIL and OPG molecules (summarized in Figure 2). In particular, although conclusive evidence is still lacking, both *in vitro* and *in vivo* data indicate that TRAIL may acts as a molecule with potential anti-inflammatory activity in vascular physiopathology. Although the scenario is very

complex, since the presence of leukocytes expressing membrane-bound TRAIL has been documented in atherosclerotic plaques and a potential pathogenetic role of these TRAIL-expressing leukocytes in inducing the destabilization of atherosclerotic plaques has been proposed, the ability of soluble recombinant TRAIL to induce Akt/eNOS pathway in endothelial cells, to inhibit leukocyte adhesion to endothelial cells, is suggestive of a potential anti-atherosclerotic activity of TRAIL (Figure 2). This hypothesis has been supported by our recent findings obtained in animal models of atherosclerosis, treated with recombinant TRAIL. Emerging evidence indicates that also OPG, besides its well-characterized anti-osteoclast activity, is involved in vascular homeostasis by acting in a paracrine or autocrine manner. However, controversial roles for OPG have been proposed. In one hand, OPG has been proposed as a protective factor for vascular diseases and it has been hypothesized that the increased serum OPG levels in patients with vascular pathologies are a compensatory self-defensive response to the progression of atherosclerosis. On the other hand, several emerging *in vitro* and *in vivo* evidences supports the concept that an increased production of OPG may be involved in the development of vascular dysfunction (Figure 2) by multiple potential mechanisms, some of which through its heparin-binding domain that is distinct from the domain interacting with RANKL and/or TRAIL. Nevertheless, although the ability of OPG to counteract the biological activity of TRAIL *in vivo* needs to be elucidated, it is clear that the relative concentrations and the expression patterns of TRAIL and OPG in the local microenvironment are key determinant in the TRAIL/OPG interactions and their reciprocal balance and interplay have to be considered. Overall, these studies shed light on new properties of TRAIL and OPG that should be considered from therapeutic perspectives of vascular diseases.

6. ACKNOWLEDGEMENTS

This work was supported by grants from Ministero dell'Istruzione, Università e Ricerca (PRIN) and from the Fondazione CRT, Italy.

7. REFERENCES

1. H. Shimokawa: Primary endothelial dysfunction: atherosclerosis. *J Mol Cell Cardiol* 31, 23–37 (1999)
2. U. Landmesser, B. Hornig and H. Drexler: Endothelial function: a critical determinant in atherosclerosis? *Circulation* 109(1), 1127-1133 (2004)
3. Carmeliet P: Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 6, 389–395 (2000)
4. J. R. Jackson, M. P. Seed, C. H. Kircher, D. A. Willoughby and J. D. Winkler: The codependence of angiogenesis and chronic inflammation. *FASEB J* 11, 457–465 (1997)
5. A. W. Griffioen and G. Molema: Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases and chronic inflammation. *Pharmacol Rev* 52, 237-268 (2000)
6. C. R. Mackay: Chemokines: immunology's high impact factors. *Nat. Immunol* 2, 95–101 (2001)
7. A. Naldini, A. Pucci, C. Bernini and F. Carraro: Regulation of angiogenesis by Th1- and Th2-type cytokines. *Curr Pharm Des* 9, 511–519 (2003)
8. G. Bergers, K. Javaherian, K. M. Lo, J. Folkman and D. Hanahan: Effects of angiogenesis inhibitors on multistage carcinogenesis in mice. *Science* 284, 808-812 (1999)
9. T. Alon, I. Hemo, A. Itin, J. Pe'er, J. Stone and E. Keshet: Vascular endothelial growth factor acts as a survival factor for newly formed retinal vessels and has implications for retinopathy of prematurity. *Nat Med* 1, 1024-1028 (1995)
10. A. Karsan, E. Yee, G. G. Poirier, P. Zhou, R. Craig and J. M. Harlan: Fibroblast growth factor-2 inhibits endothelial cell apoptosis by Bcl-2-dependent and independent mechanisms. *Am J Pathol* 151, 1775–1784 (1997)
11. A. J. Hayes, W. Q. Huang, J. Mallah, D. Yang, M. E. Lippman and L. Y. Li: Angiopoietin-1, and its receptor Tie-2 participate in the regulation of capillary-like tubule formation and survival of endothelial cells. *Microvasc Res* 58, 224–237 (1999)
12. H. J. Kwak, J. N. So, S. J. Lee, I. Kim and G. Y. Koh: Angiopoietin-1 is an apoptosis survival factor for endothelial cells. *FEBS Lett* 448, 249-253 (1999)
13. F. Dignat-George and J. Sampol: Circulating endothelial cells in vascular disorders: new insights into an old concept. *Eur J Haematol* 65, 215-220 (2000)
14. J. Davignon and P. Ganz: Role of endothelial dysfunction in atherosclerosis. *Circulation* 109, 27-32 (2004)
15. Carmeliet P: Angiogenesis in health and disease. *Nat Med* 9, 653-660 (2003)
16. A. Tedgui and Z. Mallat: Anti-inflammatory mechanisms in the vascular wall. *Circ Res* 88, 877–887 (2001)
17. A. Mantovani, F. Bussolino and E. Dejana: Cytokine regulation of endothelial cell function. *FASEB J* 6, 2591–2599 (1992)
18. J. S. Pober: Immunobiology of human vascular endothelium. *Immunol Res* 19, 225–232 (1999)
19. G. Krishnaswamy, J. Kelley, Y. Lakshiminarayan, J. K Smith and D. S. Chi: Human endothelium as a source of multifunctional cytokines: molecular regulation and possible role in human disease. *J Interferon Cytokine Res* 19, 91-104 (1999)
20. S. R. Wiley, K. Schooley, P. J. Smolak, W. S. Din, C. P. Huang, J. K. Nicholl, G. R. Sutherland, T. D. Smith, C. Rauch and C. A. Smith: Identification and characterization of a new member of the TNF family that induces apoptosis. *Immunity* 3, 673-682 (1995)
21. R. M. Pitti, S. A. Marsters, S. Ruppert, C. J. Donahue, A. Moore and A. Ashkenazi: Induction of apoptosis by Apo-2 Ligand, a new member of the Tumor Necrosis Factor cytokine family. *J Biol Chem* 271, 12687-12690 (1996)
22. E. W. Duiker, C. H. Mom, S. de Jong, P. H. Willemse, J. A. Gietema, A. G. van der Zee and E. G. de Vries: The clinical trail of TRAIL. *Eur J Cancer* 42(14), 2233-2240 (2006)
23. H. J. Gruss: Molecular, structural, and biological characteristics of the tumor necrosis factor ligand superfamily. *Int J Clin Lab Res* 26, 143-159 (1996)

24. G. Pan, K. O'Rourke, A. M. Chinnaiyan, R. Gentz, R. Ebner, J. Ni and V. M. Dixit: The receptor for the cytotoxic ligand TRAIL. *Science* 276, 111-113 (1997)
25. S. G. Hymowitz, M. H. Ultsch, A. Hurst, K. Totpal, A. Ashkenazi, A. M. De Vos and R. F. Kelley: A unique zinc-binding site revealed by a high-resolution X-ray structure of homotrimeric Apo2L/TRAIL. *Biochemistry* 39, 633–640 (2000)
26. N. Kayagaki, N. Yamaguchi, M. Nakayama, H. Eto, K. Okumura and H. Yagita: Type I interferons (IFNs) regulate tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) expression on human T cells: A novel mechanism for the antitumor effects of type I IFNs. *J Exp Med* 189, 1451–1460 (1999)
27. A. Strebel, F. Bachmann, M. Wernli and P. Erb: Tumor necrosis factor-related, apoptosis-inducing ligand supports growth of mouse mastocytoma tumors by killing tumor-infiltrating macrophages. *Int J Cancer* 100, 627–634 (2002)
28. K. Takeda, M. J. Smyth, E. Cretney, Y. Hayakawa, N. Kayagaki, H. Yagita and K. Okumura: Critical role for tumor necrosis factor-related apoptosis-inducing ligand in immune surveillance against tumor development. *J Exp Med* 195, 161–169 (2002)
29. M. Smyth, J. K. Takeda, Y. Hayakawa, J. J. Peschon, M. R. van den Brink and H. Yagita: Nature's TRAIL–on a path to cancer immunotherapy. *Immunity* 18, 1-6 (2003)
30. S. Bouralexis, D. M. Findlay and A. Evodokiou: Death to the bad buy: targeting cancer via Apo2L/TRAIL. *Apoptosis* 10(1), 35-51 (2005)
31. A. Ashkenazi and V. M. Dixit: Death receptors: signaling and modulation. *Science* 281, 1305-1308 (1998)
32. S. Shankar and R. K. Srivastava: Enhancement of therapeutic potential of TRAIL by cancer chemotherapy and irradiation: mechanisms and clinical implications. *Drug Resist Updat* 7, 139–56 (2004)
33. P. Secchiero, M. Vaccarezza, A. Gonelli, and G. Zauli: TNF-related apoptosis-inducing ligand (TRAIL): a potential candidate for combined treatment of hematological malignancies. *Cur Pharm Des* 10, 3673-3681 (2004)
34. H. Li, H. Zhu, C. J. Xu and J. Yuan: Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. *Cell* 94, 491-501 (1998)
35. A. Gross, X. M. Yin, K. Wang, M. C. Wei, J. Jockel, C. Milliman, H. Erdjument-Bromage, P. Tempst and S. J. Korsmeyer: Caspase cleaved BID targets mitochondria and is required for cytochrome c release, while BCL-XL prevents this release but not tumor necrosis factor-R1/Fas death. *J Biol Chem* 274, 1156-1163 (1999)
36. G. R Screaton, J. Mongkolsapaya, X. N. Xu, A. E. Cowper, A. J. McMichael and J. I. Bell: TRICK2, a new alternatively spliced receptor that transduces the cytotoxic signal from TRAIL. *Curr Biol* 7, 693-696 (1997)
37. M. A. Degli-Esposti, P. J. Smolak, H. Walczak, J. Waugh, C. P. Huang, R. F. DuBose, R. G. Goodwin and C. A. Smith: Cloning and characterization of TRAIL-R3, a novel member of the emerging TRAIL receptor family. *J Exp Med* 186, 1165-1170 (1997)
38. G. Pan, J. Ni, G. Yu, Y. F. Wei and V. M. Dixit: TRUNDD, a new member of the TRAIL receptor family that antagonizes TRAIL signalling. *FEBS Lett* 424, 41-45 (1998)
39. C. Ruiz de Almodovar, A. Lopez-Rivas, J. M. Redondo and A. Rodriguez: Transcription initiation sites and promoter structure of the human TRAIL-R3 gene. *FEBS Lett 531*, 304-308 (2002)
40. M. M. Van Noesel, S. van Bezouw, P. A. Voute, J. G. Herman, R. Pieters and R. Versteeg: Clustering of hypermethylated genes in neuroblastoma. *Genes Chromosomes Cancer* 38, 226-233 (2003)
41. S. A. Marsters, J. P. Sheridan, R. M. Pitti, A. Huang, M. Skubatch, D. Baldwin, J. Yuan, A. Gurney, A. D. Goddard, P. Godowski and A. Ashkenazi: A novel receptor for Apo2L/TRAIL contains a truncated death domain *Curr Biol* 7, 1003-1006 (1997)
42. P. M. Chaudhary, M. Eby, A. Jasmin, A. Bookwalter, J. Murray and L. Hood: Death receptor 5, a new member of the TNFR family, and DR4 induce FADD-dependent apoptosis and activate the NF-kappaB pathway. *Immunity* 7(6), 821-830 (1997)
43. M. A. Degli-Esposti, W. C. Dougall, P. J. Smolak, J. Y. Waugh, C. A. Smith and R. G. Goodwin: The novel receptor TRAIL-R4 induces NF-kappaB and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain. *Immunity* 7(6), 813-820 (1997)
44. P. Schneider, M. Thome, K. Burns, J. L. Bodmer, K. Hofmann, T. Kataoka, N. Holler and J. Tschopp: TRAIL receptors 1 (DR4) and 2 (DR5) signal FADD-dependent apoptosis and activate NF-kappaB. *Immunity 7*, 831-836 (1997)
45. F. Muhlenbeck, E. Haas, R. Schwenzer, G. Schubert, M. Grell, C. Smith, P. Scheurich and H. Wajant: TRAIL/Apo2L activates c-Jun NH2-terminal kinase (JNK) via caspase-dependent and caspase-independent pathways. *J Biol Chem* 273, 33091-33098 (1998)
46. M. Barkett and T. D. Gilmore: Control of apoptosis by Rel/NF-kappaB transcription factors. *Oncogene* 18(49), 6910-6924 (1999)
47. S. J. Brauer, C. Buneker, A. Mohr and M. Zwacka: Constitutively activated nuclear factor-KB, but not induced NF-KB, leads to TRAIL resistance by up-regulation of X-linked inhibitor of apoptosis protein in human cancer cells. *Mol Cancer Res* 4, 715-728 (2006)
48. I. Herr, D. Wilhelm, E. Meyer, I. Jeremias, P. Angel and K. M. Debatin: JNK/SAPK activity contributes to TRAIL-induced apoptosis. *Cell Death Differ* 6, 130-135 (1999)
49. W. H. Hu, H. Johnson and H. B. Shu: Tumor necrosis factor-related apoptosis-inducing ligand receptors signal NF-kappaB and JNK activation and apoptosis through distinct pathways. *J Biol Chem* 274, 30603-30610 (1999)
50. M. M. Keane, S. A. Ettenberg, M. M. Nau, E. K. Russell and S. Lipkowitz: Chemotherapy augments TRAIL-induced apoptosis in breast cell lines. *Cancer Res* 59, 734-741 (1999)
51. R. Di Pietro, P. Secchiero, R. Rana, D. Gibellini, G. Visani, K. Bermis, L. Zamai, S. Miscia, and G. Zauli: Ionizing radiation sensitizes erythroleukemic cells but not normal erythroblasts to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated cytotoxicity by selective up-regulation of TRAIL-R1. *Blood* 97, 2596-2603 (2001)
52. P. Secchiero, A. Gonelli, E. Carnevale, D. Milani, A. Pandolfi, D. Zella and G. Zauli: TRAIL promotes the

survival and proliferation of primary human vascular endothelial cells by activating the Akt and ERK pathways. *Circulation* 107, 2250-2256 (2003)
53. P. Secchiero, A. Gonelli, P. Mirandola, E. Melloni, L. Zamai, C. Celeghini, D. Milani, and G. Zauli: Tumor necrosis factor-related apoptosis-inducing ligand induces monocytic maturation of leukemic and normal myeloid precursors through a caspase-dependent pathway. *Blood* 100, 2421-2429 (2002)
54. G. Zauli, A. Pandolfi, A. Gonelli, R. Di Pietro, S. Guarnieri, G. Ciabattoni, R. Rana, M. Vitale and P. Secchiero: Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) sequentially upregulates nitric oxide and prostanoid production in primary human endothelial cells. *Circ Res* 92, 732-740 (2003)
55. P. Secchiero, C. Zerbinati, E. Rimondi, F. Corallini, D. Milani, V. Grill, G. Forti, S. Capitani and G. Zauli: TRAIL promotes the survival, migration and proliferation of vascular smooth muscle cells. *Cell Mol Life Sci* 61, 1965-1974 (2004)
56. P. Secchiero, E. Melloni, M. Heikinheimo, S. Mannisto, R. Di Pietro, A. Iacone and G. Zauli: TRAIL regulates normal erythroid maturation through an ERK-dependent pathway. *Blood* 103, 517-522 (2004)
57. P. Secchiero, A. Gonelli A, C. Celeghini, P. Mirandola, L. Guidotti, G. Visani, S. Capitani, and G. Zauli: Activation of the nitric oxide synthase pathway represents a key component of tumor necrosis factor-related apoptosis-inducing ligand-mediated cytotoxicity on hematologic malignancies. *Blood* 98, 2220-2228 (2001)
58. J. G. Emery, P. McDonnell, M. B. Burke, K. C. Deen, S. Lyn, C. Silverman, E. Dul, E. R. Appelbaum, C. Eichman, R. DiPrinzio, R. A. Dodds, I. E. James, M. Rosenberg, J. C. Lee and P. R. Young: Osteoprotegerin is a receptor for the cytotoxic ligand TRAIL. *J Biol Chem* 273, 14363-14367 (1998)
59. A. Truneh, S. Sharma, C. Silverman, S. Khandekar, M. P. Reddy, K. C. Deen, M. M. McLaughlin, S. M. Srinivasula, G. P. Livi, L. A. Marshall, E. S. Alnemri, W. V. Williams and M. L. Doyle: Temperature-sensitive differential affinity of TRAIL for its receptors. DR5 is the highest affinity receptor. *J Biol Chem* 275, 23319–23325 (2000)
60. I. Holen, P. I. Croucher, F. C. Hamdy and C. L. Eaton: Osteoprotegerin (OPG) is a survival factor for human prostate cancer cells. *Cancer Res* 62, 1619-1623 (2002)
61. T. Miyashita, A. Kawakami, T. Nakashima, S. Yamasaki, M. Tamai, F. Tanaka, M. Kamachi, H. Ida, K. Migita, T. Origuchi, K. Nakao and K. Eguchi: Osteoprotegerin (OPG) acts as an endogenous decoy receptor in tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis of fibroblast-like synovial cells. *Clin Exp Immunol* 137, 430-436 (2004)
62. B. R. Gochuico, J. Zhang, B. Y. Ma, A. Marshak-Rothstein and A. Fine: TRAIL expression in vascular smooth muscle. *Am J Physiol Lung Cell Mol Physiol* 278, L1045-1050 (2000)
63. S. M. Mariani, P. H. Krammer: Differential regulation of TRAIL and CD95 ligand in transformed cells of the T and B lymphocyte lineage. *Eur J Immunol* 28, 973-982 (1998)
64. H. A. Chapman, R. J. Riese and G. P. Shi: Emerging roles for cysteine proteases in human biology. *Annu Rev Physiol* 59, 63-88 (1997)
65. N. B. Liabakk, A. Sundan, S. Torp, P. Aukrust, S. S. Froland and T. Espevik: Development, characterization and use of monoclonal antibodies against sTRAIL: measurement of sTRAIL by ELISA. *J Immunol Meth* 259, 119-128 (2002)
66. J. W. Choi: Relationships between tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) and hematopoietic activity in healthy adults. *Ann Hematol* 84(11), 728-733 (2005)
67. P. Secchiero, F. Corallini, A. Pandolfi, A. Consoli, R. Candido, Capitani S. and G. Zauli: An increased osteoprotegerin (OPG) serum release characterizes the early onset of diabetes mellitus and may contribute to endothelial cell dysfunction. *Am J Pathol* 169, 2236–2244 (Dec 2006)
68. G. Pan, J. Ni, Y. F. Wei, G. Yu, R. Gentz and V. M. Dixit: An antagonist decoy receptor and a death domain-containing receptor for TRAIL. *Science* 277, 815-818 (1997)
69. X. D. Zhang, T. Nguyen, W. D. Thomas, J. E. Sanders and P. Hersey: Mechanisms of resistance of normal cells to TRAIL induced apoptosis vary between different cell types. *FEBS Lett* 483, 193-199 (2000)
70. A. Bielawska-Pohl, C. Crola, A. Caignard, C. Gaudin, D. Dus, C. Kieda and S. Chouaib: Human NK cells lyse organ-specific endothelial cells: analysis of adhesion and cytotoxic mechanisms. *J Immunol* 174, 5573-5582 (2005)
71. S. J. Alladina, J. H. Song, S. T. Davidge, C. Hao and A. S. Easton: TRAIL-induced apoptosis in human vascular endothelium is regulated by phosphatidylinositol 3-kinase/Akt through the short form of cellular FLIP and Bcl-2. *J Vasc Res* 42(4), 337-347 (2005)
72. J. H. Li, N. C. Kirkiles-Smith, J. M. McNiff and J. S. Pober: TRAIL induces apoptosis and inflammatory gene expression in human endothelial cells. *J Immunol* 171, 1526-1533 (2003)
73. U. M. Malyankar, M. Scatena, K. L. Suchland, T. J. Yun, E. A. Clark and C. M. Giachelli: Osteoprotegerin is an alpha vbeta 3-induced, NF-kappa B-dependent survival factor for endothelial cells. *J Biol Chem* 275, 20959–20962 (2000)
74. Y. Yu and J. D. Sato: MAP kinases, phosphatidylinositol 3-kinase, and p70 S6 kinase mediate the mitogenic response of endothelial cells to vascular endothelial growth factor. *J Cell Physiol* 178, 235–246 (1999)
75. P. Secchiero, A. Gonelli, E. Carnevale, F. Corallini, C. Rizzardi, S. Zacchigna, M. Melato and G Zauli: Evidence for a proangiogenic activity of TNF-related apoptosis-inducing ligand. *Neoplasia* 6(4), 364-373 (2004)
76. G. Cantarella, N. Risuglia, R. Dell'Eva, L. Lempereur, A. Albini, G. Pennisi, G. M. Scoto, D. N. Noonan and R. Bernardini: TRAIL inhibits angiogenesis stimulated by VEGF expression in human glioblastoma cells. *Br J Cancer* 94(10), 1428-1435 (2006)
77. C. Carlo-Stella, C. Lavazza, M. Di Nicola, L. Cleris, P. Longoni, M. Milanesi, M. Magni, D. Morelli, A. Gloghini,

A. Carbone and A. M. Gianni: Antitumor activity of human CD34+ cells expressing membrane-bound tumor necrosis factor-related apoptosis-inducing ligand. *Hum Gene Therapy* 17, 1-16 (2006)
78. P. Secchiero, F. Corallini, M. G. di Iasio, A. Gonelli, E. Barbarotto and G. Zauli: TRAIL counteracts the pro-adhesive activity of inflammatory cytokines by down-modulating CCL8 and CXCL10 chemokine expression and release. *Blood* 105(9), 3413-3419 (2005)
79. E. E. Eriksson: Leukocyte recruitment to atherosclerotic lesions, a complex web of dynamic cellular and molecular interactions. *Curr Drug Targets Cardiovasc Haematol Disord* 3, 309-325 (2003)
80. L. Zhang, A. Zalewski, Y. Liu, T. Mazurek, S. Cowan, J. L. Martin, S. M. Hofmann, H. Vlassara and Y. Shi: Diabetes-induced oxidative stress and low-grade inflammation in porcine coronary arteries. *Circulation* 108, 472-478 (2003)
81. P. Hillyer, E. Mordelet, G. Flynn and D. Male: Chemokines, chemokine receptors and adhesion molecules on different human endothelia: discriminating the tissue-specific functions that affect leukocyte migration. *Clin Exp Immunol* 134, 431-441 (2003)
82. P. Romagnani, L. Lasagni, F. Annunziato, M. Serio and S. Romagnani: CXC chemokines: the regulatory link between inflammation and angiogenesis. *Trends Immunol* 25, 201-208 (2004)
83. M. Schoppet, N. Al-Fakhri, F. Franke, N. Katz, P. Barth, B. Maisch, K. T. Pressner and L. C. Hofbauer: Localization of osteoprotegerin, tumor necrosis factor-related apoptosis-inducing ligand, and receptor activator of nuclear factor-kB in Monckeberg's sclerosis and atherosclerosis. *J Clin Endocrinol Metab* 89, 104-112 (2004)
84. Y. Michowitz, E. Goldstein, A. Roth, A. Afek, A. Abashidze, Y. Ben Gal, G. Keren and J. George: The involvement of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in atherosclerosis. *J Am Col Cardiol* 45, 1018-1024 (2005)
85. K. Sato, A. Niessner, S. L. Kopecky, R. L. Frye, J. J. Goronzy and C. M. Weyand: TRAIL-expressing T cells induce apoptosis of vascular smooth muscle cells in the atherosclerotic plaque. *J Exp Med* 203, 239-250 (2006)
86. A. Niessner, K. Sato, E. L. Chaikof, I. Colmegna, J. J. Goronzy and C. M. Weyand: Pathogen-Sensing Plasmacytoid dendritic cells stimulate cytotoxic T-cell function in the atherosclerotic plaque through interferon-alpha. *Circulation* 114, 2482-2489 (2006)
87. F. Muhlenbeck, P. Schneider, J. L. Bodmer, R. Schwenzer, A. Hauser, G. Schubert, P. Scheurich, D. Moosmayer, J. Tschopp and H. Wajant: The tumor necrosis factor-related apoptosis-inducing ligand receptors TRAILR1 and TRAIL-R2 have distinct cross-linking requirements for initiation of apoptosis and are non-redundant in JNK activation. *J Biol Chem* 275**,** 32208–32213 (2000)
88. P. Secchiero, R. Candido, F. Corallini**,** S. Zacchigna, B. Barbara, E. Rimondi, B. Fabris, M. Giacca and G. Zauli: Systemic TRAIL delivery shows anti-atherosclerotic activity in apoE-null diabetic mice. *Circulation* 114(14), 1522-1530 (2006)
89. M. Schoppet, A. M. Sattler, J. R. Schaefer and L. C. Hofbauer: Osteoprotegerin (OPG) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) levels in atherosclerosis. *Atherosclerosis* 184, 446-447 (2006)
90. G. J. Blake and P. M. Ridker: C-reactive protein and other inflammatory risk markers in acute coronary syndromes. *J Am Coll Cardiol* 41, 37S-42S (2003)
91. S. Kobayashi, N. Inoue, Y. Ohashi, M. Terashima, K. Matsui, T. Mori, H. Fujita, K. Awano, K. Kobayashi, H. Azumi, J. Ejiri, K. Hirata, S. Kawashima, Y. Hayashi, H. Yokozaki, H. Itoh and M. Yokoyama: Interaction of oxidative stress and inflammatory response in coronary plaque instability: important role of C-reactive protein. *Arterioscler Thromb Vasc Biol* 23, 1398-1404 (2003)
92. W. S. Simonet, D. L. Lacey, C. R. Dunstan, M. Kelley, M. S. Chang, R. Lüthy, H. Q. Nguyen, S. Wooden, L. Bennett, T. Boone, G. Shimamoto, M. DeRose, R. Eliott, A. Colombero, H. L. Tan, G. Trail, J. Sullivan, E. Davy, N. Bucay, L. Renshaw-Gegg, T. M. Hughes, D. Hill, W. Pattison, P. Campbell, S. Sander, G. Van, J. Tarpley, P. Derby, R. Lee and W. J. Boyle: Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell* 89, 159–161 (1997)
93. E. Tsuda, M. Goto, S. I. Mochizuki, K. Yano, F. Kobayashi, T. Morinaga and K. Higashio: Isolation of a novel cytokine from human fibroblasts that specifically inhibits osteoclastogenesis. *Biochem Biophys Res Commun* 234, 137–142 (1997)
94. K. Yamaguchi, M. Kinosaki, M. Goto, F. Kobayashi, E. Tsuda, T. Morinaga and K. Higashio: Characterization of structural domains of human osteoclastogenesis inhibitory factor. *J Biol Chem* 273, 5117–5123 (1998)
95. T. J. Yun, P. M. Chaudhary, G. L. Shu, J. K. Frazer, M. K. Ewings, S. M. Schwartz, V. Pascual, L. E. Hood, E. A. Clark: OPG/FDCR-1, a TNF receptor family member, is expressed in lymphoid cells and is up-regulated by ligating CD40. *J Immunol* 161, 6113–6121 (1998)
96. S. J. Baker and E. P. Reddy: Modulation of life and death by the TNF receptor superfamily. *Oncogene* 17, 3261-3270 (1998)
97. N. Itoh and S. Nagata: A novel protein domain required for apoptosis: mutational analysis of human Fas antigen. *J Biol Chem* 268, 10932–10937 (1993)
98. A. M. Chinnaiyan, K. O'Rourke, G. L. Yu, R. H. Lyons, M. Garg, D. L. Duan, L. Xing, R. Gentz, J. Ni and V. M. Dixit: Signal transduction by DR3, a death domain–containing receptor related to TNFR-1 and CD95. *Science* 274, 990-992 (1996)
99. H. Walczak, M. A. Degli-Esposti, R. S. Johnson, P. J. Smolak, J. Y. Waugh, N. Boiani, M. S. Timour, M. J. Gerhart, K. A. Schooley, C. A. Smith, R. G. Goodwin and C. T. Rauch: TRAIL-R2: a novel apoptosis-mediating receptor for TRAIL. *EMBO J* 16, 5386–5397 (1997)
100. D. W. Leung, G. Cachianes, W. J. Kuang, D. V. Goeddel and N. Ferrara: Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 246, 1306-1309 (1989)
101. A. E. Eriksson, L. S. Cousens, L. H. Weaver and B. W. Matthews: Threedimensional structure of human basic fibroblast growth factor. *Proc Natl Acad Sci U S A* 88, 3441–3445 (1991)

102. J. D. Zhang, L. S. Cousens, P. J. Barr and S. R. Sprang: Three-dimensional structure of human basic fibroblast growth factor, a structural homolog of interleukin 1β. *Proc Natl Acad Sci U S A* 88, 3446–3450 (1991)
103. A. Tomoyasu, M. Goto, N. Fujise, S. I. Mochizuki, H. Yasuda, T. Morinaga, E. Tsuda and K. Higashio: Characterization of monomeric and homodimeric forms of osteoclastogenesis inhibitory factor. *Biochem Biophys Res Commun* 245, 382–387 (1998)
104. K. B. Tan, J. Harrop, M. Reddy, P. Young, J. Terrett, J. Emery, G. Moore and A. Truneh. Characterization of a novel TNF-like ligand and recently described TNF ligand and TNF receptor superfamily genes and their constitutive and inducible expression in hematopoietic and nonhematopoietic cells. *Gene* 204, 35–46 (1997)
105. H. Yasuda, N. Shima, N. Nakagawa, S.I. Mochizucki, K. Yano, N. Fujise, Y. Sato, M. Goto, K. Yamaguchi, M. Kuriyama, T. Kanno, A. Murakami, E. Tsuda, T. Morinaga and K. Higashio: Identity of osteoclastogenesis inhibitory factor (OCIF) and osteoprotegerin (OPG): a mechanism by which OPG/OCIF inhibits osteoclastogenesis *in vitro*. *Endocrinology* 39, 1329–1337 (1998)
106. H. Yasuda, N. Shima, N. Nakagawa, K. Yamaguchi, M. Kinosaki, S. I. Mochizuki, A. Tomoyasu, K. Yano, M. Goto, A. Murakami, E. Tsuda, T. Morinaga, K. Higashio, N. Udagawa, N. Takahashi and T. Suda: Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesisinhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A* 95, 3597–3602 (1998)
107. N. Bucay, I. Sarosi, C. R. Dunstan, S. Morony, J. Tarpley, C. Capparelli, S. Scully, H. L. Tan, W. Xu, D. L. Lacey, W.J. Boyle and W. S. Simonet: Osteoprotegerin-deficient mice develop early onset osteoporosis and arterial calcification. *Genes Dev* 12, 1260-1268 (1998)
108. A. Mizuno, N. Amizuka, K. Irie, A. Murakami, N. Fujise, T. Kanno, Y. Sato, N. Nakagawa, H. Yasuda, S. Mochizuki, T. Gomibuchi, K. Yano, N. Shima, N. Washida, E. Tsuda, T. Morinaga, K. Higashio and H. Ozawa: Severe osteoporosis in mice lacking osteoclastogenesis inhibitory factor/osteoprotegerin. *Biochem Biophys Res Comm* 247, 610-615 (1998)
109. T. J. Yun, M. D. Tallquist, A. Aicher, K. L. Rafferty, A. J. Marshall, J. J. Moon, M. E. Ewings, M. Mohaupt, S. W. Herring, E. A. Clark: Osteoprotegerin, a crucial regulator of bone metabolism, also regulates B cell development and function. *J Immunol* 166(3), 1482-1491 (2001)
110. L. C. Hofbauer and M. Schoppet: Clinical implications of the osteoprotegerin/RANKL/RANK system for bone and vascular disease. *JAMA* 292, 490–495 (2004)
111. B. J. Bennett, M. Scatena, E. A. Kirk, M. Rattazzi, R. M. Varon, M. Averill, S. M. Schwartz, C. M. Giachelli, M. E. Rosenfeld: Osteoprotegerin inactivation accelerates advanced atherosclerotic lesion progression and calcification in older ApoE-/- mice. *Arterioscler Thromb Vasc Biol* 26(9):2117-24 (2006)
112. J. Golledge, M. McCann, S. Mangan, A. Lam and M. Karan: Osteoprotegerin and osteopontin are expressed at high concentrations within symptomatic carotid atherosclerosis. *Stroke* 35, 1636-1641 (2004)
113. S. S. Cross, Z. Yang, N. J. Brown, S. P. Balasubramanian, C. A. Evans, J. K. Woodward, H. L. Neville-Webbe, J. M. Lippitt, M. W. Reed, R. E. Coleman and I. Holen: Osteoprotegerin (OPG)-a potential new role in the regulation of endothelial cell phenotype and tumour angiogenesis? *Int J Cancer* 118(8), 1901-1908 (2006)
114. W. S. Browner, L. Y. Lui and S. R. Cummings: Associations of serum osteoprotegerin levels with diabetes, stroke, bone density, fractures, and mortality in elderly women. *J Clin Endocrinol Metab* 86(2), 631-637 (2001)
115. S. Jono, Y. Ikari, A. Shioi, K. Mori, T. Miki, Hara K and Y. Nishizawa: Serum osteoprotegerin levels are associated with the presence and severity of coronary artery disease. *Circulation* 106, 1192–1194 (2002)
116. S. T. Knudsen, C. H. Foss, P. L. Poulsen, N. H. Andersen, C. E. Mogensen and L. M. Rasmussen: Increased plasma concentrations of osteoprotegerin in type 2 diabetic patients with microvascular complications. *Eur J Endocrinol* 149, 39–42 (2003)
117. L. M. Rasmussen and T. Ledet: Aortic collagen alterations in human diabetes mellitus. Changes in basement membrane collagen content and in the susceptibility of total collagen to cyanogen bromide solubilisation. *Diabetologia* 36, 445–453 (1993)
118. T. Ueland, K. Brixen, L. Mosekilde, L. Mosekilde, A. Flyvbjerg and J. Bollerslev: Age-related changes in cortical bone content of insulin-like growth factor binding protein (IGFBP)-3, IGFBP-5, osteoprotegerin, and calcium in postmenopausal osteoporosis: a cross-sectional study. *J Clin Endocrinol Metab* 88, 1014–1018 (2003)
119. A. Woods, and J.R. Couchman JR: Syndecans: synergistic activators of cell adhesion. *Trends Cell Biol* 8, 189-192 (1998)
120. P. Secchiero, D. Sun, A. De Vico, R.W. Crowley, M.S. Reitz, G. Zauli, P. Lusso, and R.C. Gallo: Evidence for a role of the extracellular domain of human herpesvirus 7 glycoprotein B in the viral binding to cell surface heparan sulfate proteoglycans. *J Virol* 71, 4571-4580 (1997)
121. K. Okuma, K.P. Dalton, L. Buonocore, E. Ramsburg, and J.K. Rose. Development of a novel surrogate virus for human T-cell leukemia virus type 1: inhibition of infection by osteoprotegerin. *J Virol* 77, 8562-8569 (2003)
122. J. Zhang, M. Fu, D. Myles, X. Zhu, J. Du, X. Cao and Y. E. Chen: PDGF induces osteoprotegerin expression in vascular smooth muscle cells by multiple signal pathways. *FEBS Lett* 521, 180–184 (2002)
123. P. Collin-Osdoby, L. Rothe, F. Anderson, M. Nelson, W. Maloney and P. Osdoby: Receptor activator of NF-kB and osteoprotegerin expression by human microvascular endothelial cells, regulation by inflammatory cytokines, and role in human osteoclastogenesis. *J Biol Chem* 276, 20659-20672 (2001)
124. A. C. Zannettino, C. A. Holding, P. Diamond, G. J. Atkins, P. Kostakis, A. Farrugia, J. Gamble, L. B. To, D. M. Findlay and D. R. Haynes: Osteoprotegerin (OPG) is localized to the Weibel-Palade bodies of human vascular endothelial cells and is physically associated with von Willebrand factor. *J Cell Physiol* 204(2), 714-723 (2005)
125. M. A. Arnaout: Cell adhesion molecules in inflammation and thrombosis: Status and prospects. *Am J Kidney Dis* 21(1), 72-76 (1993)

126. D. D. Wagner: The Weibel-Palade body: The storage granule for von Willebrand factor and P-selectin. *Thromb Haemost* 70(1), 105–110 (1993)
127. N. Giuliani, R. Bataille, C. Mancini, M. Lazzaretti and S. Barille: Myeloma cells induce imbalance in the osteoprotegerin/osteoprotegerin ligand system in the human bone marrow environment. *Blood* 98(13), 3527-3533 (2001)
128. R. Wallin, N. Wajih, G. T. Greenwood and D. C. Sane: Arterial calcification: A review of mechanisms, animal models, and the prospects for therapy. *Med ResRev* 21(4), 274–301 (2001)
129. L. B. Pritzker, M. Scatena and C. M. Giachelli: The role of osteoprotegerin and tumor necrosis factor related apoptosis-inducing ligand in human microvascular endothelial cell survival. *Mol Biol Cell* 15(6), 2834-2841 (2004)
130. M. Scatena and C. Giachelli: The alpha(v)beta3 integrin, NF-kappaB, osteoprotegerin endothelial cell survival pathway. Potential role in angiogenesis. *Trends Cardiovasc Med* 12(2), 83-88 (2002)
131. Y. Asou, S. R. Rittling, H. Yoshitake, K. Tsuji, K. Shinomiya, A. Nifuji, D. T. Denhardt and M. Noda: Osteopontin facilitates angiogenesis, accumulation of osteoclasts, and resorption in ectopic bone. *Endocrinology* 142, 1325-1332 (2001)
132. H. Min, S. Morony, I. Sarosi, C. R. Dunstan, C. Capparelli, S. Scully, G. Van, S. Kaufman, P. J. Kostenuik, D. L. Lacey, W. J. Boyle and W. S. Simonet: Osteoprotegerin reverses osteoporosis by inhibiting endosteal osteoclasts and prevents vascular calcification by blocking a process resembling osteoclastogenesis. *J Exp Med* 192, 463-474. (2000)
133. R. Boukhris and K. L. Becker: Calcification of the aorta and osteoporosis. A roentgenographic study. *J Am Med Assoc* 219, 1307-1311 (1972)
134. D. M. Kado, W. S. Browner, T. Blackwell, R. Gore and S. R. Cummings: Rate of bone loss is associated with mortality in older women: a prospective study. *J Bone Miner Res* 15, 1974–1980 (2000)
135. A. E. Hak, H. A. Pols, A. M. van Hemert, A. Hofman and J. C. Witteman: Progression of aortic calcification is associated with metacarpal bone loss during menopause: a population-based longitudinal study. *Arterioscler Thromb Vasc Biol* 20, 1926–1931 (2000)
136. K. Yano, E. Tsuda, N. Washida, F. Kobayashi, M. Goto, A. Harada, K. Ikeda, K. Higashio and Y Yamada: Immunological characterization of circulating osteoprotegerin/osteoclasto-genesis inhibitory factor: increased serum concentrations in postmenopausal women with osteoporosis. *J Bone Miner Res* 14, 518–527 (1999)
137. P. A. Price, H. H. June, J. R. Buckley and M. K. Williamson: Osteoprotegerin inhibits artery calcification induced by warfarin and by vitamin D. *Arterioscler Thromb Vasc Biol* 21, 1610–1616 (2001)
138. L. C. Hofbauer and M. Schoppet: Osteoprotegerin gene polymorphism and the risk of osteoporosis and vascular disease. *J Clin Endocrinol Metab* 87, 4078–4079 (2002)
139. L. C. Hofbauer and M. Schoppet: Osteoprotegerin: a link between osteoporosis and arterial calcification? *Lancet* 358, 257–259 (2001)

Abbreviation: TNF: Tumor Necrosis Factor, TRAIL: TNF-Related Apotosis Inducing Ligand, OPG: Osteoprotegerin, IL: Interleukin, NOS: Nitric Oxide Synthase, MAPK: mitogen-activated protein kinase, ERK: extracellular signal-regulated kinase, PI3K: phosphatidylinositol 3-kinase, NF-kB: nuclear factor-kB, RANKL: receptor activator of NF-kB ligand

Key words: TRAIL, OPG, Intracellular Pathways, Endothelium, Vascular Diseases, Review

Send correspondence to: Professor Paola Secchiero, Department of Morphology and Embryology, University of Ferrara, Via Fossato di Mortara 66, 44100 Ferrara, Italy, Fax: 39-0532-207351, Tel: 39-0532-291574, E-mail: secchier@mail.umbi.umd.edu

http://www.bioscience.org/current/vol13.htm