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From bones to blood pressure, developing novel biologic approaches targeting the osteoprotegein pathway for pulmonary vascular disease

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ABSTRACT

Osteoprotegerin (*tnfsf11b*, OPG) is a soluble member of the TNF superfamily originally described as an important regulator of osteoclastogenesis almost 20 years ago. OPG is a heparin-binding secreted glycoprotein that exists as a 55–62 kDa monomer or a 110–120 kDa disulphide-linked homodimer. Acting as a soluble decoy receptor for RANKL, OPG actively regulates RANK signalling, and thereby osteoclastogenesis. OPG has subsequently been shown to also be a decoy receptor TNF related apoptosis inducing-ligand (*tnfsf10*, TRAIL, Apo2L). TRAIL is a type II transmembrane protein that is widely expressed in a variety of human tissues, including the spleen, lung, and prostate. Through binding to TRAIL, OPG can inhibit TRAIL-induced apoptosis of cancer cells. More recently, OPG has been demonstrated to be secreted by, and influence, vascular smooth muscle cells phenotype particularly related to vascular calcification and pulmonary vascular remodelling. In pulmonary artery smooth muscle cell (PASMC) suppression of BMP, induction of 5-HT and IL-1 signalling have been shown to stimulate the release of OPG *in vitro*, which causes cell migration and proliferation. Patients with idiopathic PAH (IPAH) demonstrate increased circulating and tissue levels of OPG, and circulating serum levels predict survival. In pre-clinical models, OPG levels correlate with disease severity. Since OPG is a naturally circulating protein, we are investigating the potential of novel biologic antibody therapies to rescue PAH phenotype in disease models. Further pre-clinical and mechanistic data are forthcoming, but we believe current published data identify OPG as an exciting and novel therapeutic target in PAH.

Contents

Abbreviations: 5HT, 5 hydroxytriptamine; Apo2L, Apoprotein 2 ligand; ApoE, Apolipoprotein E; BMPR2, Bone morphogenetic protein receptor type 2; CAD, Coronary artery disease; CKD, Chronic kidney disease; DR4/5, Death receptor 4/5; DcR1/2, Decoy receptor 1/2; FGF-2, Fibroblast growth factor 2; HuDEMC, Human dermal microvascular endothelial cells; HMVEC, Human microvascular endothelial cells; HUVEC, Human umbilical cord vein endothelial cells; IL-1, Interleukin 1; kDa, Kilodalton; LDLR, Low-density lipoprotein receptor; OCIF, Osteoclastogenesis inhibitory factor; OPG, Osteoprotegerin; PAD, Peripheral artery disease; PAH, Pulmonary arterial hypertension; PASMC, Pulmonary arterial hypertension; RANK, Receptor activator of nuclear factor κ B; RANKL, Receptor activator of nuclear factor κ B ligand; TNF, Tumor necrosis factor; TRAF6, Tumor necrosis factor receptor associated factor 6.

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1. Introduction

Since its discovery nearly 20 years ago, there have been significant advances in our understanding of the role of osteoprotegerin in health and disease. Osteoprotegerin, meaning "to protect bone," was originally purified from human fibroblast conditioned media and described as the osteoclastogenesis inhibitory factor (OCIF) by Tsuda et al. (1997) because of its ability to inhibit bone reabsorption (Tsuda et al., 1997). Within the same year, Simonet et al. identified OPG as an important regulator of bone density after transgenic mice overexpressing OPG developed osteopetrosis and a decrease in osteoclast number (Simonet et al., 1997). Analysis of a foetal rat intestinal library revealed a 401-amino-acid-long, secreted cytokine with an N-terminus analogous to TNF receptor superfamily members (Simonet et al., 1997). We now know that OPG is a heparin-binding secreted glycoprotein belonging to the TNF receptor superfamily that exists as either a 55–62 kDa monomer or a 110–120 kDa disulphide-linked homodimer (Simonet et al., 1997; Zauli et al., 2007).

OPG contains a 21-amino-acid long signal peptide that is cleaved to generate the mature, 380-amino-acid-long form (Zauli et al., 2007). The OPG protein consists of seven structural domains; the function of all but one of these domains has been determined. Domains 1–4 are cysteine rich and share structural similarities with the TNF receptor extracellular domains, and are sufficient to abolish osteoclastogenesis. Domains 5 and 6 contain death domains, which share similarities with both the Fas and TRAIL death receptors. Domain 7 consists of 50 amino acids and contains the cys-400 residue that is essential for disulphide bond formation and dimerisation of OPG (Yamaguchi et al., 1998). Domain 7 may also play an important role in regulating the release and activity of OPG (Zauli et al., 2007).

2. The osteoprotegerin/receptor activator of nuclear factor κ B ligand/receptor activator of nuclear factor κ B axis in bone biology

The osteoprotegerin (OPG), receptor activator of nuclear factor κ B ligand (RANKL), receptor activator of nuclear factor κ B (RANK) axis plays an important role in bone remodelling and is critical for regulating bone density. OPG acts as a decoy receptor for RANKL to inhibit osteoclastogensis (Yasuda et al., 1998; Vitovski et al., 2007). Bone is continuously renewed through reabsorption at the trabeculae by osteoclasts, and new bone deposition by osteoblasts (Hofbauer & Schoppet, 2004; Boyce & Xing, 2007). Osteoclastogenesis requires binding of RANKL, a type 2 homotrimeric transmembrane protein expressed on mature osteoblasts, to its receptor, RANK, a type 1 homotrimeric transmembrane protein expressed on osteoclast precursor cells (Hofbauer & Schoppet, 2004; Boyce & Xing, 2007; Vitovski et al., 2007) (Fig. 1). Upon formation, this receptor ligand complex induces osteoclast formation, activation, and survival via NF-kB through recruitment of the adaptor protein, tumor necrosis factor receptor associated factor 6 (TRAF6) (Boyce & Xing, 2007) to prevent precursor differentiation into macrophages (Hofbauer & Schoppet, 2004; Boyce & Xing, 2007; Vitovski et al., 2007). NF-kB then translocates to the nucleus to induce c-Fos expression, which subsequently results in osteoclastogenic gene transcription. OPG, secreted from osteoblasts, acts as a decoy receptor for RANKL, preventing the RANKL–RANK binding, osteoclast activation, and subsequent bone reabsorption (Hofbauer & Schoppet, 2004; Vitovski et al., 2007). Post-natal OPG is critical for the maintenance of bone density and disrupted OPG expression *in vivo* results in the development of bone disorders (Bucay et al., 1998). OPG knockout mice exhibit osteoporosis due to excessive bone reabsorption (Bucay et al., 1998), and conversely, elevated OPG levels or inactive RANKL result in osteopetrosis due to reduced bone reabsorption (Simonet et al., 1997; Vitovski et al., 2007). In order to protect against excessive bone reabsorption, OPG mRNA is up-regulated during the normal process of bone formation (Tanaka et al., 2011). The critical role for the regulation of OPG has been highlighted by genetic studies showing that mutations in OPG, that affect expression levels, have been associated with juvenile Paget's disease (Whyte et al., 2002).

3. Osteoprotegerin and tumor necrosis factor (TNF)-related apoptosis-inducing ligand in tumour cell biology

As well as its role in bone biology, OPG also plays an important role in tumour cell biology as a decoy receptor for TRAIL (Emery et al., 1998). Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL, Apo2L) is a type II transmembrane protein that is widely expressed in a variety of human tissues, including the spleen, lung, and prostate. In humans, TRAIL has four transmembrane receptors: death receptor 4 (DR4, TRAIL-R1), DR5 (TRAIL-R2), decoy receptor 1 (DcR1, TRAIL-R3), DcR2 (TRAIL-R4), and the fifth, OPG. By binding to TRAIL, OPG has been shown to inhibit TRAIL-induced apoptosis of Jurkat cells, and TRAIL also represses OPG inhibition of osteoclastogenesis (Emery et al., 1998). Through interaction with TRAIL, OPG was also found to inhibit TRAIL-induced apoptosis of ovarian cancer cells (Cross et al., 2006), a process that occurs in an $\alpha_v\beta_3$ integrin and $\alpha_v\beta_5$ integrin-dependent manner (Lane et al., 2012, 2013). OPG has also been reported to prevent TRAIL-induced apoptosis of human microvascular endothelial cells (HMVECs), a process also requiring $\alpha_v\beta_3$ (Pritzker et al., 2004) (Fig. 1).

Along with cancer cell survival, OPG has also been implicated in angiogenesis, a process required for the maintenance, development, and progression of tumours (Cross et al., 2006). OPG expression was identified in the endothelium of malignant colorectal, breast, and metastatic cancer tumours, but not in the endothelium of benign tumours or normal tissue. OPG induces human dermal microvascular endothelial cells (HuDMECs) to form cord-like capillary structure (Cross et al., 2006) and induces vessel-formation *in vivo* via heparin binding (McGonigle et al., 2008). More recently, work undertaken by Benslimane–Ahmim and colleagues has shown that OPG induces the migration and differentiation of endothelial colony-forming cells into cord-like structures, promotes fibroblast growth factor-2 (FGF2)-induced neo-angiogenesis *in vivo*, and increases endothelial colony-forming cell adhesion to fibronectin *in vitro* (Benslimane-Ahmim et al., 2013).

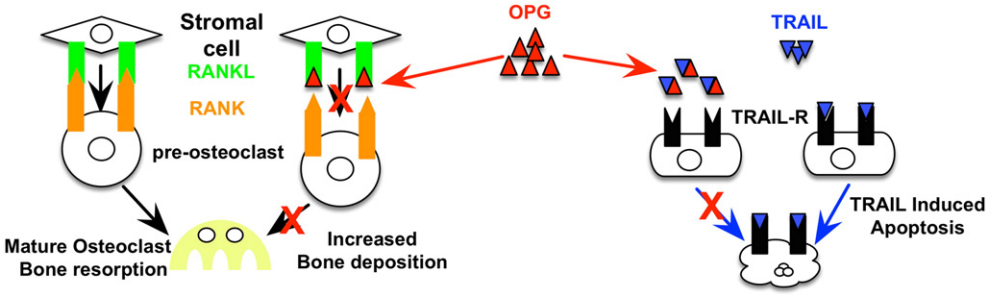

Fig. 1. *Model of OPG regulation of bone remodelling and TRAIL-induced apoptosis.* OPG binds to RANKL expressed by stromal cells to prevent RANK–RANKL binding on pre-osteoclasts to regulate osteoclastogenesis. OPG can also bind to TRAIL and inhibit TRAIL binding to TRAIL receptors expressed on tumour cells. In doing so, OPG can protect against TRAIL-induced apoptosis.