

# Recent insights into the biology of bone turnover

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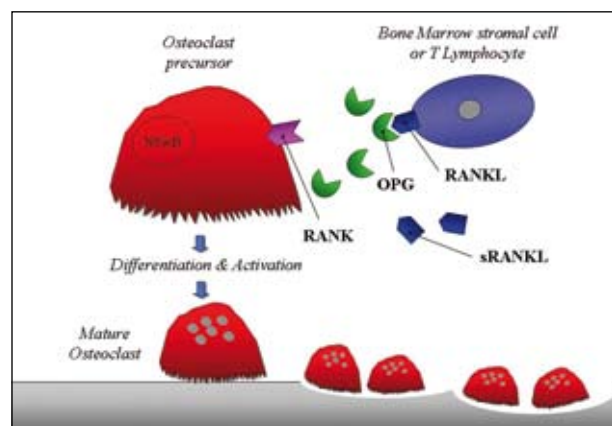
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## INTRODUCTION

The maintenance of healthy bone is an active process involving both resorption of bone by osteoclasts and the laying down of new bone by osteoblasts. It is estimated that the entire skeleton is reformed over a period of 10 to 20 years. The receptor activator of nuclear factor kappa B (RANK) pathway was identified more than ten years ago as the key regulator of osteoclast activation and cross talk between osteoblasts and osteoclasts.<sup>1,2</sup> The RANK receptor is a member of the tumour necrosis factor (TNF) receptor superfamily which is expressed on osteoclast precursors and dendritic cells, with RANK ligand (RANKL) expressed on bone marrow stromal cells and lymphocytes,<sup>3,4</sup> thereby representing an intriguing link between bone homeostasis and immune regulation.

## THE ROLE OF THE RANK PATHWAY IN THE REGULATION OF BONE TURNOVER

The RANK signalling pathway plays a critical role in regulating bone mass and bone turnover. Activation of RANK signalling in osteoclast precursors occurs on the binding of RANKL expressed by bone marrow stromal cells and activated T-cells (Figure 1), or by the binding of a soluble ligand (sRANKL) which is formed by cleavage of RANKL from the cell membrane.<sup>5</sup> This causes the activation of several intracellular signalling pathways, including the transcription factor nuclear factor kappa B (NFκB), which promote maturation and activation of osteoclasts, and consequently bone resorption. As bone marrow stromal cells mature into osteoblasts their expression of RANKL reduces, and secretion of osteoprotegerin increases, downregulating osteoclast activity and promoting bone formation.<sup>6</sup> Osteoprotegerin (OPG) is a decoy receptor for RANKL, which inhibits bone resorption by blocking its interaction with RANK (Figure 1). The essential requirement of RANK signalling for osteoclast development is demonstrated by the fact that mice with targeted inactivation of the RANK and RANKL genes develop very high bone density (osteopetrosis) due to failure of osteoclast



**FIGURE 1** The role of the receptor activator of nuclear factor kappa B (RANK) pathway in osteoclast development.

RANK ligand (RANKL) expressed by bone marrow stromal cells or T lymphocytes induces differentiation and activation of osteoclast precursors via RANK and subsequent activation of nuclear factor kappa B (NFκB). Soluble RANKL (sRANKL) is derived by cleavage from the cell membrane. Osteoprotegerin (OPG), a soluble inhibitor of RANKL, inhibits the binding of RANK by RANKL.

differentiation.<sup>7,8</sup> Although many factors have been shown to contribute to osteoclastogenesis, including interleukin-1 (IL-1); transforming growth factor (TGF) beta; TNF; IL-6; 1,25; vitamin D3; and parathyroid hormone, the effects of these agents on osteoclast activity have been shown at least in part to be mediated by alteration of the RANK signalling pathway.<sup>9</sup>

## DISTURBANCE OF THE RANK SIGNALLING PATHWAY IN DISEASE

### Osteopetrosis

Osteopetrosis is the term given to a rare group of genetic diseases characterised by defects in osteoclast differentiation or function. Patients typically develop increased bone density with a paradoxical increase in bone fragility. Examination of bone biopsies allows the identification of 'osteoclast rich' and 'osteoclast poor' subtypes. Recent studies have shown that many patients

with 'osteoclast poor' recessive osteopetrosis have loss of function mutations in the genes which encode RANK and RANKL, resulting in a failure of osteoclast differentiation.<sup>10</sup>

### Inflammation-induced bone loss

Osteoporosis is a recognised complication of autoimmune diseases such as coeliac disease,<sup>11</sup> inflammatory bowel disease<sup>12</sup> and inflammatory rheumatic diseases, including rheumatoid arthritis.<sup>13</sup> It has been attributed to multiple mechanisms, including local and systemic inflammation and corticosteroid therapy.<sup>11,14</sup> In coeliac disease specifically, reduced bone density (osteopaenia) is found in up to 40% of patients at presentation and correlates poorly with severity of disease, emphasising that this is not a simple consequence of malabsorption.<sup>11</sup> A wealth of data now exists that explains many of these effects via alteration of the RANK signalling axis. Activated T-cells express RANKL directly, and this mediates bone loss and joint destruction that can be blocked by administering recombinant OPG.<sup>15</sup> Similarly, in rheumatoid arthritis the blockade of RANKL has been shown to impede the development of bone erosions.<sup>16</sup> Inflammatory cytokines such as IL-6 or TNF are correlated with levels of RANKL or increased RANKL/OPG in rheumatoid arthritis, coeliac disease and inflammatory bowel disease.<sup>17,18</sup> Glucocorticoid therapy has been shown to directly inhibit OPG and stimulate RANKL in osteoblasts.<sup>19</sup>

Several investigators have looked for evidence of associations between circulating levels of OPG and/or sRANKL and osteoporosis associated with inflammatory disease. The results of these studies have been surprising in paradoxically showing an association between low sRANKL levels or high circulating OPG levels and osteoporosis.<sup>20,21</sup> Rationalising this difference, many authors have emphasised the paracrine rather than endocrine action of these factors on cellular function, or alternatively have suggested that this is a compensatory mechanism attempting to restore bone homeostasis. It is important to recognise that OPG and sRANKL are produced by diverse cell types, hence the circulating levels may bear little correlation with the bone microenvironment,<sup>21-23</sup> and that there exists significant variability within currently available assays for sRANKL.<sup>22</sup>

### Paget's disease

Paget's disease of bone (PDB) is a common metabolic bone disease that affects about 3% of individuals over the age of 55 in the UK.<sup>24</sup> Paget's disease is characterised by focal areas of increased osteoclastic bone resorption, coupled to increased and disorganised bone formation. The cause of PDB is incompletely understood, but accumulating evidence suggests that genetic factors play a major role.<sup>25</sup> Over the past five years it has become clear that mutations in genes within the RANK pathway play a key role in the pathogenesis of PDB and related disorders. Activating mutations of the *TNFRSF11A* gene

which encodes RANK have been identified as the cause of the rare bone dysplasias, familial expansile osteolysis, expansile skeletal hyperplasia and early onset familial PDB.<sup>26-28</sup> Similarly, loss of function mutations in the *TNFRSF11B* gene encoding OPG cause 'juvenile Paget's disease', a rare disease characterised by high bone turnover, bone deformity and multiple fractures.<sup>29</sup>

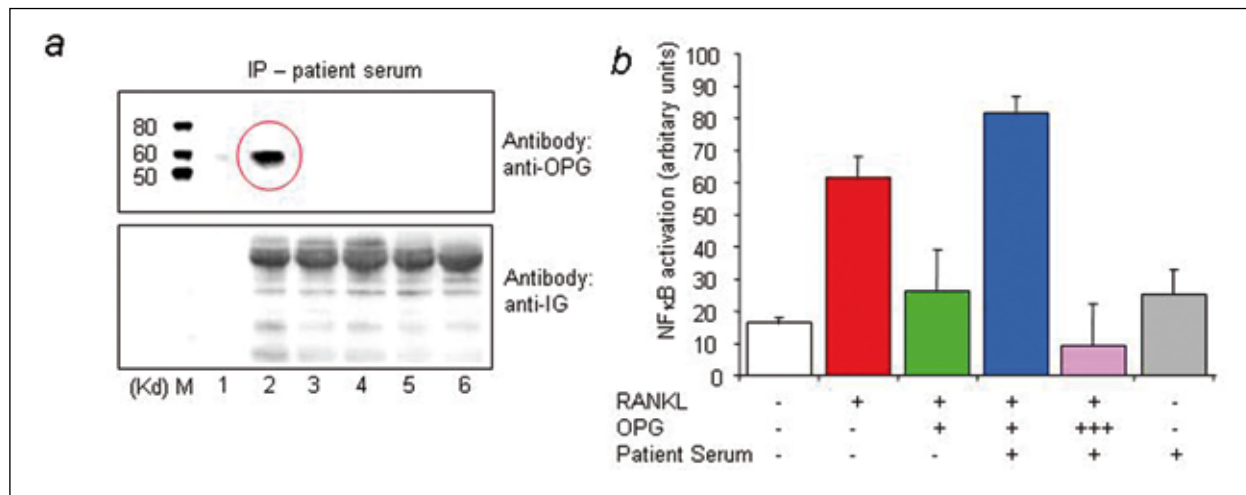
Mutations in the *SQSTM1* gene have now been identified as the most important cause of PDB, accounting for up to 40% of familial and 11% of sporadic PDB cases. *SQSTM1* encodes a scaffold protein involved in NFκB signalling downstream of RANK, explaining its effect on osteoclastic bone resorption.<sup>30</sup> Finally, mutations in the *valosin-containing protein (VCP)* gene cause the syndrome of inclusion body myopathy, PDB and fronto-temporal dementia. Once again the effect on bone can be explained by the involvement of the *VCP* gene in NFκB signalling; this appears to be mediated by targeting the inhibitor of NF kappa B (IκB) for degradation by the proteasome.<sup>30</sup>

### Osteoporosis

There is increasing evidence that common genetic variants in the *TNFRSF11A*, *TNFRSF11B* and *TNFSF11* genes encoding RANK, OPG and RANKL respectively play a role in the regulation of bone mass and susceptibility to osteoporotic fractures.<sup>31</sup> Oestrogen deficiency is a key risk factor for post-menopausal osteoporosis, which is partially explained by oestrogen's demonstrated ability to enhance OPG production by osteoblasts.<sup>32</sup>

Investigators have looked for evidence of associations between circulating levels of OPG and/or sRANKL and post-menopausal osteoporosis, but again paradoxically there are reports of high circulating OPG levels and low sRANKL levels in patients with osteoporosis.<sup>33</sup>

Recently, a novel mechanism of osteoporosis has been described due to the development of neutralising antibodies to OPG.<sup>34</sup> These antibodies block the inhibitory effect of OPG on RANK signalling (Figure 2). The index case was a 40-year-old man who presented with a low trauma fracture and severe osteoporosis (lumbar spine T score of -6.6). He was found to have coeliac disease and autoimmune hypothyroidism, but his osteoporosis deteriorated despite appropriate treatment of these conditions and calcium and vitamin D supplements. At baseline the patient was found to have markedly elevated bone turnover, with serum alkaline phosphatase levels 20 times above the upper limit of normal and collagen breakdown product deoxypyridinoline values also 20 times above the upper limit of normal in urine samples. On bone biopsy he was found to have greatly increased numbers of osteoclasts. He was eventually treated with zoledronic acid, which restored levels of bone turnover and increased bone mineral density values to within the normal range.



**FIGURE 2** Identification of neutralising antibodies to osteoprotegerin.

a) Recognition of OPG by serum from the index patient (lane 2) but not controls (lanes 3–6) demonstrated by immunoprecipitation assay. Lane 1 is a negative control. The bottom panel is probed with an anti-immunoglobulin antibody demonstrating equal loading.

b) Blockade of the inhibitory effect of OPG on RANK signalling by patient serum in vitro. RANK stimulation by RANKL (100 ng/ml) is demonstrated in human embryonic kidney-derived cells (HEK293) stably expressing a NFκB reporter vector (red). The addition of OPG (100 ng/ml) blocks this signalling (green). Patient serum (1/40 dilution) reversed this inhibition (blue), but this could be overcome by the addition of excess OPG (400 ng/ml) (purple). There was no effect on signalling in the absence of RANKL stimulation (grey).

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Autoantibodies to OPG were found in about 20% of a small cohort of patients with coeliac disease.<sup>34</sup> This raises the possibility that these antibodies might contribute to the pathogenesis of osteoporosis in coeliac disease more widely, although the clinical relevance of these findings remains to be established.

## CLINICAL IMPLICATIONS

The identification of the RANK pathway has opened up novel therapeutic targets in the management of osteoporosis. An Fc-OPG construct showed effectiveness in reducing bone turnover,<sup>35</sup> although this is no longer being developed in clinical trials. More recently denosumab, a fully humanised anti-RANKL antibody has shown effectiveness in the treatment of post-menopausal osteoporosis.<sup>36</sup> Alternatively, the signalling cascade induced by RANK binding may be targeted by small molecule inhibitors, as has been demonstrated in other members of the TNF superfamily.<sup>37</sup>

The observation that neutralising antibodies to OPG cause severe osteoporosis raises the possibility that screening for OPG antibodies, particularly in patients with autoimmune disease, may be a valuable method for detecting patients at risk of osteoporosis and guiding their subsequent management. Such patients would be expected to have high bone turnover and therefore be particularly suitable for treatment with antiresorptive drugs. It is also possible that specific treatments may be developed to block the neutralising antibody itself.

Since RANK signalling has been shown to be a critical regulator of diverse functions such as lymph node organogenesis,<sup>7</sup> thymic development<sup>38</sup> and temperature regulation,<sup>39</sup> there will need to be careful evaluation of any novel therapy targeting this pathway.

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