Review

Interaction between osteoblast and osteoclast: impact in bone disease

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Summary. The intercellular communication between osteoblasts and osteoclasts is crucial to bone homeostasis. Since Rodan and Martin proposed the control of osteoclasts by osteoblasts in the 1980s, many factors have been isolated from osteoblasts and shown to regulate the differentiation and function of osteoclasts. However, the mechanism by which osteoblasts regulate osteoclasts during bone remodelling is still unclear. On the other hand, it is well accepted that many metabolic bone diseases are associated with the disruption of the communication between osteoblast and osteoclasts. Thus, this review focuses on the cross-talk between osteoblasts and osteoclasts and its impact in bone disease.

Keywords: Osteoclast, Osteoblast, Bone disease, Osteoporosis, Interaction

Introduction

Bone is a crucial tissue that provides internal skeletal support for every organ as well as forming and structuring the entire human frame. In addition, bone is the home for the formation of haematopoietic cells and the regulation of blood calcium. Due to its importance in the human body, bone needs to be continuously replenished in order to maintain its strength and structural integrity. This replenishment, also known as bone remodelling, is controlled by two equal, but opposing, forces: bone formation by osteoblasts and bone destruction or resorption by osteoclasts. Intimate communication between these cells is an integral element in maintaining bone homeostasis. Despite the vigorous regulation and control of bone equilibrium, changes in remodelling can occur, either by defects in the cell or obstruction of the intercellular communication between the cells, which leads to debilitating bone diseases such as osteoporosis, osteopetrosis,

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osteogenesis imperfecta and even Paget's Disease. The treatment of these diseases can cost billions of dollars a year, and diseases such as osteoporosis have a relatively high incidence rate.

This review will discuss the existence of a void in osteoblast biology and explain the need to focus on the regulation of osteoblast function by osteoclasts, rather than concentrating exclusively on the osteoclast. Specifically, we will focus on the biology of osteoblasts and osteoclasts, the interaction between these two cells and the role of this "cross-talk" in bone diseases.

Osteoblasts and bone formation

Differentiation of osteoblast

Osteoblasts are specialised stromal cells that are exclusively responsible for the formation, deposition and mineralisation of bone tissue. Specifically, the mature osteoblasts synthesise and deposit calcium phosphate crystals, mainly hydroxyapatite, and various constituents of extracellular matrix, such as type 1 collagen and proteoglycans (Young et al., 1992; Robey et al., 1993; Katagiri and Takahashi, 2002).

Depending on the activation of specific transcription factors and proteins, mesenchymal cells can differentiate into four major cell types: osteoblasts, adipocytes, chondrocytes and myoblasts (Yamaguchi and Kahn, 1991; Zheng et al., 1992; Black and Olson, 1998; Rosen and Spiegelman, 2000; Katagiri and Takahashi, 2002). The program of osteoblast differentiation from mesenchymal cells is outlined in Fig. 1. Two important transcription factors, Runx and Osterix, play an essential role in driving the mesenchymal cells to the osteoblast lineage (Karsenty, 1999; Yamaguchi et al., 2000; Komori, 2003). The Runx gene codes for a small family of transcription factors consisting of three proteins, Runx 1, 2 and 3. Studies have shown that a Runx2 double knockout mouse (Runx2-/-) completely loses its ability to differentiate mesenchymal cells to osteoblastic cells (Komori et al., 1997; Otto et al., 1997). Likewise, studies with the Osterix-knockout mice further revealed an absence of bone formation, although interestingly,

these knockout mice still expressed Runx2 (Nakashima et al., 2002). It must be noted, however, that although Runx and Osterix are crucial for osteoblast differentiation, several investigations have recently demonstrated several Runx/Osterix-independent mechanisms for osteoblastogenesis. Abe et al. (2003), recently discovered that thyroid stimulating hormone could inhibit differentiation of osteoblastic cells in a Runx/Osterix-independent manner through the down-regulation of several signal transduction molecules, LRP (LDL-receptor related Protein) and Flk (tyrosine kinase receptor) (Abe et al., 2003). Moreover, Lee et al. (2003) showed that BMP-2-induced expression of Osterix, in MC3T3 cells, was Runx independent, although osterix has been proposed to be downstream of Runx (Nakashima et al., 2002; Lee et al., 2003a,b). No one can deny the importance of Runx and Osterix in the differentiation of mesenchymal cells to osteoblasts, however, these studies highlighted the realisation that there are several Runx/Osterix-independent mechanisms that are yet to be elucidated.

Upon the induction of osteoblast differentiation, osteoprogenitors under go rapid proliferation and differentiation into pre-osteoblastic cells (Fig. 1). The latter are regarded as immediate precursors to osteoblasts and are generally flatter in morphology (Aubin and Liu, 1996; Stein et al., 1996). Being precursors, however, they share similar molecular markers to osteoblasts. For instance, pre-osteoblastic cells share the same alkaline phosphatase, collagenase 3 and osteopontin markers as osteoblasts, although the expression of these markers is usually much higher in mature osteoblasts (Aubin and Liu, 1996; Stein et al., 1996). Pre-osteoblasts do not have the capacity to form bone matrix and have limited capability of dividing. Mature osteoblasts possess several unique biochemical markers and growth receptors including osteocalcin, bone sialoprotein, parathyroid hormone (PTH) receptors, insulin-like growth factor, integrins and cytokines. It is noteworthy that irrespective of what markers are expressed by the cell of the bone lineage, osteoblasts are fundamentally characterised by their primary and exclusive function: the ability to form osseous tissue identifiable as bone.

Once the bone matrix is produced by mature osteoblasts, a small proportion of these cells further differentiate and embed themselves within the newly formed extracellular bone matrix. These newly differentiated cells are termed osteocytes, and neither form nor destroy bone (Fig. 1). Instead they maintain the structural integrity of the bone and allow the bone to conform to any mechanical and chemical stimulus (mechano-transduction). Osteocytes are remarkably different to osteoblasts. They are physically smaller in size and as a consequence have smaller organelles. This means that osteocytes usually have significantly lower bone matrix protein secretion, lower metabolic and alkaline phosphatase activity and generally do not divide, unlike osteoblasts which may divide further (Nijweide et al., 1996; Knothe Tate et al., 2004). It is generally considered that osteocytes are the most differentiated cell in the osteoblast lineage and most osteocytes either remain embedded within bone matrix for several years or eventually undergo apoptosis (Nijweide et al., 1996; Knothe et al., 2004). However, recent reports have indicated that osteocytes and bone lining cells can be reactivated back to osteoblastic cells. Dobnig et al. (1995) reported that intermittent treatment with Parathyroid Hormone could recondition osteocytes back to osteoblastic cells leading to an increase in bone formation (Dobnig and Turner, 1995). Leaffer et al. (1995) also reported the same results in mice when RS-23581 peptide, which is a synthetic parathyroid hormone peptide, was injected daily into ovariectomized mice (Leaffer et al., 1995). Approximately 90% of all bone lining cells became osteoblastic when treated with RS-23581.

Skeletal development and bone formation

Skeletal development in the body can occur via two distinct pathways: Intramembranous and endochondral ossification. Intramembranous ossification occurs when the pluripotent mesenchymal stem cells condense and specifically enter the osteoblast lineage and eventually differentiate into osteoblastic cells. The newly formed cells then directly synthesise and mineralise new bone matrices (Hall and Miyake, 1992; Erlebacher et al., 1995; Aubin and Liu, 1996; Karsenty, 1999). This type of skeletal formation usually produces flat bones, such as the bone present in the skull, mandible and clavicle. For the rest of the skeletal structure, bone is formed via an alternate pathway, endochondral ossification. In this mechanism the mesenchymal stem cells condense and first differentiate into chondrocytes to form a rigid template of cartilage (Erlebacher et al., 1995; Karsenty 1999). Osteoblasts are then subsequently differentiated from the surrounding mesenchymal stem cells and eventually form bone that replaces the cartilage template
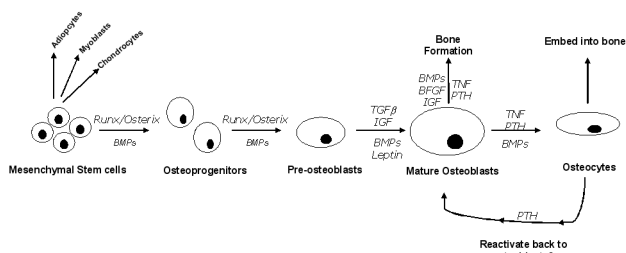

Fig. 1. Schematic representation of the osteoblast lineage from stem cell to osteocytes. Only common cytokines and growth factors have been mentioned in this diagram. BMP: Bone Morphogenic Protein; TGFß: Transforming Growth Factor Beta; IGF: Insulin-like Growth Factor; TNF: Tumour Necrosis Factor; PTH: Parathyroid Hormone; BFGF: Basic Fibroblast Growth Factor; LIF: Leukemia Inhibitory Factor.

(Erlebacher et al., 1995; Karsenty, 1999). This type of ossification produces the majority of the bones in the body, including the vertebrate. For both mechanisms, osteoblastic bone formation is the same. Osteoblasts attach themselves to the cartilage surface, for endochondral ossification, or for intramembranous ossification they begins to synthesise the matrix directly. The synthesis of the bone matrix begins with the production and deposition of type 1 collagen, which is the most abundant and important extracellular matrix protein identified in bone. Collagen 1 is a structural protein of bone that provides strength and elasticity. Furthermore, type 1 collagen provides a scaffold for the growth of bone and facilitates the deposition of other matrix proteins, such as hydroxyapatite. Other bone matrix proteins are also synthesised and deposited by the osteoblasts, including proteoglycans, glycoproteins, osteopontin and hydroxyapatite crystals.

Cytokine regulation of osteoblast

An army of cytokines and molecules have been demonstrated to facilitate the proliferation and differentiation of osteoprogenitors to pre-osteoblastic and mature osteoblastic cells. Bone Morphogenic Protein (BMP) is a family of proteins that can act on early osteoprogenitors to instigate their differentiation to pre-osteoblastic cells (Yamaguchi et al., 2000). BMPs are a part of the Transforming Growth Factor Beta (TGFß) superfamily of cytokines that has been shown to promote the proliferation, differentiation and apoptosis of a wide range of tissues and cells, including osteoblasts (Zheng et al., 1994). Yamaguchi et al. (2000) demonstrated that the addition of BMPs in pre-osteoblastic cell lines increased its alkaline phosphatase activity, as well as osteocalcin and osteopontin, all markers for mature osteoblasts (Yamaguchi et al., 2000). Yamaguchi et al. (1991) discovered that BMP increased alkaline phosphatase activity, cAMP production and osteocalcin synthesis in ROB-C26 cells, a mesenchymal stem cell line that is tri-potential; that is, has the ability to differentiate into osteoblasts, adipocytes or myoblasts (Yamaguchi and Kahn, 1991). When using MC3T3, a stem cell line committed to the osteoblast lineage, BMP further elevates alkaline phosphatase activity and osteocalcin production (Takuwa et al., 1991; Nakase et al., 1997). Likewise, addition of BMP-2 and BMP-7 to pluripotent mesenchymal C3H10T1/2 stem cells greatly increases the production of osteocalcin and activity of alkaline phosphatase (Katagiri et al., 1990; Wang et al., 1993; Chan et al., 2003; Gerstenfeld et al., 2003; Shea et al., 2003). This indicates that BMP could drive the commitment of mesenchymal cells to osteoblasts but could further induce osteoblastic differentiation. BMPs also influence the activation of the Runx2-Osterix transcription factors (Tsuji et al., 1998; Lee et al., 1999, 2003a,b). BMPs are involved in both intramembranous and endochondral ossification plus regulate the whole process of ectopic skeletal formation (Sampath et al.,

1993; Hazama et al., 1995). Likewise, they have been implicated in the organogenesis of hard and soft organs (Katagiri and Takahashi, 2002). BMP-5 has been revealed to be expressed during endochondral ossification inducing the condensation of mesenchymal cells to chondrocytes (King et al., 1994; Bailon-Plaza et al., 1999). Interestingly, when the BMP-5 gene is mutated it results in skeletal and bone abnormalities, indicating its importance in skeletal development (Kingsley et al., 1992; Storm et al., 1994; Wolfman et al., 2003). Once the cartilage template is made several BMPs are expressed including BMP-2 and BMP-7 (Lyons et al., 1995; Hogan 1996; Rosen et al., 1996). BMP-2, 4 and BMP-7 double knockout mice experiments reveal a lethal mouse model due to severe bone and skeletal defects as well as renal and eye defects (Luo et al., 1995; Zhang and Bradley 1996; Katagiri et al., 1998). Several BMPs have been reported to act as a negative regulator of ectopic skeletal formation. For example, Daluiski et al. (2001) discovered that double knockout mice of BMP-3 increased bone mass, rather than diminished skeletal growth as is usually seen in knockout mice of other BMPs (Daluiski et al., 2001).

Sonic and Indian Hedgehog are signalling proteins that play a crucial role in skeletal formation. Originally identified in Drosophila (Hammerschmidt et al., 1997; Yamaguchi et al., 2000), hedgehog is considered to be a signalling molecule that regulates tissue patterning and segmentation, including bone tissue. Additionally, hedgehog co-ordinates the condensation of mesenchymal cells, differentiation of chondrocytes and osteoblasts (Kronenberg, 2003). Kinto et al. (1997) examined whether Sonic and Indian hedgehog could regulate and induce ectopic skeletal formation by transplanting fibroblast cells with overexpression of both genes onto collagen gels (Kinto et al., 1997). The result demonstrated an induction of a cartilage template and subsequent replacement with bone matrix, indicative of endochondral bone formation. Other investigations have revealed the importance of Indian hedgehog in ectopic bone formation, especially endochondral ossification. St-Jacques et al. (1999) discovered that mice deficient in the Indian Hedgehog gene lack an osteoblast population in bone formed by endochondral ossification (St-Jacques et al., 1999). In contrast, bones formed by an intramembranous mechanism still had an osteoblast population (St-Jacques et al., 1999). The mechanism behind Indian hedgehog-dependent endochondral ossification remains to be elucidated, however it is known that Indian hedgehog is secreted by very early chondrocytes and binds to its receptor, PATCHED-1 (Kronenberg, 2003). This binding initiates the cascade of gene activations and tissue patterning for endochondral ossification (Kronenberg, 2003). Interestingly, Indian hedgehog has further been demonstrated to interact with parathyroid hormone to regulate chondrocytes proliferation and differentiation (Vortkamp et al., 1996; Weisser et al., 2002; Jemtland et al., 2003). Another report examined the role of Indian hedgehog in ectopic

bone repair and reported that an activation of Indian Hedgehog expression was observed around the site of bone fracture. The expression was subsequently down regulated once the fracture was recovered and healed (Vortkamp et al., 1996; Ferguson et al., 1999). Interestingly, Indian hedgehog was mainly seen in the cartilage template during fracture healing, again indicating its importance in endochondral ossification. Sonic Hedgehog is crucial in endochondral ossification as well since knockout mice studies have demonstrated a lethal mice model with defects in the spinal cord and eyes, and with no vertebrae formation (Chiang et al., 1996). Moreover, investigations revealed that mutations in the sonic gene cause diseases associated with skeletal abnormalities, such as Greig syndrome and Palister-Hall Syndrome (Ming et al., 1998).

Osteoclasts and bone resorption

Differentiation of osteoclast

The most interesting feature of bone is the existence of a cell that continuously destroys the very organ that the body is trying to form. This cell is called the osteoclast. Osteoclasts are large, terminally differentiated, multinucleated cells, similar to Giant Cell Tumours (GCT) of bone, and have between 5 and 12 nuclei per cell (Suda et al., 1996). Furthermore, they have well defined and developed organelles with an abundance of mitochondria and an extensive Golgi apparatus. Activated osteoclasts have slightly different morphology compared to inactive osteoclasts. Functional osteoclasts are highly polarised and contain three separate membrane domains. Non-functional osteoclasts on the other hand, do not exhibit polarisation or any distinctive membrane domains (Fig. 2). Furthermore, inactive osteoclasts tend to have a reduced cellular volume and their motility is generally decreased. Osteoclasts are usually located within the tissue layer lining the medulla of the bone (endosteal surface), or the connective tissue layer lining the surfaces of most bones (periosteal surface) (Suda et al., 1996). Regardless of their properties, or localisation, osteoclasts are fundamentally characterised by their primary and exclusive function: to degrade bone tissue.

Unlike osteoblastic cells, osteoclasts are derived from the haematopoietic cell lines of the macrophage/monocyte lineage. Osteoclast differentiation occurs within the bone microenvironment, where interaction between monocyte pre-cursors and osteoblasts allows the precursor cells to differentiate into osteoclasts. The first evidence to demonstrate that osteoclasts are derived from the haematopoietic cell line was uncovered in 1975. Walker et al. (1975) discovered that transplantation of haematopoietic cells, such as bone marrow and spleen could restore bone resorption in mice with osteopetrosis (Walker, 1973, 1975a,b). Interestingly, osteoclasts that were extracted from these mice could only exhibit bone resorptive ability in the

presence of osteoblastic cells (Owens et al., 1996), providing evidence that osteoblastic cells are essential for osteoclastic bone resorption. Presently, osteoclasts are commonly grown in co-culture with both mouse bone marrow cells and osteoblasts/spleen cells (Udagawa et al., 1990). Very recently a haematopoietic macrophage cell line (RAW264.7) was used to form osteoclasts (Xu et al., 2000). Osteoclastic cells derived from these cell lines and co-culture systems can be distinguished from normal macrophages by typical markers and receptors that are uniquely osteoclastic. These include: intense activity of tartrate resistant acid phosphatase (TRAP), upregulation of calcitonin and vitronectin receptors, and expression of vacuolar proton ATPase (V-H$^+$ATPase) (Vaananen, 1996). Another interesting facet of osteoclasts, is their ability to exhibit terminal differentiation; that is, it is unable to differentiate back to macrophage or to any other cell. In fact, once osteoclasts stop resorbing bone it is given signals to undergo apoptosis (Vaananen et al., 2000). On the other hand osteoblastic cells can be further differentiated to osteocytes.

Osteoclastic bone resorption

Bone resorption begins similarly to osteoblastic bone formation: attachment of the cell to the bone surface (Fig. 2). The osteoclast expresses several cell surface receptors that bind specifically to adhesion molecules that facilitate the attachment of the osteoclast to the bone surface. Integrin receptors are a large family of adhesion receptors involved in cell-to-cell interaction and cell-to-matrix migration. Not only are they crucial in osteoclastic migration and bone resorption, but have also been implicated in leukocyte activation and aggregation and growth of tumour cells (Vaananen, 1996). The major
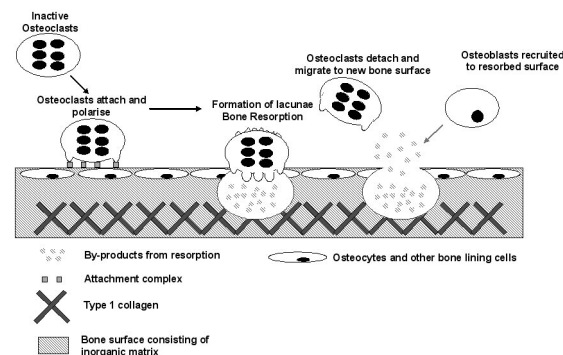

Fig. 2. A summary of the stages involved in osteoclastic bone resorption. Inactive osteoclast attaches itself to the mineralised bone surface via integrin receptors. The cell polarises into four important domains: the ruffled border, the basolateral domain, the functional secretory domain and the sealing zone. Osteoclast resorbs bone, detaches when finished and then migrate along to a new bone surface. Osteoblasts are then recruited to the site of resorption to form new bone matrix.

integrin expressed by the osteoclasts is the $\alpha_v\beta_3$ integrin. These receptors allow the attachment of the osteoclast to the surface by binding to special adhesion ligands on the bone surface, such as vitronectin. Chambers et al. (1986) first demonstrated the crucial role of $\alpha_v\beta_3$ integrins in osteoclastic mediated attachment to the bone surface, when antibodies raised against these receptors inhibited osteoclastic bone resorption (Chambers et al., 1986). Further investigations revealed that the vitronectin ligand contains a special RGD (Arginine, Glycine, Aspartic Acid) peptide sequence that interacts with integrins (Horton et al., 1991). These RGD-containing peptides are able to competitively bind to the integrin receptor, and inhibit attachment of osteoclasts to bone, as well as completely inhibiting osteoclastic bone resorption *in vitro* (Sato et al., 1990; Horton et al., 1991; Fisher et al., 1993; Engleman et al., 1997). Recently, McHugh et al. (2000) demonstrated that mice, deficient in the v3 integrins had osteosclerosis indicating that the osteoclasts in these mice were dysfunctional (McHugh et al., 2000). Despite the plethora of studies showing v3 involvement in osteoclastic attachment, the molecular mechanism behind this phenomena is yet to be elucidated. Several reports have postulated that the binding of $\alpha_v\beta_3$ integrins to vitronectin causes cellular rearrangement in osteoclasts, especially alterations in the actin cytoskeleton (Lakkakorpi and Vaananen, 1991; Duong and Rodan, 2001). Interestingly, studies have further hypothesised that v3 integrins can further interact with osteopontin, suggesting osteoblasts can indirectly induce the attachment of osteoclasts onto bone (Flores et al., 1992; Ruoslahti, 1996). v3 integrins have also been shown to induce other molecular events within osteoclasts, including elevating intracellular calcium (Zimolo et al., 1994; Paniccia et al., 1995), and activating PI3K and c-Src (Hruska et al., 1995; Duong and Rodan, 2001). Other integrins involved in the process of attachment include $\alpha_v\beta_1$, $\alpha_2\beta_1$ and $\alpha_v\beta_5$ (Duong and Rodan, 2001).

Once the osteoclast attaches to the bone surface, the cell undergoes specific morphological changes (Fig. 2). This process of polarisation allows the functional osteoclasts to produce three different membrane domains, each having their own particular function. The first is the sealing zone. This tight cell-to-matrix interaction "seals" the bone surface from the external environment and prepares the attached osteoclast for bone resorption. The sealing zone is a unique compartment in osteoclasts, since it lacks organelles, but consists of a large amount of filamentous actin (F-actin). The tight seal between the cell and bone surface, as well as the presence of F-actin in the sealing zone forms a ring structure (F-actin ring) around the resorption compartment (Vaananen and Horton, 1995; Vaananen, 1996). The molecular mechanism behind the formation of the sealing zone is not very well understood. There are conflicting investigations that demonstrate the involvement of $\alpha_v\beta_3$ integrins in the formation of the sealing zone. Hultenby et al. (1993) discovered that v3

integrins were enriched within the sealing zone of osteoclasts (Hultenby et al., 1993). In contrast, other studies revealed that $\alpha_v\beta_3$ integrins were only localised in the basolateral membrane and ruffled border of the osteoclasts, not within the sealing zone (Lakkakorpi et al., 1993). More recently, however, Nakamura et al. (1999) used a v3 integrin inhibitor called echistatin, and demonstrated that the sealing zone became dysfunctional if $\alpha_v\beta_3$ integrins were inhibited (Nakamura et al., 1999).

The polarisation of the sealing zone facilitates the formation of two more membrane domains: the basolateral domain, and more importantly, the ruffled border. The basolateral domain is the cellular membrane that is exposed to the external environment and is the "body" of the osteoclasts. Recent studies have identified a theoretical region that is part of the basolateral membrane called the functional secretory domain (Salo et al., 1996, 1997; Nesbitt and Horton, 1997). Osteoclasts, infected with influenza A virus have been shown to target the virus' glycoprotein to this theoretical domain (Salo et al., 1996). Although more research will be needed in order to ascertain the presence of this extra membrane domain, it indicates that the basolateral domain may not be homogenous, as first thought and that it may be divided into multiple domains that are as yet, unidentified. The most functionally important membrane domain is the ruffled border, the resorbing organelle of the osteoclasts. This membrane domain consists of convoluted infoldings formed via the fusion and subsequent insertion of intracellular acidic vesicles from the osteoclast plasma membrane. This fusion process reorganises the plasma membrane to form long, finger like projections that penetrate the bone matrix. This penetration forms the resorption lacunae or the resorbing compartment of osteoclasts. The ruffled border is uniquely osteoclastic and has not been identified in any other membrane of other cells.

Once polarised, the osteoclasts begin the process of bone degradation, with the first step being the dissolution of hydroxyapatite. This is demineralised by the acidification of intracellular vesicles and secretion of acids, such as hydrochloric acid, which ultimately leads to the maintenance of a low acidic pH in the resorption lacunae (Baron et al., 1985). Carbonic anhydrase II (Gay and Mueller, 1974) generates protons, which is then pumped into the resorbing compartment by the Vacuolar Proton ATPase channel (Blair and Schlesinger, 1990; Vaananen et al., 1990; Zheng et al., 1993, 1994). It has been demonstrated that V-type ATPases are highly expressed in the ruffled border of osteoclastic cells (Kawasaki-Nishi et al., 2003). Interestingly, the presence of V-ATPase subunits within the ruffled border has provided researchers pathways to control bone diseases. For instance, the use of specific inhibitors that bind to the ATPase subunits and control bone resorption *in vitro* (van Hille et al., 1995; Li et al., 1996; Visentin et al., 2000; Takami et al., 2003). Other inhibitors, such as bafilomycin, have been demonstrated to target V-ATPase directly to inhibit osteoclastic bone resorption and cause

osteopetrosis *in vivo* (Sundquist et al., 1990; Okahashi et al., 1997; Xu et al., 2003). Moreover, inhibition of CAII in rat-cultured osteoclasts led to the suppression of bone resorption (Laitala and Vaananen, 1994; Baron and Tsouderos, 2002). Furthermore, humans deficient in CAII have osteopetrosis and produce non-active osteoclasts (Sly and Hu, 1995; Borthwick et al., 2003). The continuous generation and pumping of protons, by CAII and V-ATPase, can alter the electroneutrality of the osteoclasts. However, a mechanism has been put into place to counterbalance the flow of protons. Chloride anions, present in the basolateral compartment, are channelled through special chloride pumps present on the ruffled border, and into the resorption lacunae, allowing maintenance of the electroneutrality of the compartment (Blair and Schlesinger, 1990). However, this mechanism changes the electroneutrality of the basolateral membrane. To counteract this, investigations have identified a special chloride-bicarbonate (Cl^-/HCO_3^{3-}) exchanger that removes excess bicarbonate from the basolateral membrane and restores electroneutrality (Teti et al., 1989a,b). The co-ordinate action of V-ATPase, chloride-bicarbonate exchanger and carbonic anhydrase II allows the preservation of a low acidic pH, and secretion of acids such as hydrochloric acid, which eventually leads to the dissolution of the inorganic matrix.

After the dissolution of crystalline hydroxyapatite, the osteoclasts begin to resorb the organic matrix. The organic phase comprises a large portion of the bone tissue, with most bones having around 90% of type 1 collagen (Vaananen, 1996). The degradation of this organic phase has not been fully elucidated but it is clear that several proteases and proteolytic enzymes are involved in the dissolution. The main player is thought to be the matrix metalloproteinases (MMPs) (Delaisse et al., 2003). Although the mechanism in which the MMPs degrade bone has yet to be elucidated, it is clear that several MMPs may be expressed by the osteoclasts and secreted into the resorbing compartment, such as MMP-9 and MMP-1 (Delaisse et al., 1993, 2003; Okada et al., 1995). Additionally, MMP-9 has been demonstrated to be essential in the recruitment of osteoclasts on long bones and its subsequent resorption (Engsig et al., 2000). Recently, knockout mice of MT1-MMP (MMP-14) have shown severe osteopenia and arthritis due to a defect in collagen turnover (Holmbeck et al., 1999). These observations indicate the importance of MMPs in bone resorption. Apart from MMPs, the dissolution of the organic phase is further controlled by the lysosomal cysteine proteinases. The most well studied member is cathepsin K; a serine-threonine protease that can cleave type 1 collagen. Investigations have revealed that cathepsin K is expressed by osteoclastic cells and secreted into the bone resorption lacunae (Drake et al., 1996). Moreover, mice deficient in cathepsin K had osteopetrosis indicating that this enzyme is crucial in bone resorption (Reinheckel et al., 2001). Interestingly, a mutation within the capthesin K gene causes an osteopetrotic-like disease known as pycnodystosis (Gelb et al., 1996; Johnson et al., 1996). It has been suggested that most of the by-products of bone resorption are endocytosed to the basolateral membrane, where it is secreted to the external environment (Vaananen, 1996, 2000; Salo et al., 1997; Teitelbaum 2000).

Interaction and cross talk between osteoblasts and osteoclasts

Osteoblasts control the ontogeny and function of osteoclasts

It has been well established that osteoblasts plays a pivotal role in the differentiation and function of osteoclasts by producing several factors that directly bind to osteoclastic precursors (Rodan and Martin, 1981). Until recently, it was unclear how osteoclast differentiation was controlled. However, Yoshida et al. (1990) partially solved this enigma by examining osteoclastogenesis through their osteopetrotic mice model (Yoshida et al., 1990). Osteoblastic cells, extracted from these osteopetrotic mice, could not induce osteoclastogenesis when cultured together with normal osteoclast precursor cells *in vitro*. However osteoclast precursor cells extracted from the same mice could generate into osteoclasts when exposed to normal osteoblastic cells. This reinforced the importance of osteoblasts in osteoclastogenesis. Furthermore, the osteopetrotic mice model allowed the characterisation of a paracrine factor secreted by osteoblasts, known as macrophage colony stimulating factor (M-CSF), that would bind to its receptor, c-fms, on osteoclastic precursors (Wiktor-Jedrzejczak et al., 1990). Their experiments demonstrated osteopetrotic mice that lacked M-CSF had very few osteoclasts. Interestingly, the osteopetrotic phenotype was reduced when Bcl-2, an anti-apoptotic gene, was over-expressed in these mice, indicating that M-CSF may regulate the survival of osteoclastic precursors (Lagasse and Weissman, 1997). Although the importance of M-CSF in bone remodelling can not be denied, this factor, alone, can not induce osteoclastogenesis. Therefore, the search was on for a potent "hypothetical" molecule, expressed by osteoblasts, which could directly bind to osteoclastic precursors and directly differentiate them into mature bone resorbing osteoclasts.

A different molecule was discovered, one that inhibited osteoclastogenesis. Osteoprotegerin, OPG, also known as osteoclast inhibitory factor (OCIF), is a secreted protein that is part of the TNF superfamily (Simonet et al., 1997; Yasuda et al., 1998a). It is expressed by a wide range of cells including those in the heart, kidney, lung and especially by osteoblastic cells (Simonet et al., 1997). Studies revealed that overexpression of OPG induces severe osteopetrosis in transgenic mice (Simonet et al., 1997) with completely absent osteoclasts, while mice deficient in OPG had osteoporosis (Bucay et al., 1998; Mizuno et al., 1998).

Furthermore, OPG strongly inhibited osteoclast formation induced by $1,25(OH)_2$ vitamin D_3 and parathyroid hormone *in vitro* (Simonet et al., 1997; Tsuda et al., 1997). Interestingly, when OPG was added to osteoblast-osteoclast co-culture, osteoclast formation was further inhibited (Fuller et al., 1998). These reports demonstrated that OPG is a potent negative regulator of osteoclastogenesis. To further characterise OPG, researchers looked towards its receptor. This search for the receptor came to an end when Lacey et al. (1998) and Yasuda et al. (1998b) discovered a molecule called osteoprotegerin ligand (OPGL) that could bind to OPG (Lacey et al., 1998). Further characterisation of this molecule revealed very surprising results. OPGL, also known as receptor activator of NFκB ligand (RANKL) and TNF related activation induced cytokine (TRANCE), is a part of the TNF superfamily of ligands. RANKL is expressed in a wide range of tissues, including the skin, kidney and heart, although the function of RANKL in these tissues is unknown (Kartsogiannis et al., 1999). Structurally, RANKL is smaller then OPG and naturally exists as a transmembrane protein on the surface of osteoblastic cells, although it can be cleaved to a secreted form by MMPs (Lum et al., 1999). RANKL, unlike OPG, is a potent, positive regulator of osteoclastogenesis, by binding to its own receptor, RANK (Hsu et al., 1999). For instance, soluble recombinant RANKL, together with M-CSF, can directly induce the formation of osteoclasts *in vitro*, even in the absence of osteoblasts and $1,25(OH)_2$ vitamin D_3 (Quinn et al., 1998; Burgess et al., 1999). This function was further reinforced by injection of soluble recombinant RANKL into mice, which caused osteoporosis due to the increased activation of osteoclastogenesis (Karsenty, 1999). On the other hand, mice deficient in RANKL revealed an osteopetrotic phenotype and no osteoclasts (Kong et al., 1999). Thus, RANKL was the so-called potent "hypothetical" molecule that could bind directly to osteoclastic precursors and induce their differentiation. These finding revitalised bone biology. The discovery of the OPG-RANK-RANKL axis defined the hallmark mechanism of osteoclastogenesis. RANKL, expressed on osteoblasts, forces the cell to physically interact with osteoclastic precursors in order for RANKL to bind to its receptor RANK. To negatively regulate this mechanism, OPG is expressed by osteoblasts and acts as a decoy receptor to compete with RANK for RANKL. It is important to note that the common theme for the OPG- and RANKL-mediated osteoclastogenesis is that osteoblasts directly regulate osteoclasts both positively and negatively.

The interaction of RANKL with RANK activates a deluge of signal transduction pathways that allow the commitment of osteoclastic precursors to mature bone-resorbing osteoclasts. The interaction of RANKL and RANK induces the recruitment of the TNF receptor activating factor (TRAF) family. Investigations have demonstrated that several TRAF molecules, especially

TRAF 6, are recruited and interact with the C-terminal of the RANK receptor, as well as its cytoplasmic region (Darnay et al., 1998; Wong et al., 1998). Kadono et al. (2001) discovered that TRAF6, compared with TRAF 1-3, can induce osteoclastogenesis independently of other TRAFs and therefore is essential for osteoclastogenesis (Kadono et al., 2001). Furthermore, mice deficient in TRAF6 have severe osteopetrosis (Lomaga et al., 1999; Naito et al., 1999). The recruitment of TRAF to RANK induced the activation of the NfκB and mitogen-activated protein kinase (MAPK) pathway. Both pathways are essential for cellular survival, growth and apoptosis, and especially the induction of osteoclast differentiation (Troen, 2003). Darnay et al. (1998) have revealed that over-expression of RANK stimulates the NfκB and JNK (regulator of MAPK) pathway (Darnay et al., 1998). Interestingly, other studies have further demonstrated that RANK can induce activation of the Erk and Akt pathway (Wong et al., 1999).

Several other molecules, expressed by osteoblasts, can also facilitate the regulation of osteoclastogenesis via the RANKL-RANK pathway. TNFα and TNFß play an important role in osteoclastogenesis. Early investigations demonstrated that TNF can induce DNA synthesis of osteoclasts (Canalis, 1987). Further studies reported that TNFα, and to a lesser extent TNFß, increased bone resorption when bone explants were treated with TNF (Bertolini et al., 1986; Canalis, 1987; Stashenko et al., 1987). *In vivo* studies also reported that the TNF family was the major cytokine constituent and the blockage of this family resulted in lower bone resorption (Kimble et al., 1997; Chabaud and Miossec, 2001). Johnson et al. (1989) discovered that cells over-expressed with TNFα when injected into nude mice increased osteoclast number, as well as bone resorption (Johnson et al., 1989). TNFα has also been shown to induce the expression of the RANK receptor on osteoclasts, leading to higher activation of RANKL (Kobayashi et al., 2000). Interestingly, the binding of TNFα to its receptor, TNFR1, produces a signal transduction cascade that mirrors that of the RANK-RANKL interaction. For instance, TNF instigates the recruitment of TRAF molecules to TNFR1, which leads to the activation of the Nf-κB pathway and to a lesser extent, the MAPK pathway (Nanes, 2003). TNF, together with $1,25(OH)_2$ vitamin D_3 and stromal osteoblasts, has also been demonstrated to directly mediate osteoclastogenesis in bone marrow culture (Pfeilschifter et al., 1989). Interestingly, recent studies revealed that mice deficient in both TNFα and TNFß genes, does not produce an osteopetrotic phenotype (Nanes 2003). This indicates that TNFα and TNFß are involved in modulating osteoclastogenesis rather than directly controlling it.

Interleukin-1 (IL-1) is another possible regulator of osteoclastogenesis produced by osteoblasts. The effect of IL-1 was recognised when estrogen deficient mice models were used. It was demonstrated that removal of estrogen from mice elevated the number of osteoclasts,

which coincided with an increase in IL-1 activity (Spelsberg et al., 1999; Troen, 2003). Conversely, when estrogen was augmented it lowered osteoclast number by inhibiting IL-1 (Spelsberg et al., 1999; Troen, 2003). It has been postulated that estrogen affects the IL-1 receptor, which indirectly leads to an affect on IL-1 (Sunyer et al., 1999). Investigations have revealed that IL-1 can induce osteoclast differentiation *in vitro* (Vaananen, 1996), through a special domain present on the cytokine called gp130. Interestingly, despite gp130-deficient mice experiencing mild symptoms of osteopetrosis, the average number of osteoclastic cells was still high (Kawasaki et al., 1997), indicating that like TNF, interleukins are not obligatory for osteoclastogenesis but are simply involved in modulation. Reinforcing this study is the finding that specific deletion of IL-1 does not produce severe osteopetrotic phenotype (Pacifici, 1996).

The control of osteoclastogenesis by osteoblastic cells mentioned above, indirectly leads to the modulation of bone resorption. For instance studies have discovered osteoclastic bone resorption pits increase in the presence of osteoblasts due to the maintenance of osteoclast survival and induction of osteoclastogenesis by the RANKL-RANK-OPG axis (Grano et al., 1990; Teti et al., 1990). Furthermore, administration of RANKL to mice leads to heightened bone resorption due to increased activation of osteoclastogenesis (Karsenty, 1999). However, there a several molecules expressed by the osteoblast, which bind directly to mature osteoclasts and either inhibit or enhance bone resorption. M-CSF, described above as a modulator of osteoclastogenesis, has been found to have an effect on bone resorption. For instance, osteopetrotic mice that lack M-CSF also have impaired bone resorption (Wiktor-Jedrzejczak et al., 1990). Although there is increasing evidence that osteoblasts modulate bone resorption, a recent study has shown that bone resorption continues even after complete abolishment of bone formation (Corral et al., 1998). However, it must be noted that in the presence of osteoblasts, bone resorption does increase, indicating that although osteoblasts are not obligatory for bone resorption, they do modulate the process.

Can osteoclasts control the proliferation and differentiation of osteoblastic cells?

It is well established that bone resorption and formation is a coupled process: If one increases, so does the other. Logically, this leads to the conclusion that if both cells are coupled it must mean their mechanism of control is intertwined. The preceding section has revealed a snippet of the large plethora of studies into the control of osteoclastic growth, differentiation and function by osteoblast-derived molecules (Fig. 3). However, when the reverse case is examined, only a small handful of reports can be found in the current literature. Despite this apparent paradox, recent research has uncovered some osteoclast-derived molecules that

may play an important role in the control of osteoblastic growth, differentiation and function.

Hepatocyte growth factor (HGF) has recently been described as a potential paracrine regulator of osteoblastic growth, differentiation and function. HGF is a heterodimeric protein that was originally discovered in nonparenchymal hepatocytic cells (Michalopoulos et al., 1984; Nakamura et al., 1984) and has been well characterised as an inducer of cell division, motility and as a potent morphogen. Recently, HGF was identified in cells of the bone lineage, namely osteoblasts and osteoclasts (Grano et al., 1996). In this study, active HGF proteins, expressed by osteoclasts, were found to have both a paracrine and an autocrine mode of action, due to the presence of the HGF receptor for both cell types. Functionally, HGF was able to influence DNA synthesis and cellular proliferation in osteoblasts and osteoclasts (Grano et al., 1996). The paracrine function of HGF was further reinforced when D'lppolito et al. (2002) demonstrated that HGF, together with $1,25(OH)_2$ vitamin D_3, could induce the proliferation and differentiation of bone marrow stromal cells (D'Ippolito et al., 2002). Furthermore, the treatment of both HGF and $1,25(OH)_2$ vitamin D_3 in bone marrow stromal cells elevated bone mineralisation but not with $1,25(OH)_2$ vitamin D_3 or HGF alone. Together these observations demonstrate that HGF, expressed by osteoclasts, could specifically be used to control osteoblastic growth and differentiation. Although the results for HGF are promising there are several conflicting studies showing that HGF may not be as important in osteoclast-derived control of osteoblastic growth as first thought. Taichman et al. (2001) recently discovered that specific stromal osteoblast-like cells could secrete HGF (Taichman et al., 2001). This indicates that the original affect of HGF on
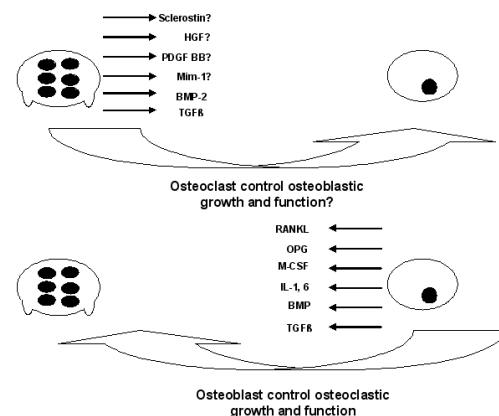

Fig. 3. Schematic representation of the proteins involved in the intercellular communication between osteoblasts and osteoclasts. A plethora of studies have been reported that the osteoblast can secrete molecules that can bind to osteoclasts and control their function and growth. The reverse case is still in question. Proteins with a question mark indicate that further studies will be needed in order to elucidate their function.

osteoblasts (Grano et al., 1996) may be due to HGF secreted by surrounding stromal osteoblastic precursor cells, rather than the osteoclast alone. If this is true, HGF may act mainly through an autocrine regulation. Furthermore, HGF seems to have a more potent affect on osteoclastic cells, especially since it can direct changes in morphology and motility in osteoclastic cells, as well as the ability to induce intracellular calcium elevation (Grano et al., 1996). Its dual affect on osteoclasts and osteoblasts may render its effectiveness as a therapeutic treatment for bone disease useless, since administration of HGF to osteoporotic patients may further increase bone resorption. To unequivocally confirm the function of HGF, *in vivo* work will need to be examined.

Sclerostin is another, recently characterised osteoclast-derived molecule that may have a specific paracrine mode of action on osteoblastic cells. A mutation within the sclerostin (SOST) gene, which codes for the Sclerostin protein, has been attributed to a type of bone disease Sclerosteosis (Balemans et al., 2001; Brunkow et al., 2001). This disease is an autosomal recessive, inherited disease characterised by abnormal hardening and thickening of developmental bone tissue (sclerosis dysplasia). Recently, Kusu et al. (2003) identified a novel protein that is expressed by osteoclastic cells that acts as a negative regulator of osteoblast differentiation (Kusu et al., 2003). In this study, sclerostin was expressed within intramembranous and endochondral bones in the mouse embryo skeleton, and colocalises within cells expressing MMP-9, a marker for osteoclasts. Due to the presence of a secretory signal on sclerostin, and the secretion of the protein from insect cells, it was characterised as an osteoclast-derived molecule (Kusu et al., 2003). Furthermore, sclerostin was demonstrated to inhibit alkaline phosphatase activity (marker for osteoblastogenesis) by binding to BMP-6 and BMP-7. Unfortunately, the results of this study were questioned when Winkler et al. (2003), discovered that sclerostin is expressed by cells in the osteoblast lineage, especially osteoblasts and osteocytes (Winkler et al., 2003). In fact, they further showed that sclerostin is not expressed in the human osteoclast. Therefore, in this study sclerostin acts as an autocrine factor that is secreted by osteoblasts, and negatively regulates osteoblastogenesis by binding to BMPs. The conflicting results produced by these two laboratories could be due to different methodology. Kusu et al. (2003) used in situ hybridisation and co-localisation with MMP-9 to characterise the expression of sclerostin in osteoclasts (Kusu et al., 2003). On the other hand Winkler et al. (2003) used specific reverse transcriptase polymerase chain reaction (RT-PCR) and examined a range of osteoclast-like and osteoblast-like cells, including specific human osteoclasts and osteoblasts. These conflicting observations, similar to the ones associated with hepatocyte growth factor, suggest that more *in vivo* work will need to be done in order to unequivocally confirm the function of this protein.

Platelet-Derived Growth Factor BB (PDGF BB) has recently been demonstrated as a potential paracrine factor that may control osteoblastogenesis. The PDGF molecule exists as a dimer composed of a variation of two subunits, A and B. PDGF AA, AB and BB have been well characterised in bone remodelling, and have been shown to have a mitogenic affect, mainly on osteoblasts and osteoblast-like cells (Canalis et al., 1989; Zhang et al., 1991; Lind et al., 1995). PDGF is expressed by platelets of the blood and osteoblastic cells. Recently, Kubota et al. (2002) recently demonstrated that the PDGF BB isoform is expressed by osteoclastic cells and has an inhibitory affect on osteoblast growth and differentiation (Kubota et al., 2002). In this study, PDGF BB was expressed and secreted by RAW264.7-derived osteoclastic cells, a macrophage cell line differentiated into osteoclasts by soluble RANKL. In addition, PDGF BB was demonstrated to inhibit alkaline phosphatase activity in MC3T3 cells, a cell line that is committed to the osteoblastic lineage. To confirm the paracrine function of PDGF BB, antibodies to the growth factor were used in order to neutralise the activity of PDGF BB. The antibodies neutralised over 83% of the PDGF BB activity (Kubota et al., 2002), suggesting that PDGF BB did have an inhibitory affect on the MC3T3 cells. Although these results are promising, further investigations will needed that take into account cells that are closer to the osteoclast and osteoblast lineage, as well as in vivo work. Recent reports have demonstrated that PDGF BB may influence the motility and attachment of osteoblastic cells to the bone surface (Ahlen et al., 2004). Although PDGF has already been implicated in cell motility (Westermark et al., 1990; Lind et al., 1995), this would be the first protein derived from osteoclasts that affects osteoblast motility. It would be interesting to see whether other studies can support this observation. It is important to note that *in vivo* work is essential for this type of study. Since PDGF BB has been ascertained to affect both osteoblasts and osteoclasts (Zhang et al., 1991), it is possible that therapeutic administration of this growth factor in osteoporotic patients may further increase bone resorption.

Apart from the above studies mentioned, several other reports have provided evidence for the intimate communication between osteoclasts and osteoblasts, and further demonstrated that osteoclasts can control osteoblastic growth and function. Transgenic overexpression of cathepsin K resulted in elevation of osteoblast cell number and mineralising surfaces as well as an increase in bone resorption (Kiviranta et al., 2001). The increase in bone resorption was expected, as cathepsin K is crucial for dissolution of the organic matrix. However, what was not expected was an increase in the number of osteoblastic cells and mineralising surface. It has been suggested that the increase in bone resorption from the overexpression of cathepsin K lead to the induction of several pro-osteoblastic growth factors, such as BMPs and RANKL (Kiviranta et al., 2001). Recently, Falany et al. (2001) discovered that

osteoclasts secrete a chemokine called myeloid protein-1 precursor (Mim-1) that can induce the proliferation of osteoblastic precursor cells (Falany et al., 2001). Although it is not known whether Mim-1 can directly bind to osteoblasts or is simply antagonising osteoblast-specific molecules, it will be interesting to see follow up studies of this cytokine.

Despite these recent discoveries into the control of osteoblasts by osteoclasts, the specific mechanism remains to be elucidated. It is noteworthy to mention that most of the proteins mentioned above have an autocrine function in addition to their proposed paracrine effect. In other words, the protein has an affect on osteoclastic cells, as well as osteoblasts. Care must be taken when using these proteins as possible anabolic treatments for bone disease, such as osteoporosis. Administration of these drugs may increase bone resorption in osteoporotic patients, further increasing the severity of the disease. For instance, parathyroid hormone (PTH) can induce bone resorption when continuously administered to the patient, while PTH increases bone formation if an intermittent dose is given (Dobnig and Turner, 1995). Presently, there is no protein that is expressed by the osteoclast that has a specific paracrine function for osteoblastic cells.

The role of the osteoclast-osteoblast interaction in bone deseases

Osteoporosis

Osteoporosis is regarded as a systemic, skeletal disorder, mostly characterised by low bone mass and high susceptibility to fractures. Despite intense research, the molecular mechanism and etiology of osteoporosis is still an enigma. Fortunately, there are several universal themes that seem to be constant in osteoporosis. Firstly, the disease is principally defined by an imbalance of bone remodelling favouring osteoclastic bone resorption. Secondly, osteoporosis is commonly associated with postmenopausal women, and to a lesser extent elderly men. For instance, a third of all men and half of all women over the age of 60 suffer from fractures due to osteoporosis. The main reason for the high incidence in postmenopausal women is the change in hormonal balance associated with menopause. Estrogen, a potent endocrine hormone linked to osteoporosis, has been demonstrated to bind to osteoblastic cells and suppress the expression of several paracrine pro-osteoclast factors, such as IL-1, IL-6 and TNFα (Troen, 2003; Zaidi et al., 2003). Moreover, estrogen can further increase OPG (Hofbauer et al., 1999; Khosla et al., 2002) and inhibit RANKL expression (Shevde et al., 2000; Srivastava et al., 2001). Therefore, since menopause is associated with a decreased level of estrogen, the above hormonal action is reversed, leading to an increase in osteoclast formation, and thus bone resorption. For example, a study demonstrated that estrogen could inhibit the transcription factor Egr-1,

which is involved in increasing production of M-CSF (Srivastava et al., 1999; Zaidi et al., 2003). In this case, the menopausal state increases Egr-1 activation leading to higher M-CSF production, eventually leading to elevated bone resorption. Studies have now shown that osteoporotic (ovariectomized) mice that are deficient in IL-1, IL-6, M-CSF, TNF or combinations of these cytokines recover from osteoporosis (Pacifici, 1996; Zaidi et al., 2003). Thus, estrogen-mediated bone resorption is accomplished via these osteoblastic-derived cytokines. Interestingly, all of these molecules are expressed and secreted by osteoblasts, which bind to osteoclasts. This indicates that estrogen indirectly affects the osteoclast by affecting the intercellular communication between the two cell types. This theory has one fundamental flaw: absence of osteoblast-osteoclast coupling. If osteoclastic bone resorption is increased, osteoblastic bone formation should be further activated to counteract the heightened level of resorption. Obviously this is not seen in osteoporotic patients. It is possible that estrogen withdrawal may further have an effect on osteoblast function via the inhibition of pro-osteoblastic factors expressed by osteoclasts. Clearly, researchers still do not know the true effect of estrogen. Furthermore, it is unlikely that our understanding of this disease will ever become clear if research focuses on osteoclastic bone resorption and production of pro-osteoclastic factors by osteoblasts, rather than bone formation and production of pro-osteoblastic factors from osteoclasts.

Osteopetrosis

Osteopetrosis is the general name given to a group of diseases where the rate of bone formation is higher than the rate of bone resorption. Due to the varying pathology, etiology and clinical features of these patients, osteopetrosis is categorised into specific disease groups. Carbonic Anhydrase II-deficient osteopetrosis has been largely established as a specific disease associated with osteoclast defects (Key and Ries, 1996). Patients with this type of disease exhibit short stature, frequent fractures, developmental delays, and in severe cases, stillbirths (Key and Ries, 1996). The abnormal bone formation in these patients can often block the respiratory path in the body, leading to severe lung complications. Infantile malignant osteopetrosis is one of the most severe forms of osteopetrosis, with most infants that inherit this disease dying before birth unless a bone marrow transplant can be performed (de Vernejoul and Benichou, 2001). Like carbonic anhydrase II-deficient osteopetrosis, the infantile form can have severe complications with breathing, due to abnormally large bone formation. Infantile malignant osteopetrosis has been associated with a defect in osteoclastic bone resorption (de Vernejoul and Benichou, 2001). For instance, osteoclastic precursor cells, extracted from patients with malignant osteopetrosis, can be differentiated into mature osteoclasts in the presence of

osteoblastic cells (Flanagan et al., 2000). However, these osteoclasts lack the ability to resorb bone, indicating that lower bone resorption may be a common factor in osteopetrosis. Unfortunately, the disease gene(s) that causes malignant osteopetrosis has yet to be elucidated. Recent studies have demonstrated the possibility of osteopetrosis being caused exclusively by a defect in the intercellular communication between osteoblasts and osteoclasts, rather than attributing it solely to osteoclasts. Lajeunesse et al. (1996) extracted and cultured osteoblast-like cells from patients with osteopetrosis (Lajeunesse et al., 1996). These osteoblast cells had normal expression of alkaline phosphatase, but no expression of osteocalcin when compared to controls. Since osteocalcin is a marker for mature osteoblasts, the results indicate that osteopetrosis occurs due to a defect in the differentiation of early osteoblasts to mature osteoblasts, rather than differentiation of mesenchymal cells to early osteoblasts. The study further demonstrated that osteopetrotic-derived osteoblasts did not significantly induce M-CSF secretion. Inhibition of M-CSF causes the down-regulation of osteoclastogenesis, which eventually leads to lower bone resorption. Lajeunesse et al. (1996) then offered a unique twist in their study. They gave the osteopetrotic patients bone marrow transplants to reverse the osteopetrotic phenotype. Osteoblastic cells extracted from these patients revealed normal osteoblast activity as well as normal expression of alkaline phosphatase and osteocalcin. Furthermore, the production and secretion of M-CSF was also normal. The proposed hypothesis is that the original defect in osteopetrosis was the osteoclast, possibly a mutation in a gene that regulates the production of pro-osteoblastic factors from osteoclastic cells. This lead to the creation of defective osteoblastic precursors that were unable to mature into proper osteocalcin-producing osteoblasts. Replacement of the defective osteoclast precursors and osteoclast population with normal ones, by bone marrow transplant, corrected the defective intercellular communication between the two cells (Lajeunesse et al., 1996). The osteoblasts formed normally again and normal bone formation followed. Although more studies are required to confirm this report, it shows promise that osteoclasts do control the growth and function of osteoblastic cells.

Osteogenesis imperfecta

Osteogenesis Imperfecta is an inherited, usually autosomal dominant, bone disease caused by defects within the type 1 collagen matrix the most abundant protein in bone tissue. Due to these defects, patients with this disease are often characterised by lower bone mass, skeletal deformities and re-occurring fractures due to the fragility of the bone tissue (Roughley et al., 2003). The clinical severity of osteogenesis imperfecta ranges from being asymptomatic to mild localised skeletal deformities to morbidity at birth. Due to the variability

of the symptoms, patients with osteogenesis imperfecta are clinically categorised into four types. Type 1 is the least severe with infrequent fractures and low probability of having congenital skeletal deformities. Small proportions of children with this type of disease exhibit deafness during early adulthood, although they are physically normal. Type II increases in severity with a small proportion of infants being born prematurely or dying before birth. Those that do survive have shorter limbs, fragile skulls and mandible (dentinogenesis) and frequently have fractures. Type III is similar to type II, however, patients exhibit more signs of skeletal deformities and, due to defects in the growth plate, are usually shorter. Type IV patients frequently have hearing loss and fractures in the mandible and skull area. All four types further exhibit blue sclera or blue discolouration of the sclera within the eyeball. Despite all the varying signs and symptoms, all of these four groups are caused by mutations within the Type 1 collagen gene, which can result in either complete abolishment of collagen synthesis or structural deficiencies in the collagen protein (Roughley et al., 2003). Recently, three new types of osteogenesis imperfecta have been described, and all of them are believed to be caused by defects in osteoblastic cells, and possibly by defects in the intercellular mechanism between osteoblasts and osteoclasts (Glorieux et al., 2000, 2002; Ward et al., 2002). Type V, VI and VII do not have mutations within type 1 collagen, although they share similar symptoms to other types of osteogenesis imperfecta (Roughley et al., 2003). Histomorphometric studies in patients with Type V have been shown to have normal rates of bone formation and resorption in the general skeleton, compared to healthy control individuals (Glorieux et al., 2000). However, localised sites within the skeleton had abnormal bone formation activity, with lower rate of bone matrix deposition. Additionally, biochemical results show higher alkaline phosphatase activity but normal osteocalcin levels in these patients. This suggests that the defect may be in osteoblast differentiation, rather than bone formation directly (Glorieux et al., 2000). The same biochemistry and histomorphometric results are seen for Type VI and, to a lesser extent, Type VII (Glorieux et al., 2002; Labuda et al., 2002; Ward et al., 2002). It is not unreasonable to hypothesise that there could also be defects within the intercellular communication between osteoblasts and osteoclasts, since disruption of osteoblast differentiation alone cannot produce the varying symptoms seen in osteogenesis imperfecta. Obviously more work will need to be done in order to characterise the pathology of Type V, VI and VII osteogenesis imperfecta. Furthermore, work is under way to scan families of cytokines and growth factors that may be involved in regulating osteoblastogenesis to determine whether these proteins may be involved in the pathogenesis of this disease (Glorieux et al., 2000). It will be interesting to see whether any novel osteoclast-derived osteoblastic proteins are discovered as a result of

this work.

Paget's disease

Paget's Disease is usually characterised by localised areas of abnormally large and expanded bone growth, usually weaker and more sensitive to fractures than other bones in the skeleton. The severity of Paget's disease also called osteitis deformans, ranges from asymptomatic to more severe bone deformity, and pain. The more severe patients often have secondary diseases, such as arthritis. The cause and etiology of Paget's disease is not known, although Paget's disease has been described as a specific disease of the osteoclast (Bender, 2003; Siris and Roodman, 2003). The osteoclasts are larger than normal and contain many more nuclei. This, however, does not rule out the possibility of a defect in osteoblastic cells. For instance, one of the clinical features of Paget's disease is very high alkaline phosphatase activity, indicating unusually high osteoblast activity (Bender, 2003; Siris and Roodman, 2003). However, it is the disruption of the osteoclast-osteoblast interaction that may be the defining cause of Paget's disease. The disease process occurs when signals are given to osteoclasts to increase bone resorption. Due to the coupling nature of osteoclasts and osteoblasts, the increase in bone degradation induces the activation and subsequent elevation of bone formation. The dual activation of these two forces, and the abnormality of the osteoclast, give rise to the large but weak bone growth seen by patients with this disease. Recent studies, however, are just beginning to unravel the molecular mechanism, and as expected it involves the osteoclast-osteoblast interaction (Buckley and Fraser, 2002). Unusually high RANKL mRNA transcripts have been discovered in osteoblastic-like cell lines in Pagetic lesions and marrow (Menaa et al., 2000). In addition, osteoclastic precursors extracted from patients with Paget's disease are much more sensitive to RANKL stimulation compared to cells from normal patients (Neale et al., 2000). Interestingly, most patients with Paget's disease have significantly lower amounts of OPG, which inhibits osteoclastogenesis (Buckley and Fraser, 2002). Therefore, it is possible that a defect in the RANKL-OPG system results in abnormal osteoclast activity. The abnormal osteoclast may further secrete its own factors to increase osteoblastic activity, in order to compensate for the elevation in bone resorption. Interestingly, only a few patients have been reported to have a specific mutation in the RANKL gene (Sparks et al., 2001). This may suggest that the specific defect or mutation may be in other genes related to the intercellular communication between osteoblasts and osteoclasts. For instance, studies have implicated p62 or sequestasome-1 in Paget's disease, an important signal transducer of NfkB that is activated via the RANK-RANKL-TRAF interaction (Laurin et al., 2002). Other investigations have implicated the role of IL-6, a potent cytokine expressed and secreted by the osteoblasts, in Paget's Disease. The abnormal activities of the osteoclast result in increased activity of IL-6 from the osteoblasts that bind to osteoclasts, which increases their activities further (Manolagas and Jilka, 1995). In addition, reports have ascertained high levels of IL-6 in Pagetic lesions (Manolagas and Jilka, 1995). The pathogenesis of Paget's disease still remains an enigma, despite all the recent research. However, it is possible that our understanding of this disease will become clearer once the focus of bone biology shifts towards the production of pro-osteoclastic factors by osteoblasts.

Periodontitis

Periodontitis, and to a lesser extent gingivitis, is the most common cause of tooth loss in human adults. Both diseases have a common etiology, with gingivitis often viewed as a pre-cursor stage during the evolution of periodontitis (Mercado et al., 2003). Infection and accumulation of bacteria on the gingival tissues (gums) initiate the pathology of periodontitis. The progressive nature of this disease results in expansion of the bacterial infection from gums to ligaments and bone that support the teeth. The inflammatory response accompanies the spread of infection. Bacterial invasion of the supporting bone structure elevate osteoclastic bone resorption which reduces the rigidity and strength of bone, ultimately leading to tooth loss (Wiebe et al., 1996; Mercado et al., 2003). Several investigations have identified the molecular players in periodontitis and as expected it involves the intercellular communication between osteoblasts and osteoclasts. Bacterial infection of bone induces the expression and secretion of several autocrine factors by osteoclasts, such as IL-1 and TNF (Graves 1999). Moreover, IL-1 has been demonstrated to bind osteoblasts and increase production of pro-osteoclastic molecules, such as monocyte chemoattractant protein-1 (MCP-1). Interestingly, MCP-1 does not directly activate osteoclasts, rather it stimulate and attract monocytes and pro-inflammatory leuokocytes to the site of infection, which results in further osteoclastic bone resorption (Wysocki et al., 1996; Graves, 1999). In this model the over-expression of pro-osteoclastic factors outweighs the growth and function of osteoblasts, leading to heightened levels of bone resorption. Interestingly, the inflammations of gingival tissue and bone have resulted in the identification of a potential link between periodontitis and rheumatoid arthritis (Mercado et al., 2003). Furthermore, since RANKL expression is high in both diseases (Buckley and Fraser, 2002; Liu et al., 2003), several investigations have postulated the use of recombinant OPG as a therapeutic approach to treating these disorders. Currently the common preventative treatment for periodontitis is proper oral hygiene, while palliative treatments are performed by the specific removal of dental plaque from inflamed gums, which is often uncomfortable. The use of OPG to possibly treat periodontitis will open a new avenue of therapeutic approaches for this disease. However, more clinical

research will be needed to ascertain whether OPG can be used in this manner.

Osteoarthritis

Osteoarthritis is a chronic joint disease that can lead to severe pain and sclerosis of the subchondral bone. Currently, there are no direct treatments for sufferers of this affliction, however, new insights into the pathology of osteoarthritis as well as rheumatoid arthritis, are revealing promising results. Osteoarthritis is initiated by inflammation of the joints and possibly by over activation of T-cells, although the latter mechanism is more commonly attributed to rheumatoid arthritis. Several key players have been discovered, most of which play an important role in osteoclast function. Recently, Clements et al. (2003) ascertained that MMP-3 knockout mice demonstrated accelerated articular cartilage breakdown compared to wild type (Clements et al., 2003). Additionally, it has been shown that MMP-3 plays an important role in the breakdown of aggregan, an important constituent of articular cartilage and bone (Tortorella et al., 2000; Tortorella and Malfait, 2003). Several investigations have implicated an indirect role of RANKL mediated arthropathy and have suggested the utilisation of recombinant OPG as a therapeutic agent to inhibit osteoclast bone resorption in the joint area (Buckley and Fraser, 2002). More clinical investigations will be needed in order to ascertain whether OPG can be used in this manner.

Aseptic loosening

Aseptic loosening is a common condition that occurs due to the failure of the bone and implant interface, used in joint replacement therapy such as total hip arthoplasty. The loss of prosthetic implants cost several millions dollars a year due to the need for replacement. The current view is that aseptic loosening is due to the presence of wear particles and surrounding resorptive macrophages. Wear particles, consisting mainly of ultra-high polyethylene, lead to the activation of specific pro-osteoclastic cytokines from osteoblasts such as TNF and IL-1. This results in an elevation of osteoclastic resorption. Several investigations have utilised bisphosphonates and BMP-2 to augment bone formation and prevent osteolysis-induced aseptic loosening (Shanbhag and Rubash, 1998; Kenney et al., 2000). This is particularly novel since a considerable number of reports have established bisphosphanates as an inhibitor of osteoclastic bone resorption. It would be interesting to see whether more clinical trials can ascertain if increasing bone formation directly can alleviate aseptic loosening of prosthetic joint implants.

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