ORAL DISEASES

Oral Diseases (2011) 17, 129–142 doi:10.1111/j.1601-0825.2010.01718.x

www.wiley.com

INVITED MEDICAL REVIEW

Osteoclasts and odontoclasts: signaling pathways to development and disease

Z Wang[1], LK McCauley[2,3]

[1]*Department of Orthodontics and Pediatric Dentistry,* [2]*Department of Periodontics and Oral Medicine, School of Dentistry,* [3]*Department of Pathology, Medical School, University of Michigan, Ann Arbor, MI, USA*

Osteoclasts are cells essential for physiologic remodeling of bone and also play important physiologic and pathologic roles in the dentofacial complex. Osteoclasts and odontoclasts are necessary for tooth eruption yet result in dental compromise when associated with permanent tooth internal or external resorption. The determinants that separate their physiologic and pathologic roles are not well delineated. Clinical cases of primary eruption failure and root resorption are challenging to treat. Mineralized tissue resorbing cells undergo a fairly well characterized series of differentiation stages driven by transcriptional mediators. Signal transduction via cytokines and integrin-mediated events comprise the detailed pathways operative in osteo/odontoclastic cells and may provide insights to their targeted regulation. A better understanding of the unique aspects of osteoclastogenesis and osteo/odontoclast function will facilitate effective development of new therapeutic approaches. This review presents the clinical challenges and delves into the cellular and biochemical aspects of the unique cells responsible for resorption of mineralized tissues of the craniofacial complex.

Oral Diseases (2011) **17**, 129–142

Keywords: osteoclast; odontoclast; tooth eruption; root resorption; receptor signaling; integrin

Introduction and clinical scenarios

Tooth eruption and exfoliation are complicated processes that involve tightly programmed events coordinating functions of osteoblasts, osteoclasts, dental follicle cells, and periodontal ligament cells (Wise *et al*, 2002). During pre-emergent tooth eruption, the rate of resorption of overlying structures is critical to direct eruption, and hence support the erupting tooth to move along the appropriate path (Proffit and Frazier-Bowers, 2009). Osteoclasts are essential for developing the eruption pathway, which is independent of the pressure from the erupting tooth (Cahill, 1969). Osteoclast precursors are recruited to the dental follicle upon the initiation of tooth eruption. The most well-studied molecules involved are colony stimulating factor 1 (CSF-1) and monocyte chemotactic protein-1 (MCP-1) secreted by dental follicle cells (Wise *et al*, 1999; Grier *et al*, 1998). CSF-1 and MCP-1 attract monocytes, which differentiate and fuse to form osteoclasts. The timing for osteoclast function during eruption is critical and if osteoclast number is altered at that time, tooth eruption will be delayed or inhibited (Yoshino *et al*, 2003). Clinically there are several dental abnormality scenarios that may be related to altered osteoclast development or function.

Primary eruption failure

Primary eruption failure is a clinical condition where a tooth fails to erupt in the absence of secondary obstruction and in addition does not respond to orthodontic treatment (Proffit and Vig, 1981). It may be syndrome or non-syndrome associated and affects both primary and permanent teeth, bilaterally or unilaterally. Primary eruption failure predominantly involves posterior teeth and is familial in about 45% of cases (Ahmad *et al*, 2006). In primary eruption failure, all teeth distal to the most mesial involved tooth have defective eruption. Permanent molars with primary eruption failure tend to become ankylosed, especially if they have undergone orthodontic treatment. The mechanism of primary eruption failure is not very well understood. In humans, eruption failure can be associated with genetic disorders associated with mutant runt-related transcription factor 2 (Runx2), TNF receptor associated factor (TRAF) 6 and fibroblast growth factor receptor (FGFR)1-3 (Ahmad *et al*, 2006). Recently mutations in parathyroid hormone receptor -1 (PTHR1) have been identified in familial nonsyndromic primary

Correspondence: Professor Laurie K. McCauley, Department of Periodontics and Oral Medicine, University of Michigan School of Dentistry, 1011 N University Avenue, Ann Arbor, MI 48109-1078, USA. Tel: +1 734 647 3206, E-mail: mccauley@umich.edu
Received 1 March 2010; revised 26 March 2010; accepted 26 March 2010

eruption failure in a dominant transmission pattern (Decker *et al*, 2008; Frazier-Bowers *et al*, 2010). These mutations make abnormal precursor proteins that undergo premature proteolytic degradation resulting in a functionless receptor, suggesting that haploinsufficiency rather than gain-of-function of PTHR1 is an underlying mechanism of nonsyndromic primary eruption failure. Clinical management of primary eruption failure is a challenge. To improve prediction and early intervention strategies, further research is needed to elucidate the mechanism of primary eruption failure, including the role of osteoclasts.

Ankylosis

Ankylosis is a clinical condition that prevents further tooth eruption due to obliteration of the periodontal ligament and subsequent fusion of the tooth and osseous structure. The prevalence of ankylosis of primary molars has been estimated at be 3.7%, with highest incidence in children between seven and eleven years of age (Krakowiak, 1978). The mandibular first primary molar is most frequently affected versus other teeth in younger children. The frequency of ankylosis of the second primary molar increases in older children. It is thought that early ankylosis may be due to congenital reduction of bone resorption (Popoff and Marks, 1990). Animal studies in op/op mice showed ankylosis between the root dentin and proliferating bone trabeculae was a common feature, very likely subsequent to a deficiency of osteoclasts and bone remodeling (Kawata *et al*, 1999). However, the exact mechanism is not clear. A clinical case of ankylosed primary molar is shown in Figure 1.

Root resorption

The precise events initiating and controlling apical root resorption in primary teeth have been elusive (Harokopakis-Hajishengallis, 2007). The presence of the erupting permanent tooth is likely a critical contributing factor. The dental follicle, rather than the tooth itself, is more critical in controlling the tooth eruption process (Marks and Cahill, 1984). The underlying tooth bud contributes to primary tooth root resorption either through direct pressure (Sahara, 2001), or via genetically programmed signals sent from the dental follicle independent of pressure. This is evidenced by the findings that conditioned media from dental follicles promotes osteoclastogenesis and the tightly controlled temporal expression of receptor activator for nuclear factor κB ligand (RANKL) in the dental follicle (Kawakami *et al*, 2000; Liu *et al*, 2005).

Odontoclasts and resorption

Odontoclasts are thought to differentiate from circulating progenitor cells (Sahara *et al*, 1996). Such progenitor cells reside in the dental pulp and periodontal ligament, sharing similar characteristics with osteoclasts such as the expression of cathepsin K, cathepsin D, tartrate-resistant acid phosphatase (TRAP), matrix metalloproteinases (MMP)-9, H^+-ATPase, membrane type 1 (MT1)-MMP expression and the formation of a clear zone and ruffled border (Gotz *et al*, 2000; Linsuwanont-Santiwong *et al*, 2006). In physiological primary tooth resorption, mature odontoclast formation only occurs after attachment to the resorption surface (Sahara *et al*, 1992). Given that the extracellular matrix protein distribution pattern is different in primary teeth versus permanent teeth, osteoclast/odontoclast activation may be extracellular matrix dependent (Lee *et al*, 2004). In the case of over-retention of primary teeth, decreased odontoclast activity is observed (Bimstein *et al*, 2000).

Cells from periodontal ligament, dental follicle, cementum, and dental pulp are all important in odontoclast/osteoclastogenesis and root resorption. All origins of osteoclastogenic cells involve the orchestration of essential molecules in the osteoprotegerin (OPG)/RANK/-RANKL system. RANKL is expressed in periodontal ligament (PDL) cells from actively resorbing primary teeth, but not non-resorbing teeth or permanent teeth

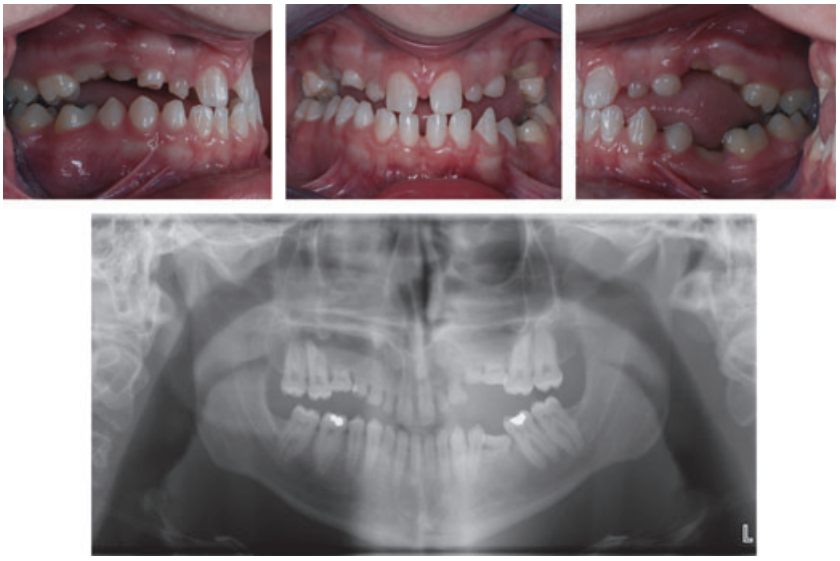

Figure 1. Ankylosis of primary teeth. An 18-year-old male presenting with retained and ankylosed primary molars. Note the posterior open bite and resorption of roots of primary molars even in the absence of permanent premolars (kindly provided Dr. Scott Conley)

(Fukushima *et al*, 2003; Hasegawa *et al*, 2002). RANKL is also expressed in pulp fibroblasts, odontoblasts, cementoblasts, and ameloblasts (Lossdorfer *et al*, 2002; Rani and MacDougall, 2000; Berry *et al*, 2006). Similarly, RANKL and CSF-1 are significantly more highly expressed in primary dental pulps than in pulpal tissues of permanent teeth, supporting the preferential resorption of primary teeth (Yildirim *et al*, 2008). PDL cells from patients having *RUNX2* mutations resulting in the genetic condition of cleidocranial dysostosis are defective in their support of osteoclastogenesis *in vitro,* and may partially account for the delayed tooth eruption in those patients (Lossdorfer *et al*, 2009). Both PDL and dental pulp spontaneously induce the differentiation of osteoclasts *in vitro* without osteotropic factors (Uchiyama *et al*, 2009). In addition, PDL cells can also mediate systemic regulation of osteoclastogenesis. For example, parathyroid hormone (PTH) increases RANKL expression in PDLs and induces Notch signaling through Jagged 1 expression in PDLs to promote osteo/odontoclastogenesis (Nakao *et al*, 2009; Nohutcu *et al*, 1993).

In conditions of periapical periodontitis, osteoclasts and a variety of inflammatory cytokines are present at the lesion site. The number of osteoclasts present correlates with gene expression for MMP-2, MMP-9 (Corotti *et al*, 2009), c-*fos* (Yang and Peng, 2008), and RANKL (Zhang and Peng, 2005) along with activation of p38 mitogen-activated protein kinases (MAPK) (Zhang *et al*, 2008). In contrast, osteoclast number is negatively associated with interleukin-17 (IL-17) expression (Xiong *et al*, 2009).

External root resorption is a relatively common and adverse complication of orthodontic treatment (Yamaguchi, 2009). About 80% of orthodontic patients will have mild root resorption at the end of treatment (Harris *et al*, 2001), 30% will have greater than 3 mm resorption, and 5% will have more than 5 mm resorption (Killiany, 1999). Risk factors for root resorption include patient gender, severity of malocclusion, apical displacement, treatment mechanics and duration, as well as genetic disposition (Segal *et al*, 2004; Harris *et al*, 2001; Lopatiene and Dumbravaite, 2008). Root resorption is a complex, sterile inflammatory process that involves mechanical force, tooth and bone tissue, cells of the surrounding matrix, and certain known biologic messengers (Brezniak and Wasserstein, 2002). The process involves two pathways (Hartsfield, 2009; Hartsfield *et al*, 2004). The first involves activation of odontoclastic cells through the ATP/P2XR7/IL-1β inflammation modulation pathway. Local damage of tissue may result in ATP release, which can activate the receptor P2xR7 on macrophages or other cell types leading to further release of cytokines including IL-1β. Such cytokines can recruit more monocytes and macrophages to eliminate apoptotic cells and prevent further necrosis. Both P2xR7 and IL-1β knockout mice have significantly greater root resorption than wild type controls when undergoing experimental orthodontic treatments (Viecilli *et al*, 2009; Hartsfield, 2009). Similarly, patients with IL-1β polymorphisms also are more susceptible to external resorption associated with orthodontic treatment (Al-Qawasmi *et al*, 2003).

The second pathway involves the RANK/RANK-L/OPG osteoclast program. Not surprisingly, excessive osteoclast activity induced by the inflammatory process will exacerbate root resorption. On the other hand, orthodontically induced root resorption may be associated with defective alveolar resorption and/or turnover along with tooth movement or force application, resulting in prolonged stress and strain (Hartsfield *et al*, 2004). Therefore, any risk factors that interfere with osteoclast function may actually contribute to more root resorption. Increased local OPG can inhibit orthodontic tooth movement and bone modeling (Dunn *et al*, 2007; Kanzaki *et al*, 2001). Recently, an association of a single nucleotide polymorphism of the OPG gene with root resorption in orthodontic treated patients was reported (Hartsfield, 2009). Targeting osteoclast activation can modulate root resorption. Such inhibition was observed in osteopontin (OPN) deficient mice (Chung *et al*, 2008) and when the $\alpha v\beta 3$ integrin receptor was inhibited by echistatin, an RGD containing peptide in a rat model (Talic *et al*, 2006; Dolce *et al*, 2003). Further research needs to be done to elucidate how osteoclast activation can be modulated during orthodontic treatment to prevent root resorption. Several clinical scenarios of root resorption are illustrated in Figure 2.

Osteoclast biology

Osteoclastogenesis

Osteoclasts originate from hematopoietic precursor cells and undergo a multistep differentiation scheme that occurs nearly exclusively in the bone marrow microenvironment. Mononuclear osteoclast precursors have been estimated to comprise 1–4% of circulating monocytic cells (Fujikawa *et al*, 1996). Myeloid precursors go through a well-characterized series of differentiation stages to develop into active bone resorbing osteoclasts (Soltanoff *et al*, 2009). The transcription factor PU.1 is present in early monocytic cells followed by Mitf, AP-1 and NFATc1 transcriptional mediators.

The two essential factors that most positively impact osteoclast differentiation are RANKL and macrophage colony stimulating factor (M-CSF) (Boyle *et al*, 2003). M-CSF is a secreted cytokine that directs hematopoietic progenitor cells along the macrophage and osteoclast lineage by binding to its receptor c-fms (CD115). M-CSF is produced by monocytes, endothelial cells, and granulocytes where its production is stimulated by IL-1, platelet derived growth factor (PDGF), interferon gamma (IFNγ) and GM-CSF (Ross and Teitelbaum, 2005). M-CSF facilitates osteoclast differentiation by promoting cell survival and proliferation of osteoclast precursors, inducing RANK on hematopoietic cells so they can respond to RANKL, and regulating cytoskeletal changes associated with bone resorption (Negishi-Koga and Takayanagi, 2009).

RANKL is a type II transmembrane glycoprotein consisting of a membrane-anchoring domain, a connecting stalk and a receptor-binding ectodomain (Kong

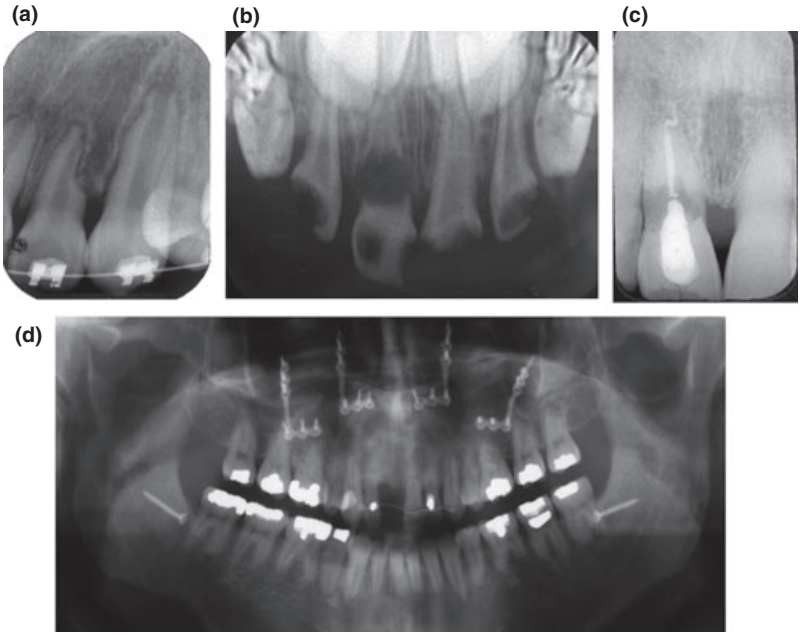

Figure 2. Root resorption. (**a**) Localized external root resorption secondary to transient mesially erupting canine in a 12-year-old female. (**b**) Internal resorption in primary incisor #E secondary to extensive caries in a 4-year-old female. (**c**) External resorption. 77-year-old female with central incisor previously endodontically treated (50 years earlier). Tooth was asymptomatic and had no signs of caries. (**d**) Generalized external root resorption secondary to comprehensive orthodontic treatment in a 35-year-old male (kindly provided by Dr. Sunil Kapila)

et al, 1999a; Lam *et al*, 2001). Although constructed as a membrane-anchored protein it can be released from the cell surface through proteolytic cleavage by metalloprotease disintegrine TNF-alpha convertase (TACE). RANKL is expressed largely by osteoblastic, stromal, dendritic cells and activated lymphocytes (Yasuda *et al*, 1998; Josien *et al*, 1999; Teng *et al*, 2000). The receptor for RANKL is RANK, which is found on the surface of hematopoietic cells. The binding of RANKL to RANK is an essential step in the promotion of osteoclast differentiation and bone resorption. Activation of RANKL initiates multiple signal pathways in osteoclast precursors. RANKL mutant mice lack tooth eruption and also have defects in lymph node development (Kong *et al*, 1999b). Most agents that stimulate osteoclastogenesis do so via increases in RANKL in stromal or lymphocytic cells. Examples include IL-1, IL-6, prostaglandin E2, and parathyroid hormone (McCauley *et al*, 1991; Hofbauer *et al*, 1999; Kanematsu *et al*, 2000; Lee and Lorenzo, 1999; O'Brien *et al*, 1999).

There are numerous endogenous and exogenous osteoclast inhibitors (McCauley and Nohutcu, 2002). Calcitonin is a 32 amino acid protein produced by the C cells of the thyroid gland. Osteoclasts have the G-protein linked calcitonin receptor and respond rapidly and profoundly by flattening and withdrawing from the mineralized surface (Chambers and Magnus, 1982). Osteoprotegerin (OPG) is a soluble decoy receptor for RANKL (Lacey *et al*, 1998; Kostenuik and Shalhoub, 2001). OPG inhibits RANKL mediated osteoclastogenesis.

Sex steroids and their exogenous pharmacologic counterparts SERMS (selective estrogen receptor modulators) and SARMS (selective androgen receptor modulators) act indirectly to inhibit osteoclast activity (Evans and Turner, 1995; Lindsay and Cosman, 1997). They reduce monocytic and marrow stromal cell production of the pro-osteoclastic cytokines such as IL-1, TNFα, IL-6, GM-CSF and M-CSF, increase the osteoblast derived pro-apoptotic factor TGFβ, and alter the RANKL/OPG ratio to reduce osteoclastogenesis (Hughes *et al*, 1996; Riggs, 2000).

Bisphosphonates are a class of pharmacologic antiresorptive drugs that are a mainstay of osteoporosis therapeutics (Russell and Rogers, 1999). The basis of these compounds are two phosphate groups flanking a central carbon atom. Various compounds with this structure have been brought forward for *in vivo* evaluation and use. Those with nitrogen containing side chains are the most potent antiresorptives. Bisphosphonates inhibit osteoclastic bone resorption via their ability to restrict osteoclast protein prenylation with resulting apoptosis. Bisphosphonates have been found to delay tooth eruption in animal models and children treated for osteogenesis imperfecta (Grier and Wise, 1998; Kamoun-Goldrat *et al*, 2008). Recent interest has centered on bisphosphonates and their association with the unique condition, osteonecrosis of the jaw (ONJ) (Khosla *et al*, 2007). ONJ appears to correlate with dose and potency of the anti-resorptive actions of the bisphosphonates (Palaska *et al*, 2009).

Interferon gamma (IFNγ) is a T-cell derived cytokine with potent *in vitro* and *in vivo* osteoclast inhibitor actions (McCauley *et al*, 1989; Takayanagi *et al*, 2000). IFNγ signals through the STAT1 pathway with inhibition of the osteoclast mediator TRAF6 (Stark *et al*, 1998). IFNβ is also a strong inhibitor of osteoclast differentiation via the inhibition of RANKL signaling and downstream expression of the AP-1 transcription factor c-*fos* (Takayanagi *et al*, 2002). Macrophage migration inhibitory factor (MIF) is a macrophage and T-cell derived cytokine expressed early during inflammation. It has notable osteoclast inhibitory actions *in vitro* yet the entirety of its actions are not yet clarified *in vivo* (Jacquin *et al*, 2009).

The ephrin receptor family is the largest family of protein tyrosine kinase receptors and was originally described in the neural system (Bruckner and Klein, 1998). Ephrins comprise a family of bidirectional signaling molecules of the A and B class. Their ability to signal in a forward or reverse manner requires cell-to-cell contact. Recently there is evidence that ephrins play critical roles in bone and specifically in the signaling between osteoblasts and osteoclasts (Zhao *et al*, 2006). Osteoclasts express ephrin B2 and osteoblasts express the ephrinB4 receptor. Reverse signaling of ephrin B4 receptor from osteoblasts through ephrin B2 in osteoclasts results in an inhibition of osteoclast differentiation via an inhibition of NFATc1 and c-*fos* (Zhao *et al*, 2006). Interestingly, mutations in ephrin B1 in humans have been found to be responsible for a craniofacial abnormality called craniofrontalnasal syndrome that includes hypertelorism, a nasal groove, craniosynostosis, and cleft lip and palate (Twigg *et al*, 2004). Ephrin B1 is downregulated in response to the PTH and may function to regulate signaling of the PTH1R (Berry *et al*, 2008).

Human conditions of altered osteoclast function: osteopetroses

Osteoclasts are essential for normal physiologic bone remodeling. Compromise in their function results in the pathologic condition of osteopetrosis (Tolar *et al*, 2004; Blair and Zaidi, 2006; Everts *et al*, 2009). Osteopetrosis is also called 'marble bone disease' due to the exaggerated bone density. The osteopetroses can be generally segregated into two clinical forms; the autosomal dominant, adult (benign) type (ADO) and the autosomal recessive, infantile (malignant) form (ARO) that is profoundly more severe, however, there are other forms that are associated with other organ systems (Villa *et al*, 2009). Numerous genes have been identified for their association with compromised osteoclast function (see Figure 3 and Table 1). Four genes are most widely linked to human osteopetroses (Figure 3). Generally speaking the severity of the osteoclast compromise is directly related to the severity of the phenotypic presentation of the condition.

Carbonic anhydrase (CAII) is an enzyme responsible for catalyzing the reaction of water and carbon dioxide to bicarbonate and hydrogen ion. The hydrogen ions are then transported to the ruffled border membrane to the V-type proton ATPase pump that exports hydrogen ions to the extracellular space resulting in the lowered pH, which is essential for mineral dissolution (Whyte, 1993). Mutations in CAII were among the first found to be associated with osteopetrosis (Sly *et al*, 1983). The gene encoding the proton pump is TCIRG1 (T-cell, immune regulator 1), which has been associated with osteopetrotic mutations in humans (Frattini *et al*, 2000).

Chloride channel 7 (CLCN7) is one of many chloride channels with specialized cellular membrane function. Chloride channel 7 is critical for facilitating acidification of lysosomes and the resorption lacuna in osteoclasts. After attaching to the osseous matrix, osteoclasts undergo polarization, form the ruffled membrane via the fusion

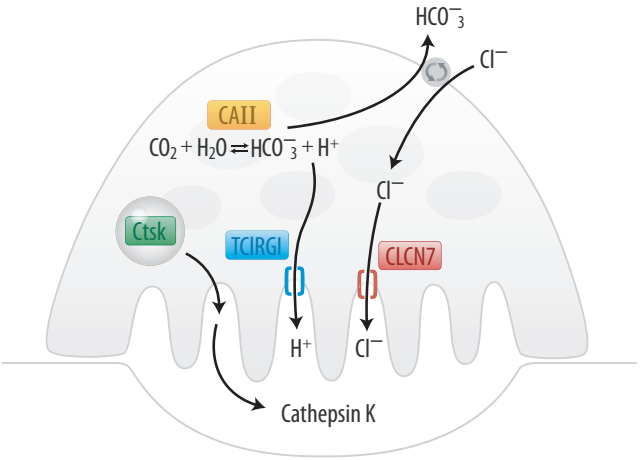

Figure 3. Four genes widely linked to human osteopetroses and their site of action in the osteoclast. Carbonic anhydrase II (CAII) is the enzyme that catalyzes the reaction of water and carbon dioxide to liberate bicarbonate and the hydrogen ions that are exported to the extracellular space to facilitate lowered pH. T-cell, immune regulator 1 (TCIRG1) encodes for a component of the proton pump. Cathepsin K (Ctsk) is a cysteine protease responsible for the degradation of collagen and matrix proteins. Chloride channel 7 (CLCN7) is critical for facilitating electroneutrality of the acidification of lysosomes and the resorption lacuna in osteoclasts

of acidic intracellular vesicles, and acidify the extracellular matrix zone. The ruffled membrane contains Cl^- channels that ensure electroneutrality of the acidification (Henriksen *et al*, 2009). Mutations in CLCN7 have been linked to osteopetrosis and account for approximately 50% of the known cases (Kornak *et al*, 2001; Cleiren *et al*, 2001). Associated with the chloride channel 7, mutations in osteopetrosis associated transmembrane protein 1 (OSTM1) are reported in human autosomal resessive osteopetrosis (Chalhoub *et al*, 2003).

Cathepsin K (Ctsk) is a cysteine protease highly expressed in osteoclasts and responsible for the degradation of collagen and matrix proteins (Inaoka *et al*, 1995). Mutations in Ctsk are associated with an osteopetrotic condition known as pycnodystosis (Gelb *et al*, 1996). Cathepsin K is under investigation as a potential target for metabolic bone disease (Henriksen *et al*, 2007).

Other mutations that have been noted, but are less well characterized relative to numbers of cases, include mutations in RANKL (TNFSF11) and RANK (TNFSF11A) (Sobacchi *et al*, 2007; Guerrini *et al*, 2008). Pleckstrin homology domain containing family M (with RUN domain) member 1 (PLEKHM1) encodes a protein implicated in late osteoclast vesicular trafficking and is associated with the immediate form of osteopetrosis (Van Wesenbeeck *et al*, 2007). A more recent uncommon genetic mutation associated with osteopetrosis involves kindlin 3, which has a close relationship to integrin function (Plow *et al*, 2009). A few other potential gene mutations have been discussed and cases remain with unidentified mutations (Segovia-Silvestre *et al*, 2009).

Interestingly, a high proportion of patients with osteopetrosis present with osteomyelitis in association

Table 1 Osteopetroses

Gene defect	*Function*	*Inheritance*	*Characteristics*
CLCN7	Chloride channel (acts as a chloride conductor allowing vacuolar proton-ATPase to acidify the subcellular resorptive area)	Autosomal Dominant	'Bone within a bone' appearance, high fracture rate, osteomyelitis, visual loss, clinical phenotype worsens over time
TCIRG1 (ATP6i, OC116)	Proton pump (Vaculoar proton-ATPase)	Autosomal Recessive	Severe osteopetrosis during infancy; diffuse sclerosis of spine and long bones; visual impairment, bone marrow failure
CLCN7	Chloride channel	Autosomal Recessive	Increased bone density but less severe than TCIRG1 mutations
CAII	Carbonic anhydrase (catalyzes the production of carbonic acid and proton for acidification of the subcellular space)	Autosomal Recessive	Renal tubular acidosis, increased frequency of fractures, short stature, cranial-nerve compression; no hematologic deficiency
OSTM1	Type I transmembrane protein which localizes to intracellular vesicles	Autosomal Recessive	Severe brain abnormalities, epilepsy
NEMO	NF-kB essential modulator	Autosomal Recessive	Osteopetrosis with anhidrotic ectodermal dysplasia and immunodeficiency
PLEKHM1	Vesicular transport, colocalizes with Rab7 to late endosomal/lysosomal vesicles	Autosomal Recessive	Not as severe as TCIRG1; very rare form
RANKL	Osteoclastogenesis	Autosomal Recessive	Not well described

with dental infections, suggesting that osteoclasts are critical for normal healing in the oral cavity. Several reports have compared this presentation to that of bisphosphonate associated osteonecrosis of the jaw (Leite *et al*, 2006; Vance, 2007).

Murine models of osteoclast functional defects

Gene targeted murine models have been extremely valuable in mapping molecular mechanisms of osteoclast-associated genes relevant for physiological and pathologic bone remodeling. Several murine models demonstrate defective craniofacial development and/or tooth eruption abnormalities (Table 2). Genes important for osteoclastogenesis and function can be categorized based on their roles as receptors or decoy receptors (IL-1R, OPG, vascular endothelial growth factor (VEGF)-R1), signaling molecules (c-Src, TRAF6), growth factors (M-CSF) and transcriptional factors (c-fos, RUNX2). The genetic change may affect osteoclasts directly or indirectly through osteoblast function or other mechanisms.

Osteoclast activation/signaling pathways

The morphologic hallmarks of osteoclast activation are the presence of a ruffled membrane and formation of the actin ring. Cytoskeletal rearrangements lead to the formation of an isolated microenvironment between the osteoclast and the bone surface to achieve a localized acidic compartment. It is not exactly clear how mature osteoclasts initiate bone resorption, or what intracellular changes are involved in this activation, however, there have been numerous advances in this area over the past ten years. Bone matrix and growth factors are known to play important roles in this process through receptor and ligand binding.

Integrins and receptor tryosine kinases

Integrins, a group of heterodimeric receptors composed of alpha and beta subunits, are important for cell and extracellular matrix interactions. Integrin mediated signaling events regulate cellular functions including proliferation, development, differentiation, cell adhesion, migration, apoptosis and cancer metastasis. Limited studies of integrin expression on osteoclasts show that αv, α2, β1 and β3 units are expressed in osteoclasts from human bone tissue and osteoclastomas (Nesbitt *et al*, 1993; Clover *et al*, 1992). The αvβ3 integrin is the most abundantly expressed and highly studied integrin in osteoclasts. This integrin recognizes the RGD (Arg-Gly-Asp) motif, which is present in osteopontin, vitronectin, and bone sialoprotein. The role of αvβ3 integrins in osteoclast function was illustrated by β3 integrin knockout mice (McHugh *et al*, 2000). The osteoclast number is increased in β3 deficient mice but they are dysfunctional, hence these mice manifest osteosclerosis as they age. Additional *in vitro* experiments expressing a mutant β3 unit in osteoclasts also demonstrate the β3 integrin is essential for formation of the actin ring and ruffled membrane, suggesting αvβ3 is indispensable for cytoskeletal rearrangement and osteoclast activation (Feng *et al*, 2001). The β3 integrin Leu33pro polymorphism that enhances the outside-in signaling of β3 integrin is associated with increased hip fracture, suggesting that activated integrin may contribute to increased osteoclast activity (Honda *et al*, 1995; Vijayan *et al*, 2000; Tofteng *et al*, 2007). Beyond its role in mature osteoclasts, the β3 integrin can also regulate osteoclast-like activity in myeloma cells (Tucci *et al*, 2009).

Integrins can be regulated through outside-in and inside-out signaling. Most studies on β3 integrin in osteoclasts have focused on outside-in signaling, looking at downstream signaling events and cell cytoskeletal change upon αvβ3 activation. Faccio *et al*'s work on two different αvβ3 integrin conformations provided the first evidence that the β3 integrin can also undergo inside-out regulation from resting to an active conformation upon M-CSF stimulation in osteoclasts (Faccio *et al*, 2002). Further studies need to be done on how

Table 2 Mouse models of altered osteoclast function

Name	*Mutation or genetic manipulation*	*Bone phenotype*	*OC activity change*	*Dental phenotype*	*References*
PTHrP	Col II-PTHrP transgenic, PTHrP-null homozygous animals	Cranial chondrodystrophy	Normal osteoclast differentiation, possible functional defect	Tooth eruption failure	Philbrick *et al* (1998)
c-fos	c-fos null homozygous	Growth-retardation, osteopetrosis, deficiencies in bone remodeling	Impaired osteoclast development and differentiation	Tooth eruption failure	Wang *et al* (1992)
IL-1R	IL1-R−/−	Skeletal growth impairment and low bone mass	Increased osteoclast number	Tooth eruption delayed	Huang and Wise (2000), Bajayo *et al* (2005)
	IL1-ra transgenic	Low bone mass, high trabecular bone turnover	Increased osteoclast number and bone formation rate		
RUNX2/CBFA1	Mutation of one allele	Femoral remodeling is normal	Decreased osteoclast number in eruption pathway	Supernumerary teeth, impaction and delayed tooth eruption	Komori *et al* (1997), Yoda *et al* (2004)
c-Src	Homozygous	Deficient bone remodeling, osterpotrosis	Defective osteoclast activation and function	Tooth eruption failure	Soriano *et al* (1991), Lowe *et al* (1993), Tiffee *et al* (1999)
op/op	M-CSF−/−	Excessive bone accumulation, restriction of bone remodeling	Defective osteoclast formation, abnormal cytoplasmic distribution of the lysosomal enzyme acid phosphatase	Tooth eruption failure, malformed tooth germ	Marks and Lane (1976), Ida-Yonemochi *et al* (2002), Tiffee *et al* (1999)
Membrane-type 1 matrix metalloproteinase (MT1-MMP)	MT1-MMP−/−	Dwarfism, osteopenia, and craniofacial abnormalities	Increased osteoclast number and activity, compensating for impaired soft tissue remodeling	Defective eruption and short root length	Beertsen *et al* (2002), Holmbeck *et al* (1999)
TRAF6	TRAF6−/−	Failure in bone modeling Osteopetrosis	Comparable numbers of osteoclasts, lack of contact with bone surfaces, defective function	Tooth eruption failure	Lomaga *et al* (1999)
OPGL (RANKL)	OPGL−/−	Severe osteopetrosis	Absence of osteoclasts	Tooth eruption failure	Kong *et al* (1999c)
MMP2	MMP2−/−	Decreased bone mineralization, joint erosion Defective bone remodeling	Decreased osteoclast number	Early and persistent craniofacial defects	Mosig *et al* (2007)
Msx2	Msx2−/− transgenic	Generalized osteopetrosis in young animals	Defective osteoclast differentiation, decreased osteoclast number	Amelogenesis imperfecta, dentinogenesis imperfecta and periodental osteopetrosis	Aioub *et al* (2007)
VEGF-R1	$Flt1^{TK}$−/−	Mild reduction of trabecular bone	Mild reduction of osteoclasts	Not described	Niida *et al* (2005)
VEGF-R1/op	op/op$Flt1^{TK}$−/−	Severe bone marrow cavity occlusion, myelofibrosis	Decreased osteoclast number	'Toothless'	Niida *et al* (2005)

αvβ3 itself is regulated to provide new insights on modulation of osteoclast activation.

The role of collagen integrin α2β1 in osteoclast adhesion and function is controversial. Beta1 integrin may also be involved in binding to other matrix proteins such as bone sialoprotein (Horton *et al*, 1995). Early studies showed this integrin together with its matrix collagen were only minimally involved in osteoclast binding to bone (Ross *et al*, 1993). However, more recent studies support the importance of this integrin. The α2β1 integrin binding to collagen is RGD dependent, and this integrin is also localized in the ruffled boarder of resorbing osteoclasts (Helfrich *et al*, 1996). Antibodies or anti-sense oligos against α2β1 can inhibit osteoclast binding to collagen as well as osteoclast resorptive function (Helfrich *et al*, 1996; Townsend *et al*, 1999).

Other than integrins, receptor tyrosine kinases also contribute to mature osteoclast activation. Cytokine or growth factor binding triggers tyrosine kinase activity and subsequent cellular responses. Receptor kinases are composed of an extracellular domain, tryosine kinase domain, transmembrane domain and one or more regulatory domains. Based on their structure, receptor tyrosine kinases are categorized into two groups: receptors with cystine-rich regions in the extracellular domain and an uninterrupted kinase domain, and receptors with immunoglobulin-like variable and constant regions having a regulatory region insertion into the kinase domain (Yarden and Ullrich, 1988). Several receptor tyrosine kinase activating cytokines such as M-CSF, hepatocyte growth factor (HGF) and fibroblast growth factor (FGF) not only contribute to osteoclastogenesis but can also activate mature osteoclasts. The receptor tyrosine kinase receptor for M-CSF is c-fms. Addition of M-CSF and RANKL to differentiated osteoclasts induces integrin αvβ3 redistribution and conformation change, which modulate osteoclast survival, migration and chemotaxis. Data from human primary osteoclasts support that M-CSF/c-fms interaction can also increase osteoclastic bone resorption (Faccio *et al*, 2002), while other data from murine osteoclast cultures showed decreased bone resorption, probably due to M-CSF's differential regulation on osteoclast apoptosis and resorbing activity (Fuller *et al*, 1993).

Hepatocyte growth factor (HGF), binding to its receptor tyrosine kinase c-met, also promotes osteoclastogenesis and osteoclast activation. Although this is directed more to osteoclast precursors (Sato *et al*, 1995; Fuller *et al*, 1995), it also affects osteoclast migration, integrin activation and the PI3 kinase pathway (Faccio *et al*, 2002).

Among the four fibroblast growth factor receptors (FGFR1-4) only FGFR-1 is expressed in osteoclasts, while others are expressed in osteoblasts. FGF-2 stimulates mature osteoclasts through direct FGFR1 activation involving the P42/44 MAP kinase pathway at low concentrations. When added in high concentration, FGF2 stimulates osteoblasts, which in turn produce COX-2 and promote bone resorption indirectly (Kawaguchi *et al*, 2000).

Ron/Stk RTK and its ligand macrophage-stimulating protein (MSP) directly promote osteoclast resorptive activity through alterations in cytoplasmic contraction, ruffled border formation, redistribution of Src and pit formation rather than via actions on osteoclastogenesis (Fuller *et al*, 1993; Kurihara *et al*, 1996). Similarly, Tyro 3 and its ligand Gas6 or protein S can also directly activate osteoclasts through the ERK (Kawaguchi *et al*, 2004) MAPK p42/44 pathway and lead to pit formation (Katagiri *et al*, 2001). Among the receptor tyrosine kinases, Tyro 3 is unique because it is expressed only in mature osteoclasts and rarely in hematopoietic stem cells or osteoclast progenitor cells, indicating its action relative to mature osteoclast activation rather than osteoclastogenesis (Nakamura *et al*, 1998b). Other receptor tyrosine kinase-associated genes like Tie, c-kit, INS-R and Axl are also expressed in mature osteoclasts but their roles in osteoclast activation need further investigation (Nakamura *et al*, 1998c).

In general, receptor tyrosine kinase activity is important in growth factor or cytokine regulation of osteoclast activation. Strategies for inhibition or promotion of these kinase activities may provide new insights in the regulation of osteoclast function.

Signaling pathways

Downstream signaling pathways in osteoclasts have been highlighted through the study of osteoclast function and activation in a series of signaling molecule-deficient mice. The function of each molecule has been demonstrated through the characterization of osteoclast function and/or the skeletal phenotype in gene-targeted murine models. The best-studied of these pathways are the αvβ3 integrin outside-in pathway and the c-Fms pathway (Figure 4). There are a lot of similarities in downstream pathways of these two receptors. Indeed, after c-Fms phosphorylation, this receptor can be recruited to and associate with the αvβ3 integrin. Upon M-CSF stimulation, these two receptors co-localize in the podosomal actin ring but not in the sealing zone (Elsegood *et al*, 2006). In addition, M-CSF can also turn on inside-out signaling to activate the αvβ3 integrin (Faccio *et al*, 2002). This indicates that the αvβ3 integrin and growth factor receptors coordinately regulate osteoclast adhesion, migration and membrane activities (Elsegood *et al*, 2006).

Upon αvβ3 activation through adhesion to vitronectin or other ligands, or c-Fms binding to M-CSF, Src is phosphorylated at Tyr416. This activates its kinase activity and interaction with other molecules to form a signaling complex, consisted of PYK2, p130Cas and c-Cbl (Duong *et al*, 1998; Sanjay *et al*, 2001; Lakkakorpi *et al*, 1999). PYK2 has both kinase and autophosphorylation activity, but only the latter is required for association with Src (Lakkakorpi *et al*, 2003). PYK2 forms a stable association with p130 (Cas) (Crk-associated subrate), which is also phosphorylated by Src (Nakamura *et al*, 1998a) and is co-localized in the sealing zone (Lakkakorpi *et al*, 1999). Src also binds and phosphorylates c-Cbl and Cbl-b. The Cbl proteins can recruit and activate additional signaling effectors,

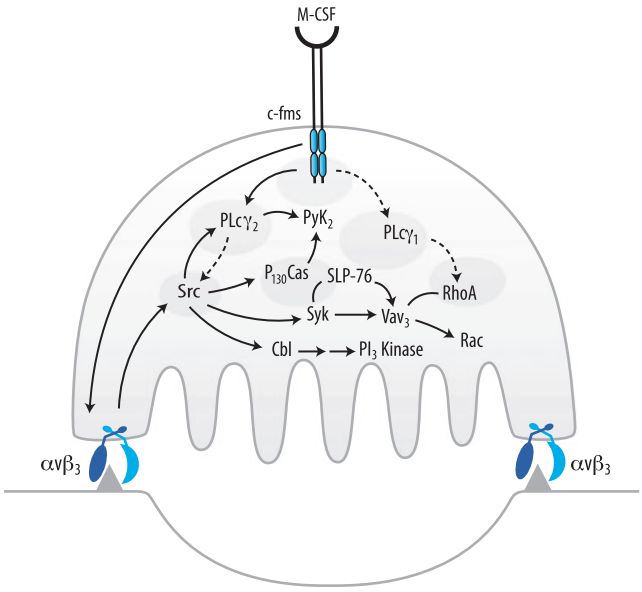

Figure 4. Model for downstream pathways of $\alpha v\beta 3$ integrin and crosstalk with M-CSF receptors in osteoclast morphology change. Ligand binding to $\alpha v\beta 3$ integrin initiates outside-in signaling involving a cascade of tyrosine phosphorylation of downstream signaling molecules. M-CSF binding to c-fms triggers inside-out signaling that leads to $\alpha v\beta 3$ activation. Activated $\alpha v\beta 3$ and c-fms work together leading to small GTPase Rac and Rho activation as well as cytoskeleton rearrangements

including phosphatidylinositol 3-kinase and dynamin (Horne *et al*, 2005). Within this signaling complex, tyrosine phosphorylation of p130Cas depends more on $\alpha v\beta 3$ integrin-mediated cell adhesion rather than M-CSF and c-fms interaction, while c-Cbl phosphorylation is predominantly activated by M-CSF and is independent of cell adhesion (Nakamura *et al*, 2003). Other than serving as adaptor proteins and active signaling molecules in osteoclasts, Cbl is also an E3 ubiquitin ligase, whose activity is confirmed in osteoclasts as to mediate Syk ubiquitination (Zou *et al*, 2009). Therefore, Cbl proteins function in two different aspects in regulating osteoclast function.

Downstream of Src, the 72-kDa nonreceptor tyrosine kinase Syk can be phosphorylated by Src and is essential for osteoclast function but not differentiation *in vitro* and *in vivo* (Zou *et al*, 2007). Syk can also trigger downstream signaling molecules and their phosphorylation including SLP-76, PLCγ2 and Vav3. The non-phosphorylated mutant Syk (Y317) can bind to Cbl to inhibit M-CSF- and integrin-stimulated Syk ubiquitination mediated by Cbl (Zou *et al*, 2009). SLP-76 is phosphorylated in a Syk-dependent manner and is critical for integrin-mediated phosphorylation of Vav3, the osteoclast cytoskeleton-organizing guanine nucleotide exchange factor (Reeve *et al*, 2009). PLCγ2 is also important in adhesion-induced PYK2 and Src phosphorylation. It mediates the interaction of Src with $\beta 3$ integrin and PYK2 complex (Epple *et al*, 2008). Vav3, a Rho family guanine nucleotide exchange factor, is essential for stimulated osteoclast activation and bone density *in vivo*. Vav3-deficient osteoclasts show defective actin cytoskeleton organization and resorption function (Faccio *et al*, 2005). Vav3 can regulate Rho GTPase activation and in turn regulate osteoclast cytoskelton and morphology.

The $\alpha v\beta 3$ integrin and c-fms have many similarities but also have differences in their signal pathways. For example, Src function is essential in integrin mediated but not in M-CSF stimulated signaling as evidenced by the findings of M-CSF stimulated osteoclast spreading and migration in Src−/− osteclasts (Nakamura *et al*, 2001). In addition, Cbl phosphorylation could also be Src independent in M-CSF/c-fms signaling (Nakamura *et al*, 2003).

Conclusion

Osteoclast and odontoclast function are closely related to physiological and pathological clinical scenarios including craniofacial abnormalities, tooth eruption and root resorption. Understanding the complex mechanisms that control osteoclast/odontoclast development and activation will provide insights in early detection and management of clinical challenges. Future translational studies should be carried out to elaborate how to modulate osteoclast/odontoclast function at the molecular level, and develop therapeutic strategies to turn on or off osteoclastogenesis/odontoclastogenesis activation pathways, and hence provide therapeutics for promoting bone remodeling or inhibiting bone resorption.

Acknowledgements

This research was supported in part by the National Institutes of Health (DE007057-32 ZW and DE019359 LM). We thank Chris Jung for illustration assistance, and Dr. Scott Conley, Dr. Sunil Kapila, and Dr. Philip McCauley for providing clinical cases and beneficial discussion.

References

Ahmad S, Bister D, Cobourne MT (2006). The clinical features and aetiological basis of primary eruption failure. *Eur J Orthod* **28:** 535–540.

Aioub M, Lezot F, Molla M *et al* (2007). Msx2−/− transgenic mice develop compound amelogenesis imperfecta, dentinogenesis imperfecta and periodental osteopetrosis. *Bone* **41:** 851–859.

Al-Qawasmi RA, Hartsfield JK Jr, Everett ET *et al* (2003). Genetic predisposition to external apical root resorption. *Am J Orthod Dentofacial Orthop* **123:** 242–252.

Bajayo A, Goshen I, Feldman S *et al* (2005). Central IL-1 receptor signaling regulates bone growth and mass. *Proc Natl Acad Sci U S A* **102:** 12956–12961.

Beertsen W, Holmbeck K, Niehof A *et al* (2002). On the role of MT1-MMP, a matrix metalloproteinase essential to collagen remodeling, in murine molar eruption and root growth. *Eur J Oral Sci* **110:** 445–451.

Berry JE, Ealba EL, Pettway GJ *et al* (2006). JunB as a downstream mediator of PTHrP actions in cementoblasts. *J Bone Miner Res* **21:** 246–257.

Berry JE, Pettway GJ, Cordell KG, Jin T, Datta NS, McCauley LK (2008). JunB as a potential mediator of PTHrP actions: new gene targets Ephrin B1 and VCAM-1. *Oral Dis* **14:** 713–726.

Bimstein E, Soskolne WA, Lustmann J (2000). Histomorphologic changes in the gingiva and pulp of overretained primary teeth. *ASDC J Dent Child* **67:** 403–7.

Blair HC, Zaidi M (2006). Osteoclastic differentiation and function regulated by old and new pathways. *Rev Endocr Metab Disord* **7:** 23–32.

Boyle WJ, Simonet WS, Lacey DL (2003). Osteoclast differentiation and activation. *Nature* **423:** 337–342.

Brezniak N, Wasserstein A (2002). Orthodontically induced inflammatory root resorption. *Part II: The clinical aspects. Angle Orthod* **72:** 180–184.

Bruckner K, Klein R (1998). Signaling by Eph receptors and their ephrin ligands. *Curr Opin Neurobiol* **8:** 375–382.

Cahill DR (1969). Eruption pathway formation in the presence of experimental tooth impaction in puppies. *Anat Rec* **164:** 67–77.

Chalhoub N, Benachenhou N, Rajapurohitam V *et al* (2003). Grey-lethal mutation induces severe malignant autosomal recessive osteopetrosis in mouse and human. *Nat Med* **9:** 399–406.

Chambers TJ, Magnus CJ (1982). Calcitonin alters behaviour of isolated osteoclasts. *J Pathol* **136:** 27–39.

Chung CJ, Soma K, Rittling SR *et al* (2008). OPN deficiency suppresses appearance of odontoclastic cells and resorption of the tooth root induced by experimental force application. *J Cell Physiol* **214:** 614–620.

Cleiren E, Benichou O, Van Hul E *et al* (2001). Albers-Schonberg disease (autosomal dominant osteopetrosis, type II) results from mutations in the ClCN7 chloride channel gene. *Hum Mol Genet* **10:** 2861–2867.

Clover J, Dodds RA, Gowen M (1992). Integrin subunit expression by human osteoblasts and osteoclasts in situ and in culture. *J Cell Sci* **103:** 267–271.

Corotti MV, Zambuzzi WF, Paiva KB *et al* (2009). Immunolocalization of matrix metalloproteinases-2 and -9 during apical periodontitis development. *Arch Oral Biol* **54:** 764–771.

Decker E, Stellzig-Eisenhauer A, Fiebig BS *et al* (2008). PTHR1 loss-of-function mutations in familial, nonsyndromic primary failure of tooth eruption. *Am J Hum Genet* **83:** 781–786.

Dolce C, Vakani A, Archer L, Morris-Wiman JA, Holliday LS (2003). Effects of echistatin and an RGD peptide on orthodontic tooth movement. *J Dent Res* **82:** 682–686.

Dunn MD, Park CH, Kostenuik PJ, Kapila S, Giannobile WV (2007). Local delivery of osteoprotegerin inhibits mechanically mediated bone modeling in orthodontic tooth movement. *Bone* **41:** 446–455.

Duong LT, Lakkakorpi PT, Nakamura I, Machwate M, Nagy RM, Rodan GA (1998). PYK2 in osteoclasts is an adhesion kinase, localized in the sealing zone, activated by ligation of alpha(v)beta3 integrin, and phosphorylated by src kinase. *J Clin Invest* **102:** 881–892.

Elsegood CL, Zhuo Y, Wesolowski GA, Hamilton JA, Rodan GA, Duong le T (2006). M-CSF induces the stable interaction of cFms with alphaVbeta3 integrin in osteoclasts. *Int J Biochem Cell Biol* **38:** 1518–1529.

Epple H, Cremasco V, Zhang K, Mao D, Longmore GD, Faccio R (2008). Phospholipase Cgamma2 modulates integrin signaling in the osteoclast by affecting the localization and activation of Src kinase. *Mol Cell Biol* **28:** 3610–3622.

Evans GL, Turner RT (1995). Tissue-selective actions of estrogen analogs. *Bone* **17:** 181S–190S.

Everts V, de Vries TJ, Helfrich MH (2009). Osteoclast heterogeneity: lessons from osteopetrosis and inflammatory conditions. *Biochim Biophys Acta* **1792:** 757–765.

Faccio R, Grano M, Colucci S *et al* (2002). Localization and possible role of two different alpha v beta 3 integrin conformations in resting and resorbing osteoclasts. *J Cell Sci* **115:** 2919–2929.

Faccio R, Teitelbaum SL, Fujikawa K *et al* (2005). Vav3 regulates osteoclast function and bone mass. *Nat Med* **11:** 284–290.

Feng X, Novack DV, Faccio R *et al* (2001). A Glanzmann's mutation in beta 3 integrin specifically impairs osteoclast function. *J Clin Invest* **107:** 1137–1144.

Frattini A, Orchard PJ, Sobacchi C *et al* (2000). Defects in TCIRG1 subunit of the vacuolar proton pump are responsible for a subset of human autosomal recessive osteopetrosis. *Nat Genet* **25:** 343–346.

Frazier-Bowers SA, Simmons D, Wright JT, Proffit WR, Ackerman JL (2010). Primary failure of eruption and PTH1R: the importance of a genetic diagnosis for orthodontic treatment planning. *Am J Orthod Dentofacial Orthop* **137:** 160e–e7; discussion 160–1.

Fujikawa Y, Quinn JM, Sabokbar A, McGee JO, Athanasou NA (1996). The human osteoclast precursor circulates in the monocyte fraction. *Endocrinology* **137:** 4058–4060.

Fukushima H, Kajiya H, Takada K, Okamoto F, Okabe K (2003). Expression and role of RANKL in periodontal ligament cells during physiological root-resorption in human deciduous teeth. *Eur J Oral Sci* **111:** 346–352.

Fuller K, Owens JM, Jagger CJ, Wilson A, Moss R, Chambers TJ (1993). Macrophage colony-stimulating factor stimulates survival and chemotactic behavior in isolated osteoclasts. *J Exp Med* **178:** 1733–1744.

Fuller K, Owens J, Chambers TJ (1995). The effect of hepatocyte growth factor on the behaviour of osteoclasts. *Biochem Biophys Res Commun* **212:** 334–340.

Gelb BD, Shi GP, Chapman HA, Desnick RJ (1996). Pycnodysostosis, a lysosomal disease caused by cathepsin K deficiency. *Science* **273:** 1236–1238.

Gotz W, Quondamatteo F, Ragotzki S, Affeldt J, Jager A (2000). Localization of cathepsin D in human odontoclasts. a light and electron microscopical immunocytochemical study. *Connect Tissue Res* **41:** 185–194.

Grier RLt, Wise GE (1998). Inhibition of tooth eruption in the rat by a bisphosphonate. *J Dent Res* **77:** 8–15.

Grier RLt, Zhao L, Adams CE, Wise GE (1998). Secretion of CSF-1 and its inhibition in rat dental follicle cells: implications for tooth eruption. *Eur J Oral Sci* **106:** 808–815.

Guerrini MM, Sobacchi C, Cassani B *et al* (2008). Human osteoclast-poor osteopetrosis with hypogammaglobulinemia due to TNFRSF11A (RANK) mutations. *Am J Hum Genet* **83:** 64–76.

Harokopakis-Hajishengallis E (2007). Physiologic root resorption in primary teeth: molecular and histological events. *J Oral Sci* **49:** 1–12.

Harris EF, Boggan BW, Wheeler DA (2001). Apical root resorption in patients treated with comprehensive orthodontics. *J Tenn Dent Assoc* **81:** 30–33.

Hartsfield JK Jr (2009). Pathways in external apical root resorption associated with orthodontia. *Orthod Craniofac Res* **12:** 236–242.

Hartsfield JK Jr, Everett ET, Al-Qawasmi RA (2004). Genetic factors in external apical root resorption and orthodontic treatment. *Crit Rev Oral Biol Med* **15:** 115–122.

Hasegawa T, Kikuiri T, Takeyama S *et al* (2002). Human periodontal ligament cells derived from deciduous teeth induce osteoclastogenesis in vitro. *Tissue Cell* **34:** 44–51.

Helfrich MH, Nesbitt SA, Lakkakorpi PT *et al* (1996). Beta 1 integrins and osteoclast function: involvement in collagen recognition and bone resorption. *Bone* **19:** 317–328.

Henriksen K, Tanko LB, Qvist P, Delmas PD, Christiansen C, Karsdal MA (2007). Assessment of osteoclast number and function: application in the development of new and improved treatment modalities for bone diseases. *Osteoporos Int* **18:** 681–685.

Henriksen K, Gram J, Neutzsky-Wulff AV *et al* (2009). Characterization of acid flux in osteoclasts from patients harboring a G215R mutation in ClC-7. *Biochem Biophys Res Commun* **378:** 804–809.

Hofbauer LC, Lacey DL, Dunstan CR, Spelsberg TC, Riggs BL, Khosla S (1999). Interleukin-1beta and tumor necrosis factor-alpha, but not interleukin-6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. *Bone* **25:** 255–259.

Holmbeck K, Bianco P, Caterina J *et al* (1999). MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. *Cell* **99:** 81–92.

Honda S, Honda Y, Bauer B, Ruan C, Kunicki TJ (1995). The impact of three-dimensional structure on the expression of PlA alloantigens on human integrin beta 3. *Blood* **86:** 234–242.

Horne WC, Sanjay A, Bruzzaniti A, Baron R (2005). The role(s) of Src kinase and Cbl proteins in the regulation of osteoclast differentiation and function. *Immunol Rev* **208:** 106–125.

Horton MA, Nesbit MA, Helfrich MH (1995). Interaction of osteopontin with osteoclast integrins. *Ann N Y Acad Sci* **760:** 190–200.

Huang H, Wise GE (2000). Delay of tooth eruption in null mice devoid of the type I IL-1R gene. *Eur J Oral Sci* **108:** 297–302.

Hughes DE, Dai A, Tiffee JC, Li HH, Mundy GR, Boyce BF (1996). Estrogen promotes apoptosis of murine osteoclasts mediated by TGF-beta. *Nat Med* **2:** 1132–1136.

Ida-Yonemochi H, Noda T, Shimokawa H, Saku T (2002). Disturbed tooth eruption in osteopetrotic (op/op) mice: histopathogenesis of tooth malformation and odontomas. *J Oral Pathol Med* **31:** 361–373.

Inaoka T, Bilbe G, Ishibashi O, Tezuka K, Kumegawa M, Kokubo T (1995). Molecular cloning of human cDNA for cathepsin K: novel cysteine proteinase predominantly expressed in bone. *Biochem Biophys Res Commun* **206:** 89–96.

Jacquin C, Koczon-Jaremko B, Aguila HL *et al* (2009). Macrophage migration inhibitory factor inhibits osteoclastogenesis. *Bone* **45:** 640–649.

Josien R, Wong BR, Li HL, Steinman RM, Choi Y (1999). TRANCE, a TNF family member, is differentially expressed on T cell subsets and induces cytokine production in dendritic cells. *J Immunol* **162:** 2562–2568.

Kamoun-Goldrat A, Ginisty D, Le Merrer M (2008). Effects of bisphosphonates on tooth eruption in children with osteogenesis imperfecta. *Eur J Oral Sci* **116:** 195–198.

Kanematsu M, Sato T, Takai H, Watanabe K, Ikeda K, Yamada Y (2000). Prostaglandin E2 induces expression of receptor activator of nuclear factor-kappa B ligand/osteoprotegrin ligand on pre-B cells: implications for accelerated osteoclastogenesis in estrogen deficiency. *J Bone Miner Res* **15:** 1321–1329.

Kanzaki H, Chiba M, Shimizu Y, Mitani H (2001). Dual regulation of osteoclast differentiation by periodontal ligament cells through RANKL stimulation and OPG inhibition. *J Dent Res* **80:** 887–891.

Katagiri M, Hakeda Y, Chikazu D *et al* (2001). Mechanism of stimulation of osteoclastic bone resorption through Gas6/-Tyro 3, a receptor tyrosine kinase signaling, in mouse osteoclasts. *J Biol Chem* **276:** 7376–7382.

Kawaguchi H, Chikazu D, Nakamura K, Kumegawa M, Hakeda Y (2000). Direct and indirect actions of fibroblast growth factor 2 on osteoclastic bone resorption in cultures. *J Bone Miner Res* **15:** 466–473.

Kawaguchi H, Katagiri M, Chikazu D (2004). Osteoclastic bone resorption through receptor tyrosine kinase and extracellular signal-regulated kinase signaling in mature osteoclasts. *Mod Rheumatol* **14:** 1–5.

Kawakami M, Kuroda S, Yoshida CA, Yamashita K, Takada K (2000). Dental follicle cell-conditioned medium enhances the formation of osteoclast-like multinucleated cells. *Eur J Orthod* **22:** 675–682.

Kawata T, Tokimasa C, Nowroozi N *et al* (1999). Lack of the bone remodeling in osteopetrotic (op/op) mice associated with microdontia. *J Craniofac Genet Dev Biol* **19:** 113–117.

Khosla S, Burr D, Cauley J *et al* (2007). Bisphosphonate-associated osteonecrosis of the jaw: report of a task force of the American Society for Bone and Mineral Research. *J Bone Miner Res* **22:** 1479–14791.

Killiany DM (1999). Root resorption caused by orthodontic treatment: an evidence-based review of literature. *Semin Orthod* **5:** 128–133.

Komori T, Yagi H, Nomura S *et al* (1997). Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. *Cell* **89:** 755–764.

Kong YY, Boyle WJ, Penninger JM (1999a). Osteoprotegerin ligand: a common link between osteoclastogenesis, lymph node formation and lymphocyte development. *Immunol Cell Biol* **77:** 188–193.

Kong YY, Feige U, Sarosi I *et al* (1999b). Activated T cells regulate bone loss and joint destruction in adjuvant arthritis through osteoprotegerin ligand. *Nature* **402:** 304–309.

Kong YY, Yoshida H, Sarosi I *et al* (1999c). OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature* **397:** 315–323.

Kornak U, Kasper D, Bosl MR *et al* (2001). Loss of the ClC-7 chloride channel leads to osteopetrosis in mice and man. *Cell* **104:** 205–215.

Kostenuik PJ, Shalhoub V (2001). Osteoprotegerin: a physiological and pharmacological inhibitor of bone resorption. *Curr Pharm Des* **7:** 613–635.

Krakowiak FJ (1978). Ankylosed primary molars. *ASDC J Dent Child* **45:** 288–292.

Kurihara N, Iwama A, Tatsumi J, Ikeda K, Suda T (1996). Macrophage-stimulating protein activates STK receptor tyrosine kinase on osteoclasts and facilitates bone resorption by osteoclast-like cells. *Blood* **87:** 3704–3710.

Lacey DL, Timms E, Tan HL *et al* (1998). Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell* **93:** 165–176.

Lakkakorpi PT, Nakamura I, Nagy RM, Parsons JT, Rodan GA, Duong LT (1999). Stable association of PYK2 and p130(Cas) in osteoclasts and their co-localization in the sealing zone. *J Biol Chem* **274:** 4900–4907.

Lakkakorpi PT, Bett AJ, Lipfert L, Rodan GA, Duong le T (2003). PYK2 autophosphorylation, but not kinase activity, is necessary for adhesion-induced association with c-Src, osteoclast spreading, and bone resorption. *J Biol Chem* **278:** 11502–11512.

Lam J, Nelson CA, Ross FP, Teitelbaum SL, Fremont DH (2001). Crystal structure of the TRANCE/RANKL cytokine reveals determinants of receptor-ligand specificity. *J Clin Invest* **108:** 971–979.

Lee SK, Lorenzo JA (1999). Parathyroid hormone stimulates TRANCE and inhibits osteoprotegerin messenger ribonucleic acid expression in murine bone marrow cultures:

correlation with osteoclast-like cell formation. *Endocrinology* **140:** 3552–3561.

Lee A, Schneider G, Finkelstein M, Southard T (2004). Root resorption: the possible role of extracellular matrix proteins. *Am J Orthod Dentofacial Orthop* **126:** 173–177.

Leite AF, Figueiredo PT, Melo NS *et al* (2006). Bisphosphonate-associated osteonecrosis of the jaws. Report of a case and literature review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **102:** 14–21.

Lindsay R, Cosman F (1997). Skeletal effects of estrogen analogs. *Osteoporos Int* **7**(Suppl 1)**:** S40–S42.

Linsuwanont-Santiwong B, Takagi Y, Ohya K, Shimokawa H (2006). Expression of MT1-MMP during deciduous tooth resorption in odontoclasts. *J Bone Miner Metab* **24:** 447–453.

Liu D, Yao S, Pan F, Wise GE (2005). Chronology and regulation of gene expression of RANKL in the rat dental follicle. *Eur J Oral Sci* **113:** 404–409.

Lomaga MA, Yeh WC, Sarosi I *et al* (1999). TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling. *Genes Dev* **13:** 1015–1024.

Lopatiene K, Dumbravaite A (2008). Risk factors of root resorption after orthodontic treatment. *Stomatologija* **10:** 89–95.

Lossdorfer S, Gotz W, Jager A (2002). Immunohistochemical localization of receptor activator of nuclear factor kappaB (RANK) and its ligand (RANKL) in human deciduous teeth. *Calcif Tissue Int* **71:** 45–52.

Lossdorfer S, Abou Jamra B, Rath-Deschner B *et al* (2009). The role of periodontal ligament cells in delayed tooth eruption in patients with cleidocranial dysostosis. *J Orofac Orthop* **70:** 495–510.

Lowe C, Yoneda T, Boyce BF, Chen H, Mundy GR, Soriano P (1993). Osteopetrosis in Src-deficient mice is due to an autonomous defect of osteoclasts. *Proc Natl Acad Sci U S A* **90:** 4485–4489.

Marks SC Jr, Cahill DR (1984). Experimental study in the dog of the non-active role of the tooth in the eruptive process. *Arch Oral Biol* **29:** 311–322.

Marks SC Jr, Lane PW (1976). Osteopetrosis, a new recessive skeletal mutation on chromosome 12 of the mouse. *J Hered* **67:** 11–18.

McCauley LK, Nohutcu RM (2002). Mediators of periodontal osseous destruction and remodeling: principles and implications for diagnosis and therapy. *J Periodontol* **73:** 1377–1391.

McCauley LK, Rosol TJ, Capen CC, Horton JE, Shanfeld J (1989). Investigations on in vitro bone resorbing activity from athymic (nude) and euthymic mouse splenic leukocytes. *Bone* **10:** 389–394.

McCauley LK, Rosol TJ, Stromberg PC, Capen CC (1991). Effects of interleukin-1 alpha and cyclosporin A in vivo and in vitro on bone and lymphoid tissues in mice. *Toxicol Pathol* **19:** 1–10.

McHugh KP, Hodivala-Dilke K, Zheng MH *et al* (2000). Mice lacking beta3 integrins are osteosclerotic because of dysfunctional osteoclasts. *J Clin Invest* **105:** 433–440.

Mosig RA, Dowling O, DiFeo A *et al* (2007). Loss of MMP-2 disrupts skeletal and craniofacial development and results in decreased bone mineralization, joint erosion and defects in osteoblast and osteoclast growth. *Hum Mol Genet* **16:** 1113–1123.

Nakamura I, Jimi E, Duong LT *et al* (1998a). Tyrosine phosphorylation of p130Cas is involved in actin organization in osteoclasts. *J Biol Chem* **273:** 11144–11149.

Nakamura I, Tanaka H, Rodan GA, Duong LT (1998b). Echistatin inhibits the migration of murine prefusion osteoclasts and the formation of multinucleated osteoclast-like cells. *Endocrinology* **139:** 5182–5193.

Nakamura YS, Hakeda Y, Takakura N *et al* (1998c). Tyro 3 receptor tyrosine kinase and its ligand, Gas6, stimulate the function of osteoclasts. *Stem Cells* **16:** 229–238.

Nakamura I, Lipfert L, Rodan GA, Le TD (2001). Convergence of alpha(v)beta(3) integrin- and macrophage colony stimulating factor-mediated signals on phospholipase Cgamma in prefusion osteoclasts. *J Cell Biol* **152:** 361–373.

Nakamura I, Rodan GA, Duong le T (2003). Distinct roles of p130Cas and c-Cbl in adhesion-induced or macrophage colony-stimulating factor-mediated signaling pathways in prefusion osteoclasts. *Endocrinology* **144:** 4739–4741.

Nakao A, Kajiya H, Fukushima H *et al* (2009). PTHrP induces Notch signaling in periodontal ligament cells. *J Dent Res* **88:** 551–556.

Negishi-Koga T, Takayanagi H (2009). Ca2+-NFATc1 signaling is an essential axis of osteoclast differentiation. *Immunol Rev* **231:** 241–256.

Nesbitt S, Nesbit A, Helfrich M, Horton M (1993). Biochemical characterization of human osteoclast integrins. Osteoclasts express alpha v beta 3, alpha 2 beta 1, and alpha v beta 1 integrins. *J Biol Chem* **268:** 16737–16745.

Niida S, Kondo T, Hiratsuka S *et al* (2005). VEGF receptor 1 signaling is essential for osteoclast development and bone marrow formation in colony-stimulating factor 1-deficient mice. *Proc Natl Acad Sci U S A* **102:** 14016–14021.

Nohutcu RM, McCauley LK, Horton JE, Capen CC, Rosol TJ (1993). Effects of hormones and cytokines on stimulation of adenylate cyclase and intracellular calcium concentration in human and canine periodontal-ligament fibroblasts. *Arch Oral Biol* **38:** 871–879.

O'Brien CA, Gubrij I, Lin SC, Saylors RL, Manolagas SC (1999). STAT3 activation in stromal/osteoblastic cells is required for induction of the receptor activator of NF-kappaB ligand and stimulation of osteoclastogenesis by gp130-utilizing cytokines or interleukin-1 but not 1,25-dihydroxyvitamin D3 or parathyroid hormone. *J Biol Chem* **274:** 19301–19308.

Palaska PK, Cartsos V, Zavras AI (2009). Bisphosphonates and time to osteonecrosis development. *Oncologist* **14:** 1154–1166.

Philbrick WM, Dreyer BE, Nakchbandi IA, Karaplis AC (1998). Parathyroid hormone-related protein is required for tooth eruption. *Proc Natl Acad Sci U S A* **95:** 11846–11851.

Plow EF, Qin J, Byzova T (2009). Kindling the flame of integrin activation and function with kindlins. *Curr Opin Hematol* **16:** 323–328.

Popoff SN, Marks SC Jr. (1990). Relationship of abnormalities in dental and skeletal development in the osteopetrotic (os) rabbit. *J Oral Pathol Med* **19:** 5–12.

Proffit WR, Frazier-Bowers SA (2009). Mechanism and control of tooth eruption: overview and clinical implications. *Orthod Craniofac Res* **12:** 59–66.

Proffit WR, Vig KW (1981). Primary failure of eruption: a possible cause of posterior open-bite. *Am J Orthod* **80:** 173–190.

Rani CS, MacDougall M (2000). Dental cells express factors that regulate bone resorption. *Mol Cell Biol Res Commun* **3:** 145–152.

Reeve JL, Zou W, Liu Y, Maltzman JS, Ross FP, Teitelbaum SL (2009). SLP-76 couples Syk to the osteoclast cytoskeleton. *J Immunol* **183:** 1804–1812.

Riggs BL (2000). The mechanisms of estrogen regulation of bone resorption. *J Clin Invest* **106:** 1203–1204.

Ross FP, Teitelbaum SL (2005). alphavbeta3 and macrophage colony-stimulating factor: partners in osteoclast biology. *Immunol Rev* **208:** 88–105.

Ross FP, Chappel J, Alvarez JI *et al* (1993). Interactions between the bone matrix proteins osteopontin and bone sialoprotein and the osteoclast integrin alpha v beta 3 potentiate bone resorption. *J Biol Chem* **268:** 9901–9907.

Russell RG, Rogers MJ (1999). Bisphosphonates: from the laboratory to the clinic and back again. *Bone* **25:** 97–106.

Sahara N (2001). Cellular events at the onset of physiological root resorption in rabbit deciduous teeth. *Anat Rec* **264:** 387–396.

Sahara N, Okafuji N, Toyoki A, Suzuki I, Deguchi T, Suzuki K (1992). Odontoclastic resorption at the pulpal surface of coronal dentin prior to the shedding of human deciduous teeth. *Arch Histol Cytol* **55:** 273–285.

Sahara N, Toyoki A, Ashizawa Y, Deguchi T, Suzuki K (1996). Cytodifferentiation of the odontoclast prior to the shedding of human deciduous teeth: an ultrastructural and cytochemical study. *Anat Rec* **244:** 33–49.

Sanjay A, Houghton A, Neff L *et al* (2001). Cbl associates with Pyk2 and Src to regulate Src kinase activity, alpha(v)beta(3) integrin-mediated signaling, cell adhesion, and osteoclast motility. *J Cell Biol* **152:** 181–195.

Sato T, Hakeda Y, Yamaguchi Y *et al* (1995). Hepatocyte growth factor is involved in formation of osteoclast-like cells mediated by clonal stromal cells (MC3T3-G2/PA6). *J Cell Physiol* **164:** 197–204.

Segal GR, Schiffman PH, Tuncay OC (2004). Meta analysis of the treatment-related factors of external apical root resorption. *Orthod Craniofac Res* **7:** 71–78.

Segovia-Silvestre T, Neutzsky-Wulff AV, Sorensen MG *et al* (2009). Advances in osteoclast biology resulting from the study of osteopetrotic mutations. *Hum Genet* **124:** 561–577.

Sly WS, Hewett-Emmett D, Whyte MP, Yu YS, Tashian RE (1983). Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. *Proc Natl Acad Sci U S A* **80:** 2752–2756.

Sobacchi C, Frattini A, Guerrini MM *et al* (2007). Osteoclast-poor human osteopetrosis due to mutations in the gene encoding RANKL. *Nat Genet* **39:** 960–962.

Soltanoff CS, Yang S, Chen W, Li YP (2009). Signaling networks that control the lineage commitment and differentiation of bone cells. *Crit Rev Eukaryot Gene Expr* **19:** 1–46.

Soriano P, Montgomery C, Geske R, Bradley A (1991). Targeted disruption of the c-src proto-oncogene leads to osteopetrosis in mice. *Cell* **64:** 693–702.

Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD (1998). How cells respond to interferons. *Annu Rev Biochem* **67:** 227–264.

Takayanagi H, Ogasawara K, Hida S *et al* (2000). T-cell-mediated regulation of osteoclastogenesis by signalling cross-talk between RANKL and IFN-gamma. *Nature* **408:** 600–605.

Takayanagi H, Kim S, Taniguchi T (2002). Signaling crosstalk between RANKL and interferons in osteoclast differentiation. *Arthritis Res* **4**(Suppl 3)**:** S227–S232.

Talic NF, Evans C, Zaki AM (2006). Inhibition of orthodontically induced root resorption with echistatin, an RGD-containing peptide. *Am J Orthod Dentofacial Orthop* **129:** 252–260.

Teng YT, Nguyen H, Gao X *et al* (2000). Functional human T-cell immunity and osteoprotegerin ligand control alveolar bone destruction in periodontal infection. *J Clin Invest* **106:** R59–R67.

Tiffee JC, Xing L, Nilsson S, Boyce BF (1999). Dental abnormalities associated with failure of tooth eruption in src knockout and op/op mice. *Calcif Tissue Int* **65:** 53–58.

Tofteng CL, Bach-Mortensen P, Bojesen SE *et al* (2007). Integrin beta3 Leu33Pro polymorphism and risk of hip fracture: 25 years follow-up of 9233 adults from the general population. *Pharmacogenet Genomics* **17:** 85–91.

Tolar J, Teitelbaum SL, Orchard PJ (2004). Osteopetrosis. *N Engl J Med* **351:** 2839–2849.

Townsend PA, Villanova I, Teti A, Horton MA (1999). Beta1 integrin antisense oligodeoxynucleotides: utility in controlling osteoclast function. *Eur J Cell Biol* **78:** 485–496.

Tucci M, De Palma R, Lombardi L *et al* (2009). beta(3) Integrin subunit mediates the bone-resorbing function exerted by cultured myeloma plasma cells. *Cancer Res* **69:** 6738–6746.

Twigg SR, Kan R, Babbs C *et al* (2004). Mutations of ephrin-B1 (EFNB1), a marker of tissue boundary formation, cause craniofrontonasal syndrome. *Proc Natl Acad Sci USA* **101:** 8652–8657.

Uchiyama M, Nakamichi Y, Nakamura M *et al* (2009). Dental pulp and periodontal ligament cells support osteoclastic differentiation. *J Dent Res* **88:** 609–614.

Van Wesenbeeck L, Odgren PR, Coxon FP *et al* (2007). Involvement of PLEKHM1 in osteoclastic vesicular transport and osteopetrosis in incisors absent rats and humans. *J Clin Invest* **117:** 919–930.

Vance MA (2007). Osteonecrosis of the jaw and bisphosphonates: a comparison with white phosphorus, radium, and osteopetrosis. *Clin Toxicol (Phila)* **45:** 753–762.

Viecilli RF, Katona TR, Chen J, Hartsfield JK Jr, Roberts WE (2009). Orthodontic mechanotransduction and the role of the P2X7 receptor. *Am J Orthod Dentofacial Orthop* **135:** 694e1–16.

Vijayan KV, Goldschmidt-Clermont PJ, Roos C, Bray PF (2000). The Pl(A2) polymorphism of integrin beta(3) enhances outside-in signaling and adhesive functions. *J Clin Invest* **105:** 793–802.

Villa A, Guerrini MM, Cassani B, Pangrazio A, Sobacchi C (2009). Infantile malignant, autosomal recessive osteopetrosis: the rich and the poor. *Calcif Tissue Int* **84:** 1–12.

Wang ZQ, Ovitt C, Grigoriadis AE, Mohle-Steinlein U, Ruther U, Wagner EF (1992). Bone and haematopoietic defects in mice lacking c-fos. *Nature* **360:** 741–745.

Whyte MP (1993). Carbonic anhydrase II deficiency. *Clin Orthop Relat Res* **294:** 52–63.

Wise GE, Huang H, Que BG (1999). Gene expression of potential tooth eruption molecules in the dental follicle of the mouse. *Eur J Oral Sci* **107:** 482–486.

Wise GE, Frazier-Bowers S, D'Souza RN (2002). Cellular, molecular, and genetic determinants of tooth eruption. *Crit Rev Oral Biol Med* **13:** 323–334.

Xiong H, Wei L, Peng B (2009). Immunohistochemical localization of IL-17 in induced rat periapical lesions. *J Endod* **35:** 216–220.

Yamaguchi M (2009). RANK/RANKL/OPG during orthodontic tooth movement. *Orthod Craniofac Res* **12:** 113–119.

Yang J, Peng B (2008). Correlation between the expression of c-Fos and osteoclasts in induced periapical lesions in rats. *J Endod* **34:** 22–25.

Yarden Y, Ullrich A (1988). Growth factor receptor tyrosine kinases. *Annu Rev Biochem* **57:** 443–478.

Yasuda H, Shima N, Nakagawa N *et al* (1998). Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. *Proc Natl Acad Sci U S A* **95:** 3597–3602.

Yildirim S, Yapar M, Sermet U, Sener K, Kubar A (2008). The role of dental pulp cells in resorption of deciduous teeth. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* **105:** 113–120.

Yoda S, Suda N, Kitahara Y, Komori T, Ohyama K (2004). Delayed tooth eruption and suppressed osteoclast number in the eruption pathway of heterozygous Runx2/Cbfa1 knock-out mice. *Arch Oral Biol* **49:** 435–442.

Yoshino M, Yamazaki H, Yoshida H *et al* (2003). Reduction of osteoclasts in a critical embryonic period is essential for inhibition of mouse tooth eruption. *J Bone Miner Res* **18:** 108–116.

Zhang X, Peng B (2005). Immunolocalization of receptor activator of NF kappa B ligand in rat periapical lesions. *J Endod* **31:** 574–577.

Zhang R, Wang L, Peng B (2008). Activation of p38 mitogen-activated protein kinase in rat periapical lesions. *J Endod* **34:** 1207–1210.

Zhao C, Irie N, Takada Y *et al* (2006). Bidirectional ephrinB2-EphB4 signaling controls bone homeostasis. *Cell Metab* **4:** 111–121.

Zou W, Kitaura H, Reeve J *et al* (2007). Syk, c-Src, the alphavbeta3 integrin, and ITAM immunoreceptors, in concert, regulate osteoclastic bone resorption. *J Cell Biol* **176:** 877–888.

Zou W, Reeve JL, Zhao H, Ross FP, Teitelbaum SL (2009). Syk tyrosine 317 negatively regulates osteoclast function via the ubiquitin-protein isopeptide ligase activity of Cbl. *J Biol Chem* **284:** 18833–18839.