



Positive and negative regulators of osteoclast apoptosis

Niroshani Surangika Soysa^{a,*}, Neil Alles^b

^a Division of Pharmacology, Department of Oral Medicine and Periodontology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka

^b Department of Biochemistry, Faculty of Medicine, University of Peradeniya, Sri Lanka



ARTICLE INFO

Keywords:

Osteoclasts
Apoptosis
FAS/FASL system
TRAIL/TRAILR system
Estrogen

ABSTRACT

Survival and apoptosis are of major importance in the osteoclast life cycle. As osteoclasts have short lifespan, any alteration that prolongs their viability may cause enhanced osteoclast activity. Hence, the regulation of OC apoptosis has been recognized as a critical factor in bone remodeling. An imbalance in bone remodeling due to increased osteoclast activity leads to most adult bone diseases such as osteoporosis, rheumatoid arthritis and multiple myeloma. Therefore, manipulating osteoclast death would be a viable therapeutic approach in ameliorating bone diseases, with accelerated resorption. Over the last few decades we have witnessed the unraveling of many of the intracellular mechanisms responsible for osteoclast apoptosis. Thus, an understanding of the underlying mechanisms by which osteoclasts undergo programmed cell death and the regulators that modulate that activity will undoubtedly provide an insight into the development of pharmacological agents to treat such pathological bone diseases.

1. Introduction

The term ‘apoptosis’ derived from the Greek language meaning, “dropping off or falling off of petals from flowers, or leaves from trees in autumn” has been coined by Kerr, Wyllie and Currie (Kerr et al., 1972) to describe the active and well-defined process of self-destruction of cells which is characterized by morphological changes such as nuclear and cytoplasmic condensation, formation of apoptotic bodies and their phagocytosis and degradation by other neighboring cells in tissue homeostasis. Apoptosis (type I cell death) is a distinctive mode of controlled programmed cell death (PCD) (other forms of PCD include autophagy, pyroptosis and oncosis) which is genetically determined and undergoes characteristic morphological and biochemical changes. Apoptosis results in the removal of cells, with little tissue disruption. It plays a complementary, yet an opposite role to mitosis in the regulation of animal cell population and occurs at a particular time point in cellular development and shows remarkable accuracy (Elmore, 2007). Apoptosis is important in maintaining the cell population: during cell injury and aging, and as a defense mechanism to eliminate damaged cells (Norbury and Hickson, 2001). Apoptosis is also involved in disease conditions such as cancer, AIDS, Parkinson’s disease and Alzheimer’s disease. Focal apoptosis is important for normal embryonic processes such as the development of the lumina of tubular structures, the fashioning of limbs and formation of interdigital clefts (Hughes and Boyce, 1997; Xing and Boyce, 2005).

Bone is a dynamic tissue and consists of cells such as osteoclasts (OCs) and osteoblasts (OBs) which interact with each other in basic multicellular units (BMUs) in which both cell types are actively involved in bone resorption and bone formation, respectively, in the process of remodeling. Physiological death of bone cells such as chondrocytes, OB precursors, OBs and OCs occurs in several situations such as during growth plate ossification, as a mean of limiting OB numbers, at the cessation of bone formation and during the reversal phase of bone remodeling, respectively (Hughes and Boyce, 1997). OCs are bone resorbing cells of hematopoietic origin. Mononuclear OC precursors (OCPs) are fused together to form the terminally differentiated multinuclear polykaryon which no longer has the capacity to proliferate. Osteoclastic bone resorption is necessary during the development of bone and in reshaping or replacement of bone during growth and following injuries. The rate of bone resorption is determined by both the number and activity of OCs. Excessive OC activity may lead to increased bone resorption and osteoporosis, whereas reduced activity will lead to an imbalance in remodeling, favoring osteopetrosis (Soysa and Alles, 2016).

The lifespan of OCs is relatively short both *in vitro* and *in vivo*, and the activated OCs in a healthy human adult usually survive approximately 2–3 weeks in the marrow (Manolagas, 2000; Weinstein and Manolagas, 2000) and then undergo apoptosis. Hence, the number of OCs is dependent upon the relative rates of cell differentiation and death. It has been demonstrated that in the presence of high levels of

* Corresponding author.

E-mail addresses: niroshanis@pdn.ac.lk (N.S. Soysa), cnraa@pdn.ac.lk (N. Alles).

<https://doi.org/10.1016/j.bonr.2019.100225>

Received 19 March 2019; Received in revised form 22 August 2019; Accepted 2 October 2019

Available online 21 October 2019

2352-1872/ © 2019 Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

extracellular calcium generated during bone resorption, OCs are subjected to negative feedback regulation through the induction of apoptosis (Lorget et al., 2000). Importantly, changes in the regulation of OC apoptosis are evident in many pathological bone diseases such as postmenopausal osteoporosis, rheumatoid arthritis (RA), osteoarthritis, hyperparathyroidism and Paget's disease of bone (PDB) (Roux and Brown, 2009). Apoptosis in OCs might be due to a lack of balance between survival factors and factors which may oppose them. While signaling through the macrophage colony stimulating factor (M-CSF), the receptor activator of nuclear factor kappa-B (NF- κ B) ligand (RANKL), tumor necrosis factor (TNF)- α and interleukin (IL)-1 via NF- κ B and Akt is necessary for cell survival, osteoprotegerin (OPG) and other factors such as estrogen cause OC apoptosis (Liu et al., 2015; Nakamura et al., 2007). Interestingly, extracellular acidosis has been identified not only as a potent stimulator of OC resorptive activity and differentiation, but also as an inhibitor of OC apoptosis, contributing to the enhancement of OC lifespan. Decreased OC apoptosis following ovariectomy (Hughes et al., 1996) may lead to enhanced bone resorption. Therefore, OC apoptosis can be considered as another strategy to reduce OC activity in conditions of accelerated bone resorption, such as RA and multiple myeloma. It can be assumed that OC activity could be controlled through regulated apoptosis. This assumption has been corroborated by a study by Wu and colleagues (Wu et al., 2008) who showed that mice lacking the solute carrier family 4 (SLC4)-A2 have a higher degree of OC apoptosis than their wild type (WT) counterparts. The HCO₃⁻/Cl⁻ anion exchanger, SLC4A, is upregulated during osteoclastogenesis through the nuclear factor of activated T Cells 1(NFATc1)-dependent pathway. *Slc4a2*-deficient mice show growth retardation and clubbing of the long bones without proper bone marrow which are suggestive of congenital osteopetrosis. Although similar numbers of OCs were observed in *Slc4a2*^{-/-} mice compared with WT counterparts, almost four times as many *Slc4a2*^{-/-} OCs displayed cytologic features of apoptosis, suggesting that the dominant role of SLC4A2 in OCs *in vivo* is to facilitate bone resorption and suppress apoptosis (Wu et al., 2008). Contrary to *Slc4a2*-deficient mice, *Src*^{-/-} mice showed increased OC numbers but osteopetrosis due to their reduced activity (Soriano et al., 1991). Though OCs undergo rapid apoptosis *in vitro* in the absence of M-CSF, OC apoptosis did not increase in *Src*^{-/-} mice *in vivo*, suggesting that other compensatory mechanisms are in place in these mice to maintain their viability despite their inactivity. Hence, the aim of this review is to examine the current literature on OC apoptosis and its regulation in a brief and concise manner.

2. Osteoclast apoptosis

The fate of OCs has been speculated for decades and was described by Arey in 1920 (Arey, 1920), who showed that after bone resorption, a part of the OCs is cut off by vacuolization and the OCs disappear due to degeneration. A subsequent study has demonstrated that during remodeling and following cessation of bone resorption, OCs are reduced in number, possibly due to their dissociation into precursor cells (Tonna, 1960). A similar study has shown that the administration of calcitonin causes OCs to separate from the bone surfaces or to reduce the extent of their contact zone with bone and a reduction in their number. They postulated that the reduction could possibly be due to fission of pre-existing OCs into undistinguishable mononucleated cells (Baron and Vignery, 1981). Contrary to the aforementioned studies (Tonna, 1960; Baron and Vignery, 1981), the study by Liu and colleagues has demonstrated that OCs undergo cell death as a result of calcium replenishment in rats which were on a calcium deficient diet for 12 days (Liu and Howard, 1991). They have also observed that OCs disintegrate or undergo degeneration as characterized by fragmentation of the existing OC cytoplasm and/or by the derangement or breakdown of nuclear material due to calcium repletion (Liu and Howard, 1991). Similarly, many subsequent studies on the effect of bisphosphonates and estrogen on OCs have described the morphological changes of

apoptosis, though the cellular changes were not recognized as apoptosis at that time.

The first reported evidence of OC apoptosis *in vitro* has been demonstrated by Fuller et al. (1993) who showed that OCs die when the cultures are depleted of M-CSF, suggesting that the survival of mature OCs occur through suppression of apoptosis. Interestingly, very low concentrations of M-CSF were adequate for OC survival (Fuller et al. 1993). This *in vitro* observation of OC apoptosis was confirmed by a subsequent *in vivo* study by Boyce and colleagues in transgenic mice following the targeting of the Simian virus 40 T (SV 40 T) antigen oncogene to the OCs using tartrate-resistant acid phosphatase (TRAP) promoter as OCs express TRAP at higher levels than other cells (Boyce et al., 1995). Contrary to the expected and despite the abnormal proliferation of OCs, these animals showed osteopetrosis instead of osteoporosis. The observation of increased apoptosis in these animals explains the reduction in bone resorption and osteopetrosis. Targeting of the anti-apoptotic gene, *bcl-xL* to the cells of the OC lineage using the same technique prevented apoptosis both *in vivo* and *in vitro* (Hentunen et al., 1998). By the aforementioned *in vitro* and *in vivo* studies, OCs showed the morphological features of apoptosis, namely loss of adhesion, cytoplasmic contraction and blebbing, nuclear chromatin condensation and nuclear fragmentation. More strikingly, the apoptotic OCs displayed strong TRAP staining and simultaneous apoptosis of all of their nuclei.

3. Osteoclast apoptosis through death receptors

The Fas/FasL system provides an important apoptotic mechanism for many cell types including immune cells and OCs. A study by Wang and colleagues (Wang et al., 2000) has showed that OBs induce OC apoptosis by the Fas/FasL pathway. However, the role of the Fas/FasL system in OCs has not been fully clarified yet. Though both RANKL and M-CSF have been shown to upregulate Fas receptors in OC progenitors (OCPs) through NF- κ B (Wu et al., 2005a), in differentiated mature OCs, RANKL reduces the levels of Fas expression and Fas-mediated apoptosis (Wu et al., 2005a). Using a Fas activating antibody it has been shown that the Fas-mediated death signal in mouse OCs involves the activation of caspase-9 in the intrinsic apoptotic pathway and the executioner caspase-3 and the release of cytochrome c from mitochondria, suggesting that Fas-mediated apoptosis is a potent mechanism in OC apoptosis (Wu et al., 2003). Interestingly, the effect of Fas in OCs is further supported by the *in vivo* data obtained using *Lpr* and *Gld* mice. These mice have a defect in Fas gene transcription and a mutation of the FasL gene, respectively. These mice have an increased number of OCs and mild osteoporosis, suggesting that the Fas/FasL system plays a pivotal role in OC apoptosis *in vivo* (Wu et al., 2003). Inflammatory cytokines such as IL-12 and IL-18 have been shown to reduce OC formation brought about by TNF- α synergistically through the upregulation of FasL, resulting in the apoptosis of the OCPs. (Kitaura et al., 2013). Similarly, IFN- γ has been shown to directly inhibit osteoclastogenesis induced by TNF- α stimulation due to accelerated apoptosis mediated by Fas/FasL signaling (Kitaura et al., 2013). Using cord blood monocyte cultures (CBMC) Roux et al. have demonstrated that Fas stimulation induces human OC apoptosis as well (Roux et al., 2005). There are lines of evidence to show that the intracellular Ca²⁺-binding protein, calmodulin (CaM) regulates OC apoptosis through the Fas/FasL pathway. It has been demonstrated that CaM binds to Fas upon Fas activation, indicating a functional significance of this interaction (Ahn et al., 2004), and *Lpr* mice that have a deficiency in Fas-mediated apoptosis, show reduced CaM binding (Ahn et al., 2004). The evidence shows that CaM exerts either an anti-apoptotic or a pro-apoptotic influence by regulating various downstream targets. In a study by Wu et al. (2005b), it has been demonstrated that interference with CaM binding to Fas by CaM antagonists, such as tamoxifen and trifluoperazine, enhances CaM antagonist-induced OC apoptosis, suggesting that CaM is protective in mature OCs and OC apoptosis by CaM

antagonists may possibly account for the CaM antagonist-induced inhibition of bone resorption as well. Further corroborating the Fas/FasL system in OC apoptosis a study by Wang et al., 2000 implicated that alendronate promotes OC apoptosis through the expression of the Fas gene. Many studies which evaluate the role of estrogen in OC apoptosis attributed cell death to the Fas/FasL system (Nakamura et al., 2007; Wang et al., 2015).

Contrary to the findings of the aforementioned studies, several reports have revealed that apoptosis by Fas/FasL is not a dominant apoptotic mechanism in OCs (Ogawa et al., 2003; Kovacic et al., 2007). Furthermore, the studies indicate that Fas/FasL has a limited role in the apoptosis of OC progenitors while having no direct effect on OC differentiation. In a similar study which could not find an association with the Fas/FasL system in murine OC apoptosis, it was however revealed that FasL enhances RANKL-induced osteoclastogenesis in mice. The addition of anti-FasL mAb to the cultures decreased osteoclastogenesis, suggesting that FasL is a positive modulator of RANKL-mediated osteoclastogenesis which is mediated through upregulated production of IL-1 and TNF- α subsequent to NF- κ B activation (Park et al., 2005). Taken together, many studies indicate a possible role of the Fas/FasL system in OC apoptosis; however, more studies are necessary to delineate the precise role of the Fas/FasL system in OC apoptosis.

TNF-related apoptosis-inducing ligand receptors (TRAIL-R) exist as a type II transmembrane protein or as a soluble receptor, and induce apoptosis by the activation of its death domain (DD)-containing receptors, TRAIL-R1 (DR4) and TRAIL-R2 (DR5). TRAIL also binds to the “decoy” receptors, TRAIL-R3 (DcR1) and TRAIL-R4 (DcR2) which lack apoptosis-inducing capability and OPG which inhibits TRAIL-mediated apoptosis. TRAIL-binding to decoy receptors prevents TRAIL-induced apoptosis in cells. Similar to the role of the Fas/FasL system, the role of TRAIL/TRAIL-R is still elusive. TRAIL receptors have been found in pre OCs as well as in fully differentiated OCs. TRAIL-deficient (TRAIL^{-/-}) mice did not display any bone abnormalities, and *in vitro* OC differentiation was normal in these mice, suggesting that these factors do not play an essential part in bone physiology under normal conditions (Sedger et al., 2002) and that the TRAIL pathway could be an additional and/or an alternative mechanism in OC apoptosis. Since TRAIL receptors are also expressed in human OCs, it is possible that the TRAIL pathway could be induced in OCs *in vivo* under pathological conditions (Roux et al., 2005). Using human and murine preosteoclastic precursors (peripheral blood mononuclear cells [PBMCs] and Raw cells, respectively) TRAIL has been shown to reduce M-CSF and RANKL-induced OC differentiation and bone resorbing activity by selectively inhibiting the activation of the p38/MAPK pathway, suggesting that TRAIL is likely to be a redundant negative regulator of physiological osteoclastogenesis because of the lack of bone abnormalities in TRAIL^{-/-} mice. In addition to the anti-osteoclastogenic activity of TRAIL, it has been shown that OC-like cells formed from CBMC express TRAIL receptors, both TRAIL-R1 and TRAIL-R2, are able to transduce apoptosis similar to FasL and TGF- β 1 (Roux et al., 2005). Moreover, using PBMCs it has been demonstrated that TRAIL is capable of up-regulating mainly TRAIL-R2/DR5 expression and activates caspase-8 and Bid in mature OCs which mediate the TRAIL-induced extracellular and mitochondrial OC apoptotic pathways (Colucci et al., 2007). However, apoptosis was not observed in OCPs during differentiation (Zauli et al., 2004; Schneider et al., 2003), which may be due to the overexpression of the decoy receptor, DcR2, compared to the other receptors (Colucci et al., 2007). Given the fact that TRAIL receptors differ in mice and humans and that they may function by different mechanisms (Schneider et al., 2003), from the foregoing it could be delineated that high concentrations of TRAIL may upregulate the expression of TRAIL death domain-containing receptors and the apoptotic pathway in human OCs. Furthermore, the ratio of TRAIL death to decoy receptors, which is determined by the stage of OC differentiation and other cells in the vicinity, may determine the sensitivity or resistance of OCs to the TRAIL apoptotic pathway. Since the methodological differences in both human and

murine studies could not be ruled out, more studies are needed to elucidate and validate the aforementioned observations. Since the literature published so far does not conclusively rule out the possibility of the involvement of the TRAIL-TRAILR pathway in OC apoptosis, there may be some redundancy in the OC apoptotic pathways.

The Bcl-2 family of proteins control and regulate the mitochondrial-mediated apoptotic events. In skeletal tissues, the anti-apoptotic member, Bcl-2 is expressed in OBs, OCs, chondrocytes and osteocytes. Bcl-2 resides on the mitochondrial outer membrane, endoplasmic reticulum, and nuclear envelope, and inhibits apoptosis by suppressing cytochrome c release from mitochondria. Bcl-2^{-/-} mice have a reduced number of OCs which are dysfunctional (Nagase et al., 2009). The same study has demonstrated that Bcl-2 promotes OC differentiation and bone resorbing activity. Bcl-2^{-/-} OCs displayed an increased expression of activated caspase-3, indicating that lack of Bcl-2 accelerates OC apoptosis, which explains the reduced number and activity of OCs in Bcl-2^{-/-} mice (Nagase et al., 2009). Bcl-xL, which is another anti-apoptotic protein, shows high expression levels in OCs than Bcl-2, and M-CSF, RANKL and TNF- α , stimulate Bcl-xL expression in mature OCs. The evidence for the role of Bcl-xL in OC apoptosis is apparent since crossing of TRAP-Bcl-xL mice with TRAP-SV40 transgenic mice rescued SV40-induced OC death and mild osteopetrosis (Hentunen et al., 1998). The expression of Bcl-xL is under the regulation of the transcription factors: NF- κ B, activator protein 1 (AP-1), and the Ets family members including Ets-1, Ets-2, and PU.1. It has also been proposed that the reduced efficacy of bisphosphonates in the treatment of chronic inflammatory bone diseases is due to the TNF-induced up-regulation of Ets-2 expression in OCPs, which in turn stimulates the transcription of the *bcl-xL* gene and makes OCs more resistant to apoptotic signals (Zhang et al., 2005). Over-expression of the dominant negative I κ B in OCs attenuates NF- κ B activation and in the presence of TNF which alone is a pro-osteoclastogenic factor, leads to OC apoptosis *in vitro*. The underlying mechanisms for the cell death includes a reduction in TRAF-6, Bcl-xL and cellular inhibitors of apoptosis (cIAP-1) expression, leading to the activation of caspases and Poly ADP-ribose polymerases (PARPs), and the upregulation of the Bax/Bcl-xL ratio (Abbas and Abu-Amer, 2003). Bcl-xL is overexpressed in inflamed joints of TNF transgenic mice and RA patients (Roux and Brown, 2009). Therefore, patients with inflamed joints show less response to bisphosphonate therapy than patients having osteoporosis (Zhang et al., 2005).

Withdrawal of M-CSF has been shown to induce rapid apoptosis of OCs with a rapid and sustained increase in the pro-apoptotic BH3-only Bcl-2 family member Bim, suggesting that Bim plays an important role in cytokine withdrawal-mediated OC apoptosis. Upregulation of Bim occurs as a result of reduced ubiquitylation and proteasomal degradation mediated by E3 ubiquitin-protein ligase c-Cbl (Akiyama et al., 2003). Further, the above study has demonstrated that OCs which were differentiated from BM cells of Bim^{-/-} animals have a prolonged survival in the absence of M-CSF (Akiyama et al., 2003).

Though the role of caspase-3 in OC differentiation is not conclusive (Szymczyk et al., 2006), apoptosis is well established (Szymczyk et al., 2006; Wakeyama et al., 2007). A study by Wakeyama et al. (2007) have demonstrated that Bim expression levels are downregulated by caspase-3 by associating Bim with c-cbl. Moreover, caspase-3 is indirectly involved in the degradation of Bim in OCs. OC bone resorption is enhanced at sites where reactive oxygen species (ROS) are high. ROS and increased oxidative stress are known factors in age-related osteoporosis. A study has demonstrated that caspase-2, which is an initiator of the intrinsic mitochondrial pathway, is induced in OCs in the presence of oxidants (Sharma et al., 2014) and they regulate OC numbers by promoting apoptosis. Loss of caspase-2 reduces the oxidative-stress-induced apoptosis of OCs and enhances their survival (Sharma et al., 2014).

Table 1
Positive and negative regulators of osteoclast apoptosis.

Factor	Effect on apoptosis	
	Positive regulators (increase apoptosis)	Negative regulators (increase survival)
Hormones	17 β -estradiol Testosterone	PTH vitamin D3 [125(OH) 2D3] Corticosteroids Calcitonin
Cytokines	IL-12 IL-18 IL-33 IL-35 IFN-g TGF- β	M-CSF RANKL IL-1 TNF- α IL-6
Transcription factors	Transcription regulators – E proteins	Pu.1 NF- κ B NFATc1
Vitamins	Vitamin K ₂	
Calcium	Ca ²⁺	
Pharmacological agents	Bisphosphonates Tamoxifen RGD peptides	

4. Regulation of OC apoptosis

It can be assumed that factors which enhance OC bone resorbing activity might inhibit OC apoptosis while factors which inhibit OC activity might enhance OC apoptosis. However, apoptosis does not follow subsequent to inhibition of bone resorption as demonstrated by calcitonin. Calcitonin, which is known to inhibit bone resorption, enhances OC survival (Kuo et al., 2012). There is a plethora of reports to suggest that OC apoptosis can be modulated by several substances as given in Table 1.

5. Negative regulation of apoptosis or OC survival

Several agents enhance OC survival by preventing OC apoptosis and include hormones such as the parathyroid hormone (PTH), 1, 25-dihydroxyvitamin D₃(1,25(OH)₂D₃) and cytokines such as M-CSF, RANKL, IL-1, TNF- α and IL-6 (Table 1).

6. RANKL

Two factors, namely RANKL and M-CSF, are sufficient for OC differentiation and bone resorbing activity. Binding of RANKL to its cognate receptor, RANK on OCPs recruits TRAF-6 and TAK1 and stimulates signaling cascades, resulting in the activation of the mitogen-activated protein kinases (MAPKs) including extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 (Fig. 1). ERK has been implicated in preosteoclastic proliferation while P38 MAPK plays an important role in RANKL-induced OC differentiation of the precursors but not in OC function. RANKL stimulation also leads to the activation and induction of the transcription factors - NF- κ B, c-Fos and NFATc1 (Soysa et al., 2012). In addition, RANK-RANKL initiates the binding of the adaptor proteins, TRAF6 and c-Src which in turn activate PI3K and Akt kinase (Fig. 1).

Akt has been shown to phosphorylate Bad and caspase-9 (Cardone et al., 1998; Wong et al., 1999), thus preventing the intrinsic apoptotic pathway. RANKL-RANK induced activation of signaling pathways, such as ERK and NF- κ B, is necessary for OC activation and survival (Miyazaki et al., 2000). Using constitutively active MEK-1 it has been demonstrated that stimulation of ERK in OCPs dramatically promotes their survival while NF- κ B is necessary for OC activation (Miyazaki et al., 2000). The anti-apoptotic agents, M-CSF, TNF- α and RANKL have been shown to converge at the level of the mammalian target of rapamycin/

p70 ribosomal protein S6 kinase (mTOR/S6K) to cause OC survival (Glantschnig et al., 2003).

7. M-CSF

The role of M-CSF in OC survival has been demonstrated by Fuller et al. who showed that the addition of M-CSF rescues OCPs from apoptosis and enhances their survival (Fuller et al., 1993). Using a co-culture system of BM cells with a ST2 stromal cell line, Jimi et al. have demonstrated that M-CSF enhances OC survival in a dose dependent manner (Jimi et al., 1995). M-CSF has been shown to increase OC survival by activating the Ras and phosphoinositide 3-kinase (PI3K) and Raf/MEK/ERK pathways (Bradley et al., 2008), and upregulates the expression of Bcl-2, Bcl-xL mRNA (Lacey et al., 2000) and the X-linked inhibitor of apoptosis protein (XIAP) (Kanaoka et al., 2000). Further, PAK1 is activated by M-CSF in a Ras-dependent manner that promotes OC survival by modulating the expression of the inhibitor of apoptosis protein (IAP) family member, Survivin (Bradley et al., 2008). A similar study by Woo and colleagues have demonstrated that M-CSF promotes the survival of OCPs through Bcl-xL-induced inhibition of caspase-9 activation in the intrinsic pathway (Woo et al., 2002). Withdrawal of M-CSF from the cultures has been shown to upregulate the pro-apoptotic BH3-only Bcl-2 family member, Bim, but this does not affect the levels of Bid, Bax or Bcl-xL. Bim^{-/-} OCPs show a marked prolongation of survival in the absence of M-CSF, compared with bim^{+/+} OCP while bim^{-/-} OCPs show an elongated lifespan *in vivo* (Akiyama et al., 2003). M-CSF-induced inhibition of OC apoptosis is also thought to increase the anti-apoptotic protein, XIAP in OCPs, which is a direct proteinaceous inhibitor of caspase-3 and caspase-7 (Kanaoka et al., 2000).

8. Transcription factors

RANKL-RANK binding has been shown to activate several transcription factors, such as PU.1, NF- κ B, NFATc1, AP-1 and MITF, which regulate genes necessary for OC proliferation, differentiation and survival. Mice lacking PU.1 and NF- κ B have been shown to develop osteopetrosis due to reduced OC numbers. PU.1 is a hematopoietic-specific transcriptional activator and PU.1-deficient fetal erythroid progenitors lose their self-renewal capacity and undergo proliferation arrest, premature differentiation and apoptosis (Back et al., 2004), suggesting that PU.1 may modulate the survival of OCPs. NF- κ B is an important transcription factor necessary for OC formation and differentiation as well as OC bone resorbing activity (Xing and Boyce, 2005; Miyazaki et al., 2000), which acts through the activation of canonical as well as non-canonical pathways (Soysa et al., 2009, 2011; Soysa et al., 2010). A study has shown that NF- κ B is pivotal in protecting the cells against TNF-induced apoptosis (Liu et al., 1996). Many cytokines, such as RANKL and IL-1, have been shown to increase OC survival through NF- κ B. AP-1 transcription factors, c-fos, Fra1 and 2, are necessary for OCP commitment and survival. C-fos acts as an essential switch between OC and macrophage differentiation from a common progenitor, and OCPs do not form in its absence. Though c-Fos is important in OC formation and activity, its role in OC survival is negligible (Xing and Boyce, 2005).

NFATc1 is considered the master transcription factor in OC differentiation. A study has demonstrated that in the presence of RANKL, ITAM-dependent costimulatory signals coming from Fc γ R and DAP12 through binding to their cognate receptors, such as OSCAR and TREM2, respectively, are necessary to activate Ca²⁺ signaling through phospholipase C gamma (Koga et al., 2004). Ca²⁺ then binds to CaM which in turn activates the CaM-dependent phosphatase, calcineurin. Dephosphorylation of NFATc1 by activated calcineurin leads to nuclear translocation and the activation of NFATc1 in OCPs for their terminal differentiation into mature OCPs. A study has shown that inhibition of calcineurin using cyclosporine A and FK506 in turn inhibits the RANKL-induced OC formation while inducing OC apoptosis, indicating that

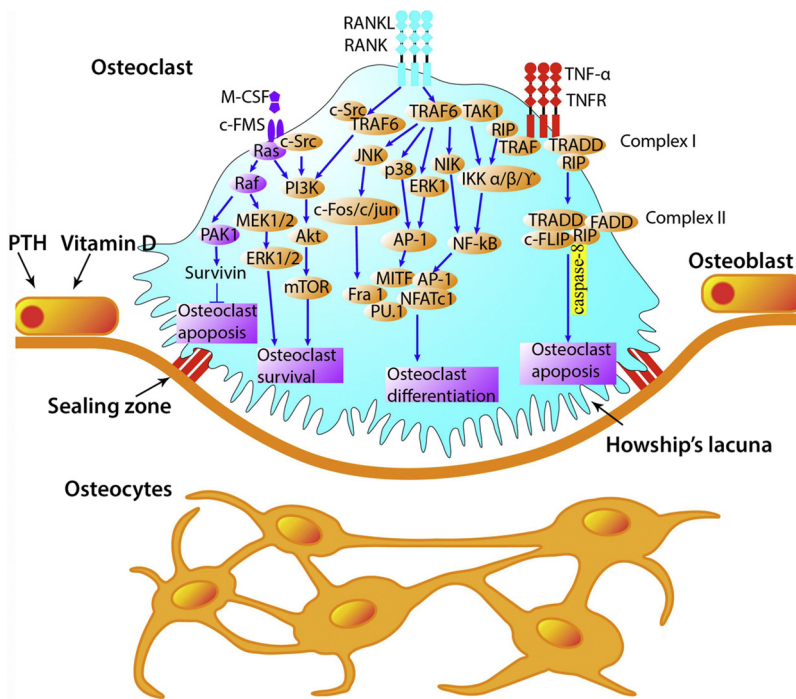


Fig. 1. Signaling pathways in osteoclast (OC) differentiation, survival and death. Binding of RANKL to RANK results in the recruitment of adaptor molecules such as the TNFR-associated factors (TRAFs) and activation of several downstream signaling pathways. The RANKL-RANK complex can activate all three MAPK pathways and the NF-κB pathway, by interacting with TRAF6 and TGF-α activated kinase (TAK)-1. Activation of the JNK pathway mobilizes a number of osteoclastogenic transcription factors such as c-Fos, Fos-related antigens (Fra-1 and 2) and the nuclear factor-activated T-cells (NFAT) c1. Both the NF-κB and the JNK signaling pathways are essential for OC differentiation. Binding of TNF-α to TNFR has a strong synergistic effect with RANK in NF-κB and JNK pathways through RIP-1/IKK activation. The RANKL-RANK-mediated interaction of TRAF6 with c-Src stimulates the PI3K-Akt pathway necessary for cell survival. M-CSF binding to its cognate receptor, c-Fms, and its association with c-Src and PI3K acts synergistically on the PI3K-Akt pathway necessary for OC survival. Binding of Ras to c-Fms activates the Ras and Raf/MEK/ERK pathways as well. PAK1 is activated by M-CSF in a Ras-dependent mechanism that promotes OC survival. PAK1 promotes OC survival by modulating expression of the IAP family member, Survivin.

NFATc1 is necessary for OC differentiation, activity and survival (Igarashi et al., 2004).

9. Interleukins (ILs)

IL-1α has been shown to enhance OC survival through activating NF-κB (Ruoslahti and Reed, 1994), suggesting that IL-1-induced survival of OCs may also play an important role in bone resorption, stimulated by pathological conditions such as RA, lymphoma and osteoporosis (Jimi et al., 1998). IL-1 has been shown to induce OC survival by acting through the PI 3-kinase/Akt and ERK signaling pathways and by reducing caspase-3 activity (Lee et al., 2002). It has been shown that OCPs and mature OCs express IL-3R which is necessary for OCP survival and commitment to OC lineage, whereas its role in OC differentiation has not been fully delineated yet (Soysa and Alles, 2018). IL-6 is a known activator of OC bone resorption activity. IL-6 has been shown to reverse the effect of rising levels of Ca^{2+} in bone resorption and attenuate OC apoptosis brought about by high calcium levels (Adebanjo et al., 1998).

10. Hormones

10.1. PTH and 1, 25-dihydroxy vitamin D3 [1, 25(OH)2D3]

It has been shown that both PTH and 1, 25(OH)2D3 are necessary for Ca^{2+} homeostasis by increasing the serum Ca^{2+} levels, enhancing Ca^{2+} absorption from intestines and by resorbing bone as well as preventing Ca^{2+} loss through kidneys via urine. Both hormones stimulate OC formation, bone resorption and OC survival through the activation of OBs and RANKL expression and by reducing the OPG expression by stromal cells. PTH and 1,25-(OH)2D3 enhances the OC survival indirectly by stimulating the production of interleukins such as IL-6 and IL-11 by OBs.

10.2. Calcitonin

According to earlier studies calcitonin which is a calcitropic hormone (a 32-amino-acid peptide), inhibits OC bone resorption by causing cell detachment, changes in cytoskeleton and mobility while

downregulating cytochrome oxidase activity, without affecting OC apoptosis. However, subsequent studies have demonstrated that calcitonin promotes OC survival and regulates the onset of apoptosis (Kuo et al., 2012). Prevention of OC apoptosis by calcitonin is postulated to occur due to the activation of protein kinase A (PKA), thereby inhibiting the activation of procaspase-9 and procaspase-3. In addition, PKA is involved in phosphorylation and inactivation of Bad which cause the elevation of functional Bcl-2 or Bcl-xL, both of which are dominant members in the prevention of mitochondrial permeability transition. Moreover, calcitonin protects OCs from the effects of nitric oxide releaser-NOC18, a highly effective apoptotic stimulus (Kanaoka et al., 2000). The aforementioned effect of the inhibition of OC apoptosis has been further confirmed by a study which showed that calcitonin prevents OC apoptosis brought about by the bisphosphonate analog, sintered dicalcium pyrophosphate (SDCP), but not bone resorption. ERK1/2 is also involved in antiapoptotic signaling induced by calcitonin in OCs. Calcitonin inhibition of OC apoptosis is postulated to occur in part by upregulating Bcl-2 expression and inhibition of caspase-3 and -9. Lack of activation of caspases-8 indicates that this inhibition does not occur through the extrinsic apoptotic pathway (Kuo et al., 2012).

10.3. Integrin binding

OCs typically exhibit high expression of transmembrane integrins such as $\alpha_v\beta_3$ heterodimer (vitronectin receptor) which recognizes the Arg-Gly-Asp (RGD) motif on the bone matrix components such as thrombospondin, fibronectin, vitronectin, osteopontin and bone sialic protein. Anoikis denotes the apoptosis of cells following detachment from the underlying extracellular matrix containing RGD peptides (Ruoslahti and Reed, 1994). It has been demonstrated that OCs cultured *in vitro* on the bone matrix survive longer than the OCs cultured on plastic, suggesting that OC adherence to the underlying bone is necessary for their survival (Hughes et al., 1995). *In vivo*, at the reversal sites in the BMU, OCs detach from the underlying bone and then undergo apoptosis. Using mature rabbit OCs, it has been shown that deprivation of adhesion promotes anoikis in OCs *in vitro* (Sakai et al., 2000). It has also been demonstrated that while unoccupied, $\alpha_v\beta_3$ integrin induces apoptosis in pre OCs by transmitting a positive death signal regulated by caspase-8 while pre OCs lacking $\alpha_v\beta_3$ integrin enhance their survival

(Zhao et al., 2005). In addition, blockade of bone matrix-integrin interaction using RGD peptides has been shown to induce apoptosis (Xing and Boyce, 2005). A study by (Villanova et al. (1999)) has demonstrated that antisense oligonucleotides to the α_v gene prevent OC adhesion and bone resorption while enhancing OC apoptosis due to reduced expression of Bcl-2 in rabbit OCs. Though proapoptotic Bax is not changed, a reduction in anti-apoptotic Bcl-2 causes a reduction in Bcl-2/Bax ratio, suggesting that a reduction in the synthesis of α_v may enhance OC apoptosis.

At focal adhesions, integrin-extracellular matrix interactions promote the assembly of the cytoskeletal and signaling molecule complexes which include the Src-family members, focal adhesion kinase (FAK), phosphatidylinositol-3'-kinase (PI3K) and phospholipase C (PLC)- γ (Miyamoto et al., 1995), and prevent anoikis-mediated OC death. The survival of OCs mediated by c-Src is thought to occur through the Akt/PKB pathway (Wong et al., 1999). Using selective c-Src inhibitors, Recchia and colleagues have demonstrated that a reduction in c-Src activity increases OC apoptosis as a result of a sustained increase in ERK1/2 phosphorylation (Recchia et al., 2004). However, other pathways may act in conjunction with Akt/PKB pathway for OC survival as lack of c-Src in Src^{-/-} mice does not show enhanced OC apoptosis (Xing et al., 2001). Nevertheless, transgenic mice lacking the entire kinase domain and regulatory tyrosine 527 have enhanced OC apoptosis, suggesting that misregulation of the Src251 protein interaction domains may have given rise to the observed OC phenotype (Xing et al., 2001).

The type II collagen N-propeptide, PIIBNP is the most abundant cartilage protein. In an attempt to delineate the effect of PIIBNP in bone cells, Hayashi and colleagues have demonstrated that PIIBNP induces cell death of OCs *via* binding through the RGD domain by detaching OCs when cultured on vitronectin-coated plates. Though PIIBNP did not detach cells from type I collagen or fibronectin, it caused apoptosis in a β_3 integrin-dependent manner, suggesting that unlike RGD peptides, PIIBNP does not cause apoptosis only by anoikis, but involves the caspase-3/-8 pathway, indicating a potential therapeutic indication for PIIBNP in bone related diseases with accelerated resorption (Hayashi et al., 2011).

10.4. Positive regulation or inducers of OC apoptosis

10.4.1. OPG

The TNF family member, osteoprotegerin (OPG) is a secreted glycoprotein that prevents RANKL-RANK binding by acting as a decoy receptor for RANKL and thus prevents RANKL-induced OC differentiation and activity. Using BM cultures it has been shown that low concentrations of OPG (20–80 ng/ml) enhances the apoptosis of OCPs in a dose-dependent manner (Liu et al., 2015). In addition, OPG is able to upregulate the expression levels of FasL in both OCs and OCPs dose-dependently. Further, OPG significantly increases the Bax/Bcl-2 ratio in both OCs and OCPs and the level of activated caspase-3 and cleaved-caspase-9. Taken together, the data of this study suggest that OPG induces the apoptosis of OCs and OCPs by triggering the downstream mitochondria caspase cascade through the Fas/FasL pathway by regulating the form of FasL either by binding to soluble FasL, or inhibiting the shedding of FasL.

Contrary to the aforementioned study, it has been shown *in vitro* that OPG reduces OC apoptosis through the TRAIL pathway (Chamoux et al., 2008). Though TRAIL mRNA expression by OCs is not affected in the presence of OPG, TRAIL levels in the culture medium as well as the level of activated caspase-8 in OCs decrease dose-dependently in the presence of OPG (50–100 ng/ml). It has been postulated that the inhibition of apoptosis by OPG may occur *via* several mechanisms including inhibition of TRAIL-induced OC apoptosis at least in part by binding of TRAIL and limiting its availability and access to cell surface TRAIL receptors (Chamoux et al., 2008). Since TRAIL is expressed and secreted by OCs, it can be assumed that the TRAIL pathway is an

autocrine signaling process that controls OC apoptosis. Since OPG acts as a decoy receptor for both RANK and TRAIL, the binding of OPG to TRAIL or RANKL may depend on the relative concentrations of these molecules in the environment. It can be assumed that in the presence of reduced concentrations of RANKL with an elevated level of OPG, OPG could bind TRAIL, thus limiting its apoptosis-inducing effect. From the foregoing it can be delineated that OPG modulates the apoptosis of OCs; however, the role of OPG as a survival factor of OCs *in vivo* remains to be verified.

Wnts, which are secreted glycol-proteins, have been shown to universally protect against apoptosis through mechanisms involving β -catenin and the activation of PI3K/Akt and other mechanisms depending on the cell type. It has been well demonstrated that Wnt proteins prevent the apoptosis of both OBPs and OBs by β -catenin-dependent and -independent pathways through the activation of Src/ERK and the phosphatidylinositol 3-Kinase/AKT pathway (Almeida et al., 2005). In OCs it has been shown that Wnt/ β -catenin signaling decreases OC differentiation by stimulating the production and secretion of OPG (Glass et al., 2005), and the deletion of β -catenin in OCs has been shown to increase OC numbers and bone resorption, thus decreasing bone mass (Wei et al., 2011). A study has unraveled a direct effect of WNT ligands on OCPs and OCs *via* an autocrine mechanism (Pederson et al., 2008). In OCPs activation of β -catenin has been shown to enhance OCP proliferation while osteoclastogenesis is inhibited by WNT3a, suggesting bi-phasic and dosage-dependent regulation of osteoclastogenesis (Wei et al., 2011). Notwithstanding the aforementioned observations, the activation of the noncanonical pathway by WNT5a has been shown to stimulate differentiation of OCPs and osteoclastogenesis (Maeda et al., 2012). However, the direct role of Wnts in OC apoptosis needs to be clarified.

10.4.2. Estrogen (17 β -estradiol)

Bone resorption by OCs and bone formation by OBs are tightly coupled to maintain a balance between these two processes. The net loss of bone following menopause, which can be overcome by estrogen replacement therapy, indicates that estrogen is important in the maintenance of this balance. Earlier studies by Liu and Howard (1991) have demonstrated that administration of estrogen to weaning mice causes a reduction in OC numbers attached to bone and changes their morphology and size. Moreover, they have observed numerous acid-phosphatase-positive fragments in the marrow space, suggesting that estrogen treatment causes the dissociation and disintegration, and thus decreases the activity of OCs. However, they did not attribute these changes to the apoptosis of OCs. In a subsequent study using ovariectomized mice Hughes et al. have showed that estrogen promotes OC apoptosis *in vivo* and *in vitro* (Hughes et al., 1996). The discovery of estrogen receptors in OBs (Eriksen et al., 1988) and OCs (Oursler et al., 1991a) suggests that estrogen may exert both indirect and direct effects through these two cell types.

Evidence of the indirect effect of estrogen on OC apoptosis comes from the study by Hughes et al. who showed that OC apoptosis can be induced by the administration of the 17 β -estradiol, tamoxifen and transforming growth factor (TGF)- β . By adding anti TGF- β antibody to the cultures they were able to inhibit TGF- β , estrogen and tamoxifen-induced OC apoptosis (Hughes et al., 1996), suggesting that TGF- β might mediate the observed effects by acting through OBs. It has been demonstrated later that TGF- β -induced production of OPG by OBs and stromal cells (Murakami et al., 1998) may explain the indirect estrogen-induced OC apoptosis. Though the foregoing may explain OC apoptosis subsequent to the secretion of TGF- β and OPG by OBs and stromal cells, this may not be the sole mechanism by which estrogen promotes OC apoptosis since in the absence of OPG, OCs still undergo apoptosis in stromal cell-depleted cultures (Akatsu et al., 1998). A similar study has shown that estrogen may elevate FasL expression in OBs, leading to OB-mediated apoptosis of the OC progenitors (Krum et al., 2008) through MMP3-induced FasL cleavage in OBs by ER α , resulting in the secretion

of soluble FasL (Garcia et al., 2013). FasL expression is down-regulated in OB progenitors/OBs from OVX mice, and OVX induces more bone loss in FasL conditional knock-out (cKO) mice. FasL cKO mice presented the lowest ratio of apoptotic OCs regardless of the sham or OVX procedures (Wang et al., 2015), indicating that estrogen may exert its effect through the Fas/FasL system *via* a paracrine mechanism. There is substantial evidence to indicate that upregulation of cytokines, such as TNF- α , IL-1, IL-6 and M-CSF, contributes to enhanced OC activity and bone resorption in postmenopausal osteoporosis. These cytokines are necessary to prime the pre-OCs for estrogen-mediated apoptosis by FasL, thus the requirement for ER α expression in OCs (Krum et al., 2008).

Several studies have demonstrated that estrogen by an estrogen receptor-mediated mechanism directly acting through OCs prevents their bone resorbing activity partially due to apoptosis (Kameda et al., 1997). Using OC-specific ER α knockout mice (Nakamura et al. (2007)), have demonstrated that *via* the induction of the Fas/FasL system the estrogen receptor, ER α induces apoptosis in an autocrine manner. The role of the Fas/FasL system in estrogen-induced apoptosis has been corroborated by an *in vitro* study using Lpr and Gld mice (Krum et al., 2008). However, the direct effect of estrogen on OCs may not be solely through the Fas/FasL system as estradiol has been shown by a similar study not to exert any effect on the expression of Fas in OCs (Kovacic et al., 2010). Furthermore, tamoxifen, which is a selective estrogen receptor modulator (SERM) having an estrogenic effect on bone, has been shown to reduce OC bone resorbing activity by enhancing OC apoptosis (Nakamura et al. (2007); Krum et al., 2008; Arnett et al., 1996). Despite the disagreements regarding the molecular basis of how ER α promotes OC apoptosis the published studies provide strong evidence that estrogen inhibits OC survival *via* direct action on OCs and OCPs.

10.4.3. TGF- β 1

TGF- β 1 is most abundant in bone and human OCs express both type I and type II TGF- β receptors (TGF- β -R1 and RII). Binding of TGF- β to its cognate receptor, TGF- β -RII recruits TGF- β -R1 and the activation of the Smad-dependent and Smad-independent pathways. The Smad-independent pathway includes the activation of MAPKs such as ERK, JNK and p38. The p38 has been shown to mediate apoptosis through the activation of caspase-9. The role of TGF- β in OC apoptosis is inconsistent and has not been fully elucidated yet. Previous studies have shown that TGF- β 1 induces apoptosis of murine OCs at least in part, by up-regulating the expression of OPG (Murakami et al., 1998). Using CBMs Houde and colleagues have demonstrated that TGF- β 1 induces human OC apoptosis by inducing the activation of Bim and caspase-9 in the intrinsic apoptotic pathway, but not in the extrinsic pathway as TGF- β receptors lack DRs (Houde et al., 2009). The intensity of apoptosis induced by TGF- β has been shown to be less in the presence of the OC survival factor, RANKL. Further, the study suggests that TGF- β acts as a coupling agent in bone remodeling. This has been postulated to occur following the release of TGF- β from the bone matrix due to OC bone resorption which in turn inhibits bone resorption partly by inducing OC apoptosis and partly by stimulating osteoblastic bone formation (Houde et al., 2009). As discussed above, estrogen has been shown to enhance the production of TGF- β , suggesting that TGF- β may cause estrogen-mediated inhibition of OC activity (Oursler et al., 1991b). This has been confirmed by a subsequent study which shows that TGF- β mediates OC apoptosis in a dose-dependent manner and also mediates the action of estrogen and tamoxifen (Hughes et al., 1996). In another similar study it has been shown that the expression of TGF- β 1 and OPG can be greatly elevated in stretched bone lining cells after force application which leads to OC apoptosis, suggesting that force-induced OC apoptosis is accompanied by an elevation of TGF- β and OPG expression (Kobayashi et al., 2000).

In contrast to the aforementioned positive regulation of OC apoptosis by TGF- β , a study has shown that TGF- β inhibits OC apoptosis in

mice by activating TAK1/MEK and the expression of pro-survival Bcl-xL along with the TAK1/MEK and SMAD pathways to induce the expression of pro-survival factor, Mcl-1 (Gingery et al., 2008). The same group have later demonstrated that TGF- β enhances OC survival by inducing the expression of leukemia inhibitory factor (LIF) and suppressor of cytokine signaling 3 (SOCS3) by activating the Akt/NF- β B and Smad pathways, respectively. Binding of LIF to its receptor in turn activates the pro-survival pathways such as Akt/NF- κ B and MEK signaling to enhance the expression of Bcl-xL and suppress the expression of Bid (Ruan et al., 2010). Therefore, due to the ambiguous nature of the data, more studies are necessary to clarify the role of TGF- β in OC apoptosis.

10.4.4. Transcription regulators and Ca²⁺

E proteins are ubiquitously expressed transcription regulators that regulate cell proliferation and survival. E proteins can activate or repress transcription by binding themselves to the promoter or the enhancer region of a gene. E protein activity is regulated by inhibitors named Id proteins (Inhibitor of DNA binding or Inhibiting Differentiation) and their role in both OBs and OCs has already been established. It has been clearly demonstrated that RANKL stimulation causes the downregulation of Id1-3 proteins and that overexpression of Id1-3 inhibits the expression of TRAP and multinucleation of OCs (Lee et al., 2006), whereas mice lacking Id have severe osteoporosis with enhanced OC numbers and activity (Chan et al., 2009). Using myeloid specific conditional knock-in mouse expressing ET2 with loss of Id proteins, Long and colleagues (Long et al., 2012) have shown that elevated E protein activity in these mice develops a high bone mass phenotype due to decreased OC numbers consequent to OC apoptosis. This was thought to occur as a result of the upregulation of CD38, an enzyme which is important in transmembrane signaling, cell adhesion and anti-osteoclastogenic activity. The anti-osteoclastogenic effects of CD38 are due to its intracellular calcium release which in turn signals the OCs to retract from bone, thus inhibiting bone resorption in mature OCs. CD38 increases the apoptosis of OCPs, pre OCs, and mature OCs (Long et al., 2012). Another study demonstrating the role of Ca²⁺ in OC apoptosis has shown that both mature OCs and pre OCs express the Ca²⁺ receptor (CaR) and that lack of a functional CaR or antagonizing endogenous CaR function through overexpression of a dominant negative form of the CaR (DN-CaR) reduces the formation of OCs and enhances the Ca²⁺-induced OC apoptosis (Mentaverri et al., 2006).

11. Interleukins

ILs such as IL-12 and IL-18 have been shown to induce apoptosis of OCPs through the activation of the Fas/FasL system (Kitaura et al., 2013). IL-33 and its receptor, ST2 are expressed by OCs (Mun et al., 2010). The treatment of BM-derived OCs with IL-33 results in decreased formation of TRAP⁺ OCs and reduced resorption pits when compared with RANKL-stimulated cells. IL-33 has been shown to increase the expression of pro-apoptotic molecules, including Bax, Fas, FasL, FADD, TRAIL and Bid, suggesting that IL-33 interaction with ST2 has anti-osteoclastogenic effects with reduced OC formation and activity by inducing OC apoptosis (Lima et al., 2015). IL-35, which is a newly identified IL, has been shown to inhibit TNF- α -induced osteoclastogenesis, and to promote the apoptosis of OCs *via* the TNFR1-TRADD-FADD pathway by activating JAK1/STAT1 while suppressing NF- κ B and MAPK (Peng et al., 2018).

12. Bisphosphonates

Bisphosphonates, potent analogues of inorganic pyrophosphate, are commonly employed in the management of Paget's disease of bone, hypercalcemia of malignancy and osteoporosis due to their inhibitory effect on bone resorption. The inhibitory effect on bone resorption is attributed to their apoptotic effect on OCs. In an earlier study, Hughes et al. have demonstrated that bisphosphonates enhance OC apoptosis *in*

vivo as well as *in vitro* (Hughes et al., 1995). However, they could not delineate the precise mechanism by which bisphosphonates induce apoptosis in OCs at that time (Hughes et al., 1995). Simple bisphosphonates (BPs) such as etidronate, have been shown to incorporate into nonhydrolysable adenosine triphosphate analogues and induce OC apoptosis. Nitrogen-containing bisphosphonates (NBPs), such as alendronate (ALN), risedronate (RIS), ibandronate and pamidronate (PAM) which are taken up by OCs inhibit farnesyl pyrophosphate (FPP) synthase, an enzyme of the mevalonate pathway of OCs. A study has demonstrated that BPs induce OC apoptosis directly by the induction of caspase-3-dependent cleavage of Mammalian Sterile 20-like kinase 1 (Mst1) to form the active 34-kDa species, which is associated with apoptosis as a result of the inhibition of FPP synthase (Reszka et al., 1999) and cause apoptosis of OCs by inhibiting the post-translational prenylation of proteins such as Ras (Luckman et al., 1998).

13. Vitamin K₂

In an attempt to delineate the role of Vitamin K₂ in the reduction of OC numbers through OC apoptosis, *op/op* mice were injected with a single M-CSF injection and a subcutaneous injection of Vitamin K₂. The maximum number of OCs was observed on day 5 after a single injection of M-CSF, which significantly reduced to 30% and 15% in mice which received Vitamin K₂ injections 12 h and 24 h previously, respectively. The same pattern of reduction of OCs was observed starting from day 2 up to until day 25 (Kawata et al., 1999). This *in vivo* reduction of OCs by apoptosis has also been confirmed by an *in vitro* study which showed that OCs in the presence of Vitamin K₂ is unable to resorb bone, which is attributed to the enhanced OC apoptosis by Vitamin K₂. (Kameda et al., 1996). On the other hand, a similar study using ovariectomized rats, dietary supplementation (50 mg/kg a day) of menatetrenone (MK-4), a vitamin K₂ has been shown to prevent OC activity without OC apoptosis (Asawa et al., 2004).

14. Glucocorticoides

It is generally believed that glucocorticoids (GCs) cause osteoporosis through a combination of decreased bone formation and increased bone resorption as glucocorticoids exert diverse effects on bone and Ca²⁺ metabolism. It has clearly been delineated that GCs induce OB apoptosis and retard bone formation in GC-induced osteoporosis. However, the role of GC in OC apoptosis is still debatable. Moreover, according to the published literature, there is a marked species difference in the response of OCs to GCs (Dempster et al., 1997) as well. Using neonatal rat OCs *in vitro*, a study has shown that GCs cause dose-dependent inhibition of bone resorption by reducing OC numbers, which was due to their death by apoptosis (Dempster et al., 1997). A similar study carried out using rat OCs has shown that hydrocortisone and dexamethasone cause dose-dependent inhibition of OC bone resorption as a result of their death, suggesting that GCs may have a role in physiology as inhibitors of OC bone resorption as a result of their apoptosis (Tobias and Chambers, 1989).

Contrary to the foregoing, a similar study using murine OC cultures has demonstrated that GCs enhance OC survival and prevent the induction of OC apoptosis by antagonizing bisphosphonate-induced activation of caspase-3, caspase-8 and caspase-9 through a mechanism via the GC receptor (Weinstein et al., 2002). The aforementioned observation has been confirmed by a subsequent study by the Teitelbaum group (Kim et al., 2007) who showed that dexamethasone in murine OC cultures enhances their longevity as well as inhibits OCP proliferation, thus maintaining normal OC number. Using human peripheral blood mononuclear cells as OCPs, a study has shown that OC apoptosis may not be affected by GCs (Sivagurunathan et al., 2005). Species differences or methods of study (e.g. incubation on plastic or bone) might play a role in producing these contrasting results. Studies have also not been clear as to whether direct effects of GCs on OCs are a monomeric

event or require dimerization of the GC receptor.

15. Summary

Most adult bone diseases, such as osteoporosis and rheumatoid arthritis occur as a result of an imbalance in bone remodeling due to enhanced osteoclast numbers and osteoclast activity. One mechanism of preventing OC activity would be to commit these cells to undergo apoptosis. Though OCs have the DRs and the associated signaling pathways, their exact role in OC apoptosis is questionable as Fas/FasL and TRAIL/DR4 have been shown to induce both osteoclastogenesis and apoptosis. Moreover, TGF-β, which has long been known to induce OC death, has been shown to increase survival as well. Therefore, the literature only provides a glimpse of the signaling pathways of OC apoptosis, and more studies are required before these cytokines and signaling molecules can be considered as viable therapeutic targets of OC apoptosis in treating pathological bone diseases.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors wish to acknowledge Ms. Harshini Pethiyagoda of the English Language Teaching Unit (ELTU) of the Faculty of Sciences, University of Peradeniya, Sri Lanka for professionally editing the language of the manuscript and Dr. Kosala Kithsiri Jayasingha of the Faculty of Dental Sciences, University of Peradeniya, Sri Lanka for providing the outline of Fig. 1.

References

- Abbas, S., Abu-Amer, Y., 2003. Dominant-negative IkappaB facilitates apoptosis of osteoclasts by tumor necrosis factor-alpha. *J. Biol. Chem.* 278, 20077–20082. <https://doi.org/10.1074/jbc.M208619200>.
- Adebanjo, O.A., Moonga, B.S., Yamate, T., Sun, L., Minkin, C., Abe, E., Zaidi, M., 1998. Mode of action of interleukin-6 on mature osteoclasts. Novel interactions with extracellular Ca²⁺ sensing in the regulation of osteoclastic bone resorption. *J. Cell Biol.* 142, 1347–1356.
- Ahn, E.-Y., Lim, S.-T., Cook, W.J., McDonald, J.M., 2004. Calmodulin binding to the Fas death domain. Regulation by Fas activation. *J. Biol. Chem.* 279, 5661–5666. <https://doi.org/10.1074/jbc.M311040200>.
- Akatsu, T., Murakami, T., Nishikawa, M., Ono, K., Shinomiya, N., Tsuda, E., Mochizuki, S., Yamaguchi, K., Kinoshita, M., Higashio, K., Yamamoto, M., Motoyoshi, K., Nagata, N., 1998. Osteoclastogenesis inhibitory factor suppresses osteoclast survival by interfering in the interaction of stromal cells with osteoclast. *Biochem. Biophys. Res. Commun.* 250, 229–234.
- Akiyama, T., Bouillet, P., Miyazaki, T., Kadono, Y., Chikuda, H., Chung, U.-I., Fukuda, A., Hikita, A., Seto, H., Okada, T., Inaba, T., Sanjay, A., Baron, R., Kawaguchi, H., Oda, H., Nakamura, K., Strasser, A., Tanaka, S., 2003. Regulation of osteoclast apoptosis by ubiquitylation of proapoptotic BH3-only Bcl-2 family member Bim. *EMBO J.* 22, 6653–6664. <https://doi.org/10.1093/emboj/cdg635>.
- Almeida, M., Han, L., Bellido, T., Manolagas, S.C., Kousteni, S., 2005. Wnt proteins prevent apoptosis of both uncommitted osteoblast progenitors and differentiated osteoblasts by beta-catenin-dependent and -independent signaling cascades involving Src/ERK and phosphatidylinositol 3-kinase/AKT. *J. Biol. Chem.* 280, 41342–41351. <https://doi.org/10.1074/jbc.M502168200>.
- Arey, L.B., 1920. The origin, growth and fate of osteoclasts and their relation to bone resorption. *Am. J. Anat.* 26, 314–345. <https://doi.org/10.1002/aja.1000260302>.
- Arnett, T.R., Lindsay, R., Kilb, J.M., Moonga, B.S., Spowage, M., Dempster, D.W., 1996. Selective toxic effects of tamoxifen on osteoclasts: comparison with the effects of oestrogen. *J. Endocrinol.* 149, 503–508.
- Asawa, Y., Amizuka, N., Hara, K., Kobayashi, M., Aita, M., Li, M., Kenmotsu, S., Oda, K., Akiyama, Y., Ozawa, H., 2004. Histochemical evaluation for the biological effect of menatetrenone on metaphyseal trabeculae of ovariectomized rats. *Bone* 35, 870–880. <https://doi.org/10.1016/j.bone.2004.06.007>.
- Back, J., Dierich, A., Bronn, C., Kastner, P., Chan, S., 2004. PU.1 determines the self-renewal capacity of erythroid progenitor cells. *Blood* 103, 3615–3623. <https://doi.org/10.1182/blood-2003-11-4089>.
- Baron, R., Vignery, A., 1981. Behavior of osteoclasts during a rapid change in their number induced by high doses of parathyroid hormone or calcitonin in intact rats. *Metab. Bone Dis. Relat. Res.* 2, 339–346. [https://doi.org/10.1016/0221-8747\(81\)90020-5](https://doi.org/10.1016/0221-8747(81)90020-5).
- Boyce, B.F., Wright, K., Reddy, S.V., Koop, B.A., Story, B., Devlin, R., Leach, R.J.,

- Roodman, G.D., Windle, J.J., 1995. Targeting simian virus 40 T antigen to the osteoclast in transgenic mice causes osteoclast tumors and transformation and apoptosis of osteoclasts. *Endocrinology* 136, 5751–5759. <https://doi.org/10.1210/endo.136.12.7588333>.
- Bradley, E.W., Ruan, M.M., Vrable, A., Oursler, M.J., 2008. Pathway crosstalk between Ras/Raf and PI3K in promotion of M-CSF-induced MEK/ERK-mediated osteoclast survival. *J. Cell. Biochem.* 104, 1439–1451. <https://doi.org/10.1002/jcb.21719>.
- Cardone, M.H., Roy, N., Stennicke, H.R., Salvesen, G.S., Franke, T.F., Stanbridge, E., Frisch, S., Reed, J.C., 1998. Regulation of cell death protease caspase-9 by phosphorylation. *Science* 282, 1318–1321.
- Chamoux, E., Houde, N., L'Eriger, K., Roux, S., 2008. Osteoprotegerin decreases human osteoclast apoptosis by inhibiting the TRAIL pathway. *J. Cell. Physiol.* 216, 536–542. <https://doi.org/10.1002/jcp.21430>.
- Chan, A.S., Jensen, K.K., Skokos, D., Doty, S., Lederman, H.K., Kaplan, R.N., Rafii, S., Rivella, S., Lyden, D., 2009. Id1 represses osteoclast-dependent transcription and affects bone formation and hematopoiesis. *PLoS One* 4, e7955. <https://doi.org/10.1371/journal.pone.0007955>.
- Colucci, S., Brunetti, G., Cantatore, F.P., Oranger, A., Mori, G., Pignataro, P., Tamma, R., Grassi, F.R., Zallone, A., Grano, M., 2007. The death receptor DR5 is involved in TRAIL-mediated human osteoclast apoptosis. *Apoptosis Int. J. Program. Cell Death* 12, 1623–1632. <https://doi.org/10.1007/s10495-007-0095-3>.
- Dempster, D.W., Moonga, B.S., Stein, L.S., Horbert, W.R., Antakly, T., 1997. Glucocorticoids inhibit bone resorption by isolated rat osteoclasts by enhancing apoptosis. *J. Endocrinol.* 154, 397–406.
- Elmore, S., 2007. Apoptosis: a review of programmed cell death. *Toxicol. Pathol.* 35, 495–516. <https://doi.org/10.1080/01926230701320337>.
- Eriksen, E.F., Colvard, D.S., Berg, N.J., Graham, M.L., Mann, K.G., Spelsberg, T.C., Riggs, B.L., 1988. Evidence of estrogen receptors in normal human osteoblast-like cells. *Science*. 241, 84–86.
- Fuller, K., Owens, J.M., Jagger, C.J., Wilson, A., Moss, R., Chambers, T.J., 1993. Macrophage colony-stimulating factor stimulates survival and chemotactic behavior in isolated osteoclasts. *J. Exp. Med.* 178, 1733–1744.
- Garcia, A.J., Tom, C., Guemes, M., Polanco, G., Mayorga, M.E., Wend, K., Miranda-Carboni, G.A., Krum, S.A., 2013. ER α signaling regulates MMP3 expression to induce FasL cleavage and osteoclast apoptosis. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 28, 283–290. <https://doi.org/10.1002/jbmr.1747>.
- Gingery, A., Bradley, E.W., Pederson, L., Ruan, M., Horwood, N.J., Oursler, M.J., 2008. TGF- β coordinately activates TAK1/MEK/AKT/NF κ B and SMAD pathways to promote osteoclast survival. *Exp. Cell Res.* 314, 2725–2738. <https://doi.org/10.1016/j.yexcr.2008.06.006>.
- Glantschnig, H., Fisher, J.E., Wesolowski, G., Rodan, G.A., Reszka, A.A., 2003. M-CSF, TNF α and RANK ligand promote osteoclast survival by signaling through mTOR/S6 kinase. *Cell Death Differ.* 10, 1165–1177. <https://doi.org/10.1038/sj.cdd.4401285>.
- Glass, D.A., Bialek, P., Ahn, J.D., Starbuck, M., Patel, M.S., Clevers, H., Taketo, M.M., Long, F., McMahon, A.P., Lang, R.A., Karsenty, G., 2005. Canonical Wnt signaling in differentiated osteoblasts controls osteoclast differentiation. *Dev. Cell* 8, 751–764. <https://doi.org/10.1016/j.devcel.2005.02.017>.
- Hayashi, S., Wang, Z., Bryan, J., Kobayashi, C., Faccio, R., Sandell, L.J., 2011. The type II collagen N-propeptide, PIIBNP, inhibits cell survival and bone resorption of osteoclasts via integrin-mediated signaling. *Bone* 49, 644–652. <https://doi.org/10.1016/j.bone.2011.06.011>.
- Hentunen, T.A., Reddy, S.V., Boyce, B.F., Devlin, R., Park, H.R., Chung, H., Selander, K.S., Dallas, M., Kurihara, N., Galson, D.L., Goldring, S.R., Koop, B.A., Windle, J.J., Roodman, G.D., 1998. Immortalization of osteoclast precursors by targeting Bel-XL and Simian virus 40 large T antigen to the osteoclast lineage in transgenic mice. *J. Clin. Invest.* 102, 88–97. <https://doi.org/10.1172/JCI2004>.
- Houde, N., Chamoux, E., Bisson, M., Roux, S., 2009. Transforming growth factor- β 1 (TGF- β 1) induces human osteoclast apoptosis by up-regulating Bim. *J. Biol. Chem.* 284, 23397–23404. <https://doi.org/10.1074/jbc.M109.019372>.
- Hughes, D.E., Boyce, B.F., 1997. Apoptosis in bone physiology and disease. *Mol. Pathol. MP.* 50, 132–137.
- Hughes, D.E., Wright, K.R., Uy, H.L., Sasaki, A., Yoneda, T., Roodman, G.D., Mundy, G.R., Boyce, B.F., 1995. Bisphosphonates promote apoptosis in murine osteoclasts in vitro and in vivo. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 10, 1478–1487. <https://doi.org/10.1002/jbmr.5650101008>.
- Hughes, D.E., Dai, A., Tiffée, J.C., Li, H.H., Mundy, G.R., Boyce, B.F., 1996. Estrogen promotes apoptosis of murine osteoclasts mediated by TGF- β . *Nat. Med.* 2, 1132–1136.
- Igarashi, K., Hirotani, H., Woo, J.-T., Stern, P.H., 2004. Cyclosporine A and FK506 induce osteoclast apoptosis in mouse bone marrow cell cultures. *Bone* 35, 47–56. <https://doi.org/10.1016/j.bone.2004.02.009>.
- Jimi, E., Shuto, T., Koga, T., 1995. Macrophage colony-stimulating factor and interleukin-1 α maintain the survival of osteoclast-like cells. *Endocrinology* 136, 808–811. <https://doi.org/10.1210/endo.136.2.7835314>.
- Jimi, E., Nakamura, I., Ikebe, T., Akiyama, S., Takahashi, N., Suda, T., 1998. Activation of NF- κ B is involved in the survival of osteoclasts promoted by interleukin-1. *J. Biol. Chem.* 273, 8799–8805.
- Kameda, T., Miyazawa, K., Mori, Y., Yuasa, T., Shiokawa, M., Nakamaru, Y., Mano, H., Hakeda, Y., Kameda, A., Kumegawa, M., 1996. Vitamin K2 inhibits osteoclastic bone resorption by inducing osteoclast apoptosis. *Biochem. Biophys. Res. Commun.* 220, 515–519. <https://doi.org/10.1006/bbrc.1996.0436>.
- Kameda, T., Mano, H., Yuasa, T., Mori, Y., Miyazawa, K., Shiokawa, M., Nakamaru, Y., Hiroi, E., Hiura, K., Kameda, A., Yang, N.N., Hakeda, Y., Kumegawa, M., 1997. Estrogen inhibits bone resorption by directly inducing apoptosis of the bone-resorbing osteoclasts. *J. Exp. Med.* 186, 489–495.
- Kanaoka, K., Kobayashi, Y., Hashimoto, F., Nakashima, T., Shibata, M., Kobayashi, K., Kato, Y., Sakai, H., 2000. A common downstream signaling activity of osteoclast survival factors that prevent nitric oxide-promoted osteoclast apoptosis. *Endocrinology* 141, 2995–3005. <https://doi.org/10.1210/endo.141.8.7603>.
- Kawata, T., Zernik, J.H., Fujita, T., Tokimasa, C., Tanne, K., 1999. Mechanism in inhibitory effects of vitamin K2 on osteoclastic bone resorption: in vivo study in osteopetrotic (op/op) mice. *J. Nutr. Sci. Vitaminol. (Tokyo)* 45, 501–507.
- Kerr, J.F., Wyllie, A.H., Currie, A.R., 1972. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. *Br. J. Cancer* 26, 239–257.
- Kim, H.-J., Zhao, H., Kitaura, H., Bhattacharyya, S., Brewer, J.A., Muglia, L.J., Patrick Ross, F., Teitelbaum, S.L., 2007. Glucocorticoids and the osteoclast. *Ann. N. Y. Acad. Sci.* 1116, 335–339. <https://doi.org/10.1196/annals.1402.057>.
- Kitaura, H., Kimura, K., Ishida, M., Kohara, H., Yoshimatsu, M., Takano-Yamamoto, T., 2013. Immunological reaction in TNF- α -mediated osteoclast formation and bone resorption in vitro and in vivo. *Clin. Dev. Immunol.* 2013, 181849. <https://doi.org/10.1155/2013/181849>.
- Kobayashi, Y., Hashimoto, F., Miyamoto, H., Kanaoka, K., Miyazaki-Kawashita, Y., Nakashima, T., Shibata, M., Kobayashi, K., Kato, Y., Sakai, H., 2000. Force-induced osteoclast apoptosis in vivo is accompanied by elevation in transforming growth factor beta and osteoprotegerin expression. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 15, 1924–1934. <https://doi.org/10.1359/jbmr.2000.15.10.1924>.
- Koga, T., Inui, M., Inoue, K., Kim, S., Suematsu, A., Kobayashi, E., Iwata, T., Ohnishi, H., Matozaki, T., Kodama, T., Taniguchi, T., Takayanagi, H., Takai, T., 2004. Costimulatory signals mediated by the ITAM motif cooperate with RANKL for bone homeostasis. *Nature* 428, 758–763. <https://doi.org/10.1038/nature02444>.
- Kovacic, N., Lukić, I.K., Grčević, D., Katavić, V., Croucher, P., Marusić, A., 2007. The Fas/Fas ligand system inhibits differentiation of murine osteoblasts but has a limited role in osteoblast and osteoclast apoptosis. *J. Immunol. Baltim. Md* 190, 3379–3389.
- Kovacic, N., Grčević, D., Katavić, V., Lukić, I.K., Grubisic, V., Mihovilovic, K., Cvijica, H., Croucher, P.I., Marusic, A., 2010. Fas receptor is required for estrogen deficiency-induced bone loss in mice. *Lab. Invest. J. Tech. Methods Pathol.* 90, 402–413. <https://doi.org/10.1038/labinvest.2009.144>.
- Krum, S.A., Miranda-Carboni, G.A., Hauschka, P.V., Carroll, J.S., Lane, T.F., Freedman, L.P., Brown, M., 2008. Estrogen protects bone by inducing Fas ligand in osteoblasts to regulate osteoclast survival. *EMBO J.* 27, 535–545. <https://doi.org/10.1038/sj.emboj.7601984>.
- Kuo, Y.-J., Tsuang, F.-Y., Sun, J.-S., Lin, C.-H., Chen, C.-H., Li, J.-Y., Huang, Y.-C., Chen, W.-Y., Yeh, C.-B., Shyu, J.-F., 2012. Calcitonin inhibits SDCP-induced osteoclast apoptosis and increases its efficacy in a rat model of osteoporosis. *PLoS One* 7, e40272. <https://doi.org/10.1371/journal.pone.0040272>.
- Lacey, D.L., Tan, H.L., Lu, J., Kaufman, S., Van, G., Qiu, W., Rattan, A., Scully, S., Fletcher, F., Juan, T., Kelley, M., Burgess, T.L., Boyle, W.J., Polverino, A.J., 2000. Osteoprotegerin ligand modulates murine osteoclast survival in vitro and in vivo. *Am. J. Pathol.* 157, 435–448. [https://doi.org/10.1016/S0002-9440\(10\)64556-7](https://doi.org/10.1016/S0002-9440(10)64556-7).
- Lee, Z.H., Lee, S.E., Kim, C.-W., Lee, S.H., Kim, S.W., Kwack, K., Walsh, K., Kim, H.-H., 2002. IL-1 α stimulation of osteoclast survival through the PI 3-kinase/Akt and ERK pathways. *J. Biochem. (Tokyo)* 131, 161–166.
- Lee, J., Kim, K., Kim, J.H., Jin, H.M., Choi, H.K., Lee, S.-H., Kook, H., Kim, K.K., Yokota, Y., Lee, S.-Y., Choi, Y., Kim, N., 2006. Id helix-loop-helix proteins negatively regulate TRANCE-mediated osteoclast differentiation. *Blood* 107, 2686–2693. <https://doi.org/10.1182/blood-2005-07-2798>.
- Lima, I.L.A., Macari, S., Madeira, M.F.M., Rodrigues, L.F.D., Colavite, P.M., Garlet, G.P., Soriani, F.M., Teixeira, M.M., Fukada, S.Y., Silva, T.A., 2015. Osteoprotegerin effects of IL-33/ST2 link to osteoclast apoptosis. *Am. J. Pathol.* 185, 3338–3348. <https://doi.org/10.1016/j.ajpath.2015.08.013>.
- Liu, C.C., Howard, G.A., 1991. Bone-cell changes in estrogen-induced bone-mass increase in mice: dissociation of osteoclasts from bone surfaces. *Anat. Rec.* 229, 240–250. <https://doi.org/10.1002/ar.1092290211>.
- Liu, Z.G., Hsu, H., Goeddel, D.V., Karin, M., 1996. Dissection of TNF receptor 1 effector functions: JNK activation is not linked to apoptosis while NF- κ B activation prevents cell death. *Cell* 87, 565–576.
- Liu, W., Xu, C., Zhao, H., Xia, P., Song, R., Gu, J., Liu, X., Bian, J., Yuan, Y., Liu, Z., 2015. Osteoprotegerin induces apoptosis of osteoclasts and osteoclast precursor cells via the Fas/Fas ligand pathway. *PLoS One* 10, e0142519. <https://doi.org/10.1371/journal.pone.0142519>.
- Long, C.L., Berry, W.L., Zhao, Y., Sun, X.-H., Humphrey, M.B., 2012. E proteins regulate osteoclast maturation and survival. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 27, 2476–2489. <https://doi.org/10.1002/jbmr.1707>.
- Lorget, F., Kamel, S., Mentaverri, R., Wattel, A., Naassila, M., Maamer, M., Brazier, M., 2000. High extracellular calcium concentrations directly stimulate osteoclast apoptosis. *Biochem. Biophys. Res. Commun.* 268, 899–903. <https://doi.org/10.1006/bbrc.2000.2229>.
- Luckman, S.P., Hughes, D.E., Coxon, F.P., Graham, R., Russell, G., Rogers, M.J., 1998. Nitrogen-containing bisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins, including Ras. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 13, 581–589. <https://doi.org/10.1359/jbmr.1998.13.4.581>.
- Maeda, K., Kobayashi, Y., Udagawa, N., Uehara, S., Ishihara, A., Mizoguchi, T., Kikuchi, Y., Takada, I., Kato, S., Kani, S., Nishita, M., Marumo, K., Martin, T.J., Minami, Y., Takahashi, N., 2012. Wnt5a-Ror2 signaling between osteoblast-lineage cells and osteoclast precursors enhances osteoclastogenesis. *Nat. Med.* 18, 405–412. <https://doi.org/10.1038/nm.2653>.
- Manolagas, S.C., 2000. Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. *Endocr. Rev.* 21, 115–137. <https://doi.org/10.1210/edrv.21.2.0395>.
- Mentaverri, R., Yano, S., Chattopadhyay, N., Petit, L., Kifor, O., Kamel, S., Terwilliger,

- E.F., Brazier, M., Brown, E.M., 2006. The calcium sensing receptor is directly involved in both osteoclast differentiation and apoptosis. *FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol.* 20, 2562–2564. <https://doi.org/10.1096/fj.06-6304.fje>.
- Miyamoto, S., Akiyama, S.K., Yamada, K.M., 1995. Synergistic roles for receptor occupancy and aggregation in integrin transmembrane function. *Science* 267, 883–885.
- Miyazaki, T., Katagiri, H., Kanegae, Y., Takayanagi, H., Sawada, Y., Yamamoto, A., Pando, M.P., Asano, T., Verma, I.M., Oda, H., Nakamura, K., Tanaka, S., 2000. Reciprocal role of ERK and NF-kappaB pathways in survival and activation of osteoclasts. *J. Cell Biol.* 148, 333–342.
- Mun, S.H., Ko, N.Y., Kim, H.S., Kim, J.W., Kim, D.K., Kim, A.-R., Lee, S.H., Kim, Y.-G., Lee, C.K., Lee, S.H., Kim, B.K., Beaven, M.A., Kim, Y.M., Choi, W.S., 2010. Interleukin-33 stimulates formation of functional osteoclasts from human CD14(+) monocytes. *Cell. Mol. Life Sci. CMLS* 67, 3883–3892. <https://doi.org/10.1007/s00018-010-0410-y>.
- Murakami, T., Yamamoto, M., Ono, K., Nishikawa, M., Nagata, N., Motoyoshi, K., Akatsu, T., 1998. Transforming growth factor-beta1 increases mRNA levels of osteoclastogenesis inhibitory factor in osteoblastic/stromal cells and inhibits the survival of murine osteoclast-like cells. *Biochem. Biophys. Res. Commun.* 252, 747–752. <https://doi.org/10.1006/bbrc.1998.9723>.
- Nagase, Y., Iwasawa, M., Akiyama, T., Kadono, Y., Nakamura, M., Oshima, Y., Yasui, T., Matsumoto, T., Hirose, J., Nakamura, H., Miyamoto, T., Bouillet, P., Nakamura, K., Tanaka, S., 2009. Anti-apoptotic molecule Bcl-2 regulates the differentiation, activation, and survival of both osteoblasts and osteoclasts. *J. Biol. Chem.* 284, 36659–36669. <https://doi.org/10.1074/jbc.M109.016915>.
- Nakamura, T., Imai, Y., Matsumoto, T., Sato, S., Takeuchi, K., Igarashi, K., Harada, Y., Azuma, Y., Krust, A., Yamamoto, Y., Nishina, H., Takeda, S., Takayanagi, H., Metzger, D., Kanno, J., Takaoka, K., Martin, T.J., Chambon, P., Kato, S., 2007. Estrogen prevents bone loss via estrogen receptor alpha and induction of Fas ligand in osteoclasts. *Cell* 130, 811–823. <https://doi.org/10.1016/j.cell.2007.07.025>.
- Norbury, C.J., Hickson, I.D., 2001. Cellular responses to DNA damage. *Annu. Rev. Pharmacol. Toxicol.* 41, 367–401. <https://doi.org/10.1146/annurev.pharmtox.41.1.367>.
- Ogawa, Y., Ohtsuki, M., Uzuki, M., Sawai, T., Onozawa, Y., Nakayama, J., Yonemura, A., Kimura, T., Matsuno, H., 2003. Suppression of osteoclastogenesis in rheumatoid arthritis by induction of apoptosis in activated CD4+ T cells. *Arthritis Rheum.* 48, 3350–3358. <https://doi.org/10.1002/art.11322>.
- Oursler, M.J., Osoby, P., Pyfferoen, J., Riggs, B.L., Spelsberg, T.C., 1991a. Avian osteoclasts as estrogen target cells. *Proc. Natl. Acad. Sci. U. S. A.* 88, 6613–6617.
- Oursler, M.J., Cortese, C., Keeting, P., Anderson, M.A., Bonde, S.K., Riggs, B.L., Spelsberg, T.C., 1991b. Modulation of transforming growth factor-beta production in normal human osteoblast-like cells by 17 beta-estradiol and parathyroid hormone. *Endocrinology* 129, 3313–3320. <https://doi.org/10.1210/endo-129-6-3313>.
- Park, H., Jung, Y.-K., Park, O.-J., Lee, Y.J., Choi, J.-Y., Choi, Y., 2005. Interaction of Fas ligand and Fas expressed on osteoclast precursors increases osteoclastogenesis. *J. Immunol. Baltim. Md* 1950 175, 7193–7201.
- Pederson, L., Ruan, M., Westendorf, J.J., Khosla, S., Oursler, M.J., 2008. Regulation of bone formation by osteoclasts involves Wnt/BMP signaling and the chemokine sphingosine-1-phosphate. *Proc. Natl. Acad. Sci. U. S. A.* 105, 20764–20769. <https://doi.org/10.1073/pnas.0805133106>.
- Peng, M., Wang, Y., Qiang, L., Xu, Y., Li, C., Li, T., Zhou, X., Xiao, M., Wang, J., 2018. Interleukin-35 inhibits TNF- α -induced osteoclastogenesis and promotes apoptosis via shifting the activation from TNF receptor-associated death domain (TRADD)-TRAF2 to TRADD-Fas-Associated death domain by JAK1/STAT1. *Front. Immunol.* 9, 1417. <https://doi.org/10.3389/fimmu.2018.01417>.
- Recchia, L., Rucci, N., Funari, A., Migliaccio, S., Taranta, A., Longo, M., Kneissel, M., Susa, M., Fabbro, D., Teti, A., 2004. Reduction of c-Src activity by substituted 5,7-diphenylpyrrolo[2,3-d]-pyrimidines induces osteoclast apoptosis in vivo and in vitro. Involvement of ERK1/2 pathway. *Bone* 34, 65–79.
- Reszka, A.A., Halasy-Nagy, J.M., Masaracha, P.J., Rodan, G.A., 1999. Bisphosphonates act directly on the osteoclast to induce caspase cleavage of mst1 kinase during apoptosis. A link between inhibition of the mevalonate pathway and regulation of an apoptosis-promoting kinase. *J. Biol. Chem.* 274, 34967–34973.
- Roux, S., Brown, J., 2009. Osteoclast apoptosis in rheumatic diseases characterized by a high level of bone resorption (osteoporosis, rheumatoid arthritis, myeloma and pagets disease of bone). *Curr. Rheumatol. Rev.* 5, 98–110. <https://doi.org/10.2174/157339709788298428>.
- Roux, S., Lambert-Comeau, P., Saint-Pierre, C., Lépine, M., Sawan, B., Parent, J.-L., 2005. Death receptors, Fas and TRAIL receptors, are involved in human osteoclast apoptosis. *Biochem. Biophys. Res. Commun.* 333, 42–50. <https://doi.org/10.1016/j.bbrc.2005.05.092>.
- Ruan, M., Pederson, L., Bradley, E.W., Bamberger, A.-M., Oursler, M.J., 2010. Transforming growth factor-(beta) coordinately induces suppressor of cytokine signaling 3 and leukemia inhibitory factor to suppress osteoclast apoptosis. *Endocrinology* 151, 1713–1722. <https://doi.org/10.1210/en.2009-0813>.
- Ruoslahti, E., Reed, J.C., 1994. Anchorage dependence, integrins, and apoptosis. *Cell* 77, 477–478.
- Sakai, H., Kobayashi, Y., Sakai, E., Shibata, M., Kato, Y., 2000. Cell adhesion is a prerequisite for osteoclast survival. *Biochem. Biophys. Res. Commun.* 270, 550–556. <https://doi.org/10.1006/bbrc.2000.2485>.
- Schneider, P., Olson, D., Tardivel, A., Browning, B., Lugovskoy, A., Gong, D., Dobles, M., Hertig, S., Hofmann, K., Van Vlijmen, H., Hsu, Y.-M., Burkly, L.C., Tschopp, J., Zheng, T.S., 2003. Identification of a new murine tumor necrosis factor receptor locus that contains two novel murine receptors for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). *J. Biol. Chem.* 278, 5444–5454. <https://doi.org/10.1074/jbc.M210783200>.
- Sedger, L.M., Glaccum, M.B., Schuh, J.C.L., Kanaly, S.T., Williamson, E., Kayagaki, N., Yun, T., Smolak, P., Le, T., Goodwin, R., Gliniak, B., 2002. Characterization of the in vivo function of TNF-alpha-related apoptosis-inducing ligand, TRAIL/Apo2L, using TRAIL/Apo2L gene-deficient mice. *Eur. J. Immunol.* 32, 2246–2254. [https://doi.org/10.1002/1521-4141\(200208\)32:8<2246::AID-IMMU2246>3.0.CO;2-6](https://doi.org/10.1002/1521-4141(200208)32:8<2246::AID-IMMU2246>3.0.CO;2-6).
- Sharma, R., Callaway, D., Vanegas, D., Bendele, M., Lopez-Cruzan, M., Horn, D., Guda, T., Fajardo, R., Abdou-Werner, S., Herman, B., 2014. Caspase-2 maintains bone homeostasis by inducing apoptosis of oxidatively-damaged osteoclasts. *PLoS One* 9, e93696. <https://doi.org/10.1371/journal.pone.0093696>.
- Sivagurunathan, S., Muir, M.M., Brennan, T.C., Seale, J.P., Mason, R.S., 2005. Influence of glucocorticoids on human osteoclast generation and activity. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 20, 390–398. <https://doi.org/10.1359/JBMR.041233>.
- 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.
- Soya, N.S., Alles, N., 2016. Osteoclast function and bone-resorbing activity: an overview. *Biochem. Biophys. Res. Commun.* 476, 115–120. <https://doi.org/10.1016/j.bbrc.2016.05.019>.
- Soya, N.S., Alles, N., 2018. The role of IL-3 in bone. *J. Cell. Biochem.* <https://doi.org/10.1002/jcb.27956>.
- Soya, N.S., Alles, N., Shimokawa, H., Jimi, E., Aoki, K., Ohya, K., 2009. Inhibition of the classical NF-kappaB pathway prevents osteoclast bone-resorbing activity. *J. Bone Miner. Metab.* 27, 131–139. <https://doi.org/10.1007/s00774-008-0026-6>.
- Soya, N.S., Alles, N., Weih, D., Lovas, A., Mian, A.H., Shimokawa, H., Yasuda, H., Weih, F., Jimi, E., Ohya, K., Aoki, K., 2010. The pivotal role of the alternative NF-kappaB pathway in maintenance of basal bone homeostasis and osteoclastogenesis. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 25, 809–818. <https://doi.org/10.1359/jbmr.091030>.
- Soya, N.S., Alles, N., Takahashi, M., Aoki, K., Ohya, K., 2011. Defective nuclear factor-kappaB-inducing kinase in aly/aly mice prevents bone resorption induced by local injection of lipopolysaccharide. *J. Periodontol. Res. Suppl.* 46, 280–284. <https://doi.org/10.1111/j.1600-0765.2010.01333.x>.
- Soya, N.S., Alles, N., Aoki, K., Ohya, K., 2012. Osteoclast formation and differentiation: an overview. *J. Med. Dent. Sci.* 59, 65–74.
- Szymczyk, K.H., Freeman, T.A., Adams, C.S., Srinivas, V., Steinbeck, M.J., 2006. Active caspase-3 is required for osteoclast differentiation. *J. Cell. Physiol.* 209, 836–844. <https://doi.org/10.1002/jcp.20770>.
- Tobias, J., Chambers, T.J., 1989. Glucocorticoids impair bone resorptive activity and viability of osteoclasts disaggregated from neonatal rat long bones. *Endocrinology* 125, 1290–1295. <https://doi.org/10.1210/endo-125-3-1290>.
- Tonna, E.A., 1960. Periosteal osteoclasts, skeletal development and ageing. *Nature* 185, 405–407.
- Villanova, I., Townsend, P.A., Uhlmann, E., Knolle, J., Peyman, A., Amling, M., Baron, R., Horton, M.A., Teti, A., 1999. Oligodeoxynucleotide targeted to the alphav gene inhibits alphav integrin synthesis, impairs osteoclast function, and activates intracellular signals to apoptosis. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 14, 1867–1879. <https://doi.org/10.1359/jbmr.1999.14.11.1867>.
- Wakeyama, H., Akiyama, T., Takahashi, K., Amano, H., Kadono, Y., Nakamura, M., Oshima, Y., Itabe, H., Nakayama, K.I., Nakayama, K., Nakamura, K., Tanaka, S., 2007. Negative feedback loop in the Bim-caspase-3 axis regulating apoptosis and activity of osteoclasts. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 22, 1631–1639. <https://doi.org/10.1359/jbmr.070619>.
- Wang, X.M., Yu, S.F., Yang, Z.P., 2000. Apoptosis of osteoclast-like cells induced by alendronate is related to Fas gene expression. *Chin. J. Dent. Res. Off. J. Sci. Sect. Chin. Stomatol. Assoc. CSA* 3, 26–32.
- Wang, L., Liu, S., Zhao, Y., Liu, D., Liu, Y., Chen, C., Karray, S., Shi, S., Jin, Y., 2015. Osteoblast-induced osteoclast apoptosis by fas ligand/FAS pathway is required for maintenance of bone mass. *Cell Death Differ.* 22, 1654–1664. <https://doi.org/10.1038/cdd.2015.14>.
- Wei, W., Zeve, D., Suh, J.M., Wang, X., Du, Y., Zerwekh, J.E., Dechow, P.C., Graff, J.M., Wan, Y., 2011. Biphasic and dosage-dependent regulation of osteoclastogenesis by β -catenin. *Mol. Cell. Biol.* 31, 4706–4719. <https://doi.org/10.1128/MCB.05980-11>.
- Weinstein, R.S., Manolagas, S.C., 2000. Apoptosis and osteoporosis. *Am. J. Med.* 108, 153–164. [https://doi.org/10.1016/s0002-9343\(99\)00420-9](https://doi.org/10.1016/s0002-9343(99)00420-9).
- Weinstein, R.S., Chen, J.-R., Powers, C.C., Stewart, S.A., Landes, R.D., Bellido, T., Jilka, R.L., Parfitt, A.M., Manolagas, S.C., 2002. Promotion of osteoclast survival and antagonism of bisphosphonate-induced osteoclast apoptosis by glucocorticoids. *J. Clin. Invest.* 109, 1041–1048. <https://doi.org/10.1172/JCI14538>.
- Wong, B.R., Besser, D., Kim, N., Arron, J.R., Vologodskaja, M., Hanafusa, H., Choi, Y., 1999. TRANCE, a TNF family member, activates Akt/PKB through a signaling complex involving TRAF6 and c-Src. *Mol. Cell* 4, 1041–1049.
- Woo, K.M., Kim, H.-M., Ko, J.S., 2002. Macrophage colony-stimulating factor promotes the survival of osteoclast precursors by up-regulating Bcl-X(L). *Exp. Mol. Med.* 34, 340–346. <https://doi.org/10.1038/emmm.2002.52>.
- Wu, X., McKenna, M.A., Feng, X., Nagy, T.R., McDonald, J.M., 2003. Osteoclast apoptosis: the role of Fas in vivo and in vitro. *Endocrinology* 144, 5545–5555. <https://doi.org/10.1210/en.2003-0296>.
- Wu, X., Pan, G., McKenna, M.A., Zayzafoon, M., Xiong, W.-C., McDonald, J.M., 2005a. RANKL regulates Fas mediated apoptosis and Fas-mediated apoptosis in osteoclasts. *J. Bone Miner. Res. Off. J. Am. Soc. Bone Miner. Res.* 20, 107–116. <https://doi.org/10.1359/JBMR.041022>.
- Wu, X., Ahn, E.-Y., McKenna, M.A., Yeo, H., McDonald, J.M., 2005b. Fas binding to calmodulin regulates apoptosis in osteoclasts. *J. Biol. Chem.* 280, 29964–29970. <https://doi.org/10.1074/jbc.M500710200>.
- Wu, J., Glimcher, L.H., Aliprantis, A.O., 2008. HCO3-/Cl- anion exchanger SLC4A2 is required for proper osteoclast differentiation and function. *Proc. Natl. Acad. Sci. U. S. A.* 105, 16934–16939. <https://doi.org/10.1073/pnas.0808763105>.
- Xing, L., Boyce, B.F., 2005. Regulation of apoptosis in osteoclasts and osteoblastic cells. *Biochem. Biophys. Res. Commun.* 328, 709–720. <https://doi.org/10.1016/j.bbrc.2005.05.019>.

- 2004.11.072.
- Xing, L., Venegas, A.M., Chen, A., Garrett-Beal, L., Boyce, B.F., Varmus, H.E., Schwartzberg, P.L., 2001. Genetic evidence for a role for Src family kinases in TNF family receptor signaling and cell survival. *Genes Dev.* 15, 241–253.
- Zauli, G., Rimondi, E., Nicolin, V., Melloni, E., Celeghini, C., Secchiero, P., 2004. TNF-related apoptosis-inducing ligand (TRAIL) blocks osteoclastic differentiation induced by RANKL plus M-CSF. *Blood* 104, 2044–2050. <https://doi.org/10.1182/blood-2004-03-1196>.
- Zhang, Q., Badell, I.R., Schwarz, E.M., Boulukos, K.E., Yao, Z., Boyce, B.F., Xing, L., 2005. Tumor necrosis factor prevents alendronate-induced osteoclast apoptosis in vivo by stimulating Bcl-xL expression through Ets-2. *Arthritis Rheum.* 52, 2708–2718. <https://doi.org/10.1002/art.21236>.
- Zhao, H., Ross, F.P., Teitelbaum, S.L., 2005. Unoccupied alpha(v)beta3 integrin regulates osteoclast apoptosis by transmitting a positive death signal. *Mol. Endocrinol. Baltim. Md.* 19, 771–780. <https://doi.org/10.1210/me.2004-0161>.