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The Differentiation and Regulatory Pathways of Osteoclasts

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Osteoclasts are multinucleated cells that are present on the surface of the bone matrix, which is made up of compact bone. Osteoclasts are responsible for the removal of parts of the calcified bone matrix, which is important for the remodeling of bones [1]. Bone remodeling is necessary for: 1) removing old and brittle bone tissue; 2) reshaping bones after a fracture; and 3) repairing micro-cracks which form during ordinary everyday activities, or micro-cracks in bones that are under stress from certain activities such as running or lifting weights [2,3]. Osteoclast activity allows for the constant turnover of bone cells during each of these three scenarios previously listed.

In order to begin resorbing bone, osteoclasts secrete lysosomal enzymes, such as collagenase. Collagenase digests the organic part of the bone matrix which consists of collagen proteins [4], and this action leads to the formation of Howship's lacunae or resorption bays, which are small wells or pits on the bone surface where osteoclasts have begun bone resorption. Cathepsin K is an example of a lysosomal enzyme and is a major protease involved in the degradation of Type I collagen of bone subjacent to osteoclasts [5]. In addition to lysosomal enzymes digesting collagen proteins, osteoclasts also produce hydrochloric acid (HCl) which dissolves hydroxyapatite into soluble calcium (Ca^{2+}) and phosphate (PO_4^{2-}) ions which are able to be released into the bloodstream [6].

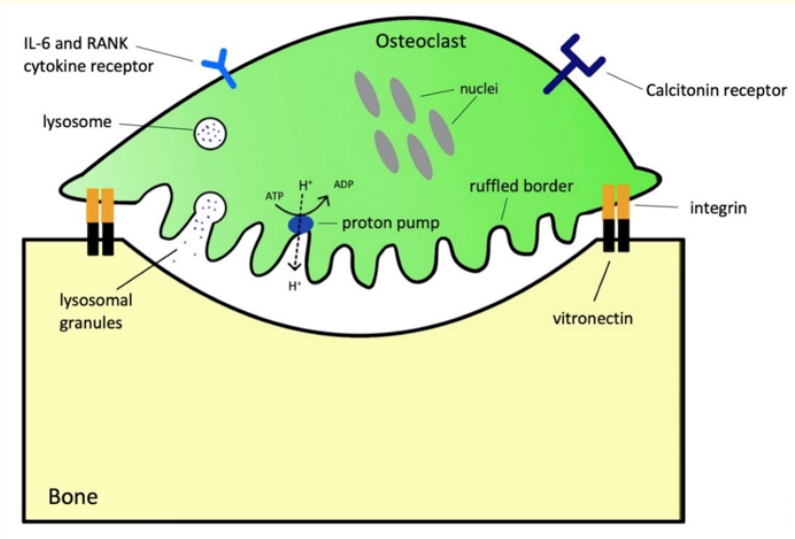

Figure 1: A visual of an osteoclast and the mechanism of resorption of bone. A tight seal is formed between the osteoclast (top) and the bone (bottom) through the binding of osteoclast integrins with vitronectin, a bone protein. The finger-like "ruffled border" utilizes an ATP-dependent proton pump that acidifies the extracellular area subjacent to the osteoclast. Also, lysosomal proteases and acid phosphatases are released which is mainly important in the breakdown of collagen (organic matrix).

The process of osteoclast activity is stimulated through a pathway that first involves osteoblasts. First, osteoblasts, which are also located in the bone matrix, sense micro-cracks at a specific spot on the bone where an osteoblast is located. The osteoblast produces a substance called Receptor Activator of Nuclear Factor κβ Ligand (RANKL). Furthermore, macrophage colony-stimulating factor (M-CSF) is essential for the differentiation and formation of osteoclasts. M-CSF binds to its receptor c-Fms, and this binding along with RANKL binding to the surface of monocytes that are nearby, causes the monocytes to differentiate into an osteoclast [6]. Specifically, when RANKL binds to the RANK receptor on the monocyte, it leads to multiple monocytes fusing together, which leads to osteoclasts having their multinucleated feature. In addition to the M-CSF and RANKL causing differentiation of monocytes into osteoclasts, M-CSF and RANKL also ensure that osteoclasts mature and become active in resorption of bones [6,7].

While osteoclasts are important in maintaining healthy bones by their action of resorption of old and brittle bone tissue, it is also important for osteoclasts to be inhibited in order to ensure that bone resorption does not occur too much. This regulation occurs in many different ways. For example, there are highly elucidated pathways that are regulated by osteoprotegerin, parathyroid hormone, calcitonin, and IL-6. It is also important to note that after osteoclasts complete their job, they inactivate themselves through apoptosis.

Osteoprotegerin binds to RANKL and prevents it from activating RANK receptors on monocytes. This decreases osteoclastogenesis and the activation of osteoclasts and therefore decreases the rate of bone resorption [8].

The parathyroid glands, located on the thyroid gland in the neck, release parathyroid hormone when blood calcium levels are low. Parathyroid hormone then travels to the bones and stimulates RANKL release from osteoblasts, which ultimately triggers bone resorption. This resorption then leads to an increase in blood calcium levels, which corrects the initial deficiency [9]. In addition, it has been found that parathyroid hormone acts on osteoblasts and increases collagenase gene transcription and synthesis [10], which further supports the claim that parathyroid hormone increases bone resorption. On the contrary, when blood calcium levels are high, the parathyroid gland releases less parathyroid hormone in order to decrease the rate of bone resorption.

Calcitonin is released from parafollicular cells in the thyroid gland, and high levels of calcitonin inhibits bone resorption which leads to lower blood calcium levels. This inhibition of bone resorption is believed to be due to several different pathways. One theory is that calcitonin disrupts the cytoskeletal organization of osteoclasts [11]. Therefore, calcitonin would decrease osteoclast stability and lead to osteoclast death or inhibition of osteoclast formation. Another theory is in regard to the effect that calcitonin has on sphingosine 1-phosphate. It has been found that calcitonin inhibits the release of sphingosine 1-phosphate from osteoclasts [12], and this is significant because sphingosine 1-phosphate could potentially play a role in the continuous turnover of bone [13].

While there is some inconsistency regarding the role Vitamin D plays in bone resorption, the literature seems to state that Vitamin D induces bone resorption. This may happen because Vitamin D can stimulate RANKL expression [14]. Increased RANKL expression can therefore increase osteoclastogenesis, which then leads to an increase in osteoclast number and activity [15].

Interleukin-6 (IL-6) is an important regulator with regards to osteoclastogenesis and pregnancy. First, IL-6 is extremely important in enhancement of osteoclastogenesis and therefore bone resorption [16]. IL-6 regulates osteoclastogenesis through sensing of cytosolic Ca^{2+} levels; when Ca^{2+} levels are high, IL-6 is inhibited in order to ensure that the Ca^{2+} levels are not raised higher because of bone resorption. Furthermore, estrogen inhibits the production of IL-6 [17]. It is important to note that when women are pregnant, they produce more estrogen than usual, and therefore their bone resorption could decrease.

It is also important to note Wolff's Law. This law states that bones that bear a lot of weight, meaning they have high mechanical stress, remodel at a higher rate [18]. This phenomenon is significant because it explains that our individual movement, and the physical impact those movements have on our bones, can help regulate the formation and activity of osteoclasts as they are needed as part of bone repair pathways.

In addition to the pathways previously outlined, there are also newer pathways that offer important insights in the future knowledge of osteoclast regulation such as the novel gene *Merlot*, Tussilagone, *Lrp1*, and even melatonin.

The novel gene *Merlot* was recently found to have two extremely impactful effects on the regulation of osteoclasts. First, *Merlot* was found to terminate osteogenesis by inducing apoptosis. This was discovered in mice that were lacking *Merlot*, because these mice were found to have low bone mass due to a lack of osteoclast apoptosis and therefore an increase in bone resorption. Second, osteoclast precursors that had increased levels of *Merlot* expression did not differentiate into osteoclasts. This shows that *Merlot* can inhibit osteogenesis. It is through these two pathways (inhibition of differentiation and simultaneous induction of apoptosis) that *Merlot* regulates osteoclast lifespan [19].

Tussilagone is a sesquiterpenoid that had similar end results as *Merlot.* Tussilagone was found to suppress the differentiation of bone marrow-derived monocytes into osteoclasts. Tussilagone also induces apoptosis in osteoclasts, and both of these findings lead to the conclusion that Tussilagone can assist in decreasing the degradation of bone by osteoclasts [20].

Melatonin has been found to play a role in the regulation of bone healing, but the specificities of the role melatonin plays are still unknown. One finding indicated that melatonin treatment can impair fracture healing in zebrafish. A fracture or stress on a bone can cause Erk signals to be transduced, and these signals promote bone repair. However, melatonin can actually suppress the Erk signals in fractured scales of zebrafish. Specifically, melatonin inhibits the differentiation of both osteoblasts and osteoclasts, and this was determined by finding a decrease in the number of mature osteoblasts and osteoclasts in zebrafish with a fractured scale [21].

Finally, low density lipoprotein receptor-related protein 1 (LRP1) was found to be required for the differentiation of monocytes into osteoclasts, and it is also required for osteoclast-osteoblast interactions. This was found using cell line RAW264, where knockdown of *Lrp1* inhibited RANKL-induced osteoclast differentiation. Furthermore, knockdown of *Lrp1* also showed suppressed osteoclast proliferation [22].

Conflict of Interest

The authors declare that they have no conflict of interest.

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