REVIEW ARTICLE

Osteoclastogenesis in Craniofacial Bone Defect: TNF-α and RANKL Interaction in Signaling Pathway at Guided Bone Regeneration as a Viable Treatment Option

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ABSTRACT

Adequate reconstruction of craniofacial bone defects is a fundamental measure to restore the function of bone that undergo various pathological processes. Guided bone regeneration (GBR) is a reconstructive treatment option for craniofacial bone defects, employing bioresorbable barrier membrane such as demineralized dentin material membrane. This approach has been extensively studied due to its potential as an effective bone-inducing matrix that prevents constant bone damage. Understanding the mechanisms behind osteoclastogenesis is crucial, as bone defects can lead to an imbalance in bone remodelling. Numerous cytokines, including TNF-α and RANKL, play significant roles in osteoclastogenesis. TNF-α influences bone resorption activity through a variety of processes that advance along the trajectory under the significance of RANKL and convey signals via mitogen-activated protein kinase and Nf-κB. Literature searches were conducted via Pubmed and Google Scholar for full-text journals published between 2015 and 2023.

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Keywords: Craniofacial bone defect; Guided bone regeneration; TNF-α; RANKL; Osteoclastogenesis.

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INTRODUCTION

Throughout the course of an individual's life, various pathological processes can initiate craniofacial bone defects, including congenital malformations, severe trauma, infections, accidents, and tumour resection (1,2). Successful reconstruction of bone abnormalities is a fundamental step to repair its function. The molecular pattern involved in bone reconstruction are achieved through natural processes called bone remodelling, wherein bone tissue maintains a regulated equilibrium between periodic deposition episodes and continual resorption (3,4). The primary cells accountable for resorption of bone are osteoclasts, which originate from the hematopoietic stem cell lineage (5). Moreover, receptor activator of nuclear factor κB ligand (RANKL) is the most crucial component in osteoclast formation during both pathological and developing bone resorption (5). There are many cell types that express RANKL including osteoblastic, chondroblastic, or T and B cells (6). RANKL promotes the differentiation of osteoclast precursors into bone-resorbing osteoclasts by binding itself to the RANK receptor on the surface of precursor cells (5). On the other hand, tumour necrosis factor-α (TNF-α) is one of the most adaptable cytokines secreted by macrophages as a by-product of the immune response (5). TNF-α is typically involved in the early stages of acute inflammation, with detectable levels appearing 24 hours post bone defect and reaching peak levels on the third day (7). Furthermore, TNF-α has been shown to raise RANKL level in osteocytes, thereby promoting the differentiation and function of osteoclasts (5,8).

Addressing the molecular pattern behind osteoclast development is crucial because bone defects can lead to an imbalance in bone remodelling, resulting

in continuous bone degradation. Additionally, understanding treatment options for reconstructing craniofacial bone defects is vital. There are various procedures can be carried out to address this issue, including the application of GBR, which is expected to promote bone cell repopulation throughout the wound healing phase because excessive soft tissue infiltration at this stage may result in alterations of bone dimensions (9). This review outlines the current understanding of the roles of TNF-α and RANKL in osteoclastogenesis, as well as the possible mechanism of demineralized dentin material membrane (DDMM) as GBR in craniofacial bone defect.

METHODS

The present study involved a literature search of various article reviews, original in vivo and in vitro research studies, and journals focused on the impact of GBR on osteoclastogenesis in craniofacial bone defect, including the underlying interaction of TNF-α and RANKL. Keywords used in the search included 'craniofacial bone defect', 'guided bone regeneration', 'TNF-α', 'RANKL', and 'osteoclastogenesis'. Literature searches were conducted via Pubmed and Google Scholar for full-text journals published between 2015 and 2023.

REVIEW

Craniofacial Bone Defect

The craniofacial area, which consists various complex tissues such as bone, cartilage, muscle, salivary glands, nerve tissue, tooth, periodontium, and mucosa, is susceptible to trauma and pathology often resulting in major bone destruction. This considerable bone loss leads to both aesthetic and functional complications for the patient. These injuries may arise from acute trauma due to accidents, congenital abnormalities, pathologies such as infections or maxillofacial tumours like ameloblastoma, or surgical interventions (2).

Large lesions surpass the body's natural regenerative capacity, resulting in major bone loss that cannot be repaired on its own (2,10). The emergence of craniofacial deformities can lead to numerous symptoms that affect individuals' quality of life, including headaches, anxiety, depression, dizziness, intolerance to vibration and noise, and an inability to concentrate (11–13). Consequently, the craniofacial region is at risk for severe injuries throughout life, which may necessitate bone grafting or particular procedures to restore their function (2).

Bone Remodelling and Its Signalling Pathway

Bone remodelling is vital to preserve bone volume, biomechanical stability, homeostatic, and structural integrity. This phase is regulated in such a way as to achieve an ideal state between osteoclasts' hard callus resorption and osteoblasts' lamella bone deposition. The three main types of cells involved in this process are osteoclasts, osteocytes, and osteoblasts. Proteins like osterix (OSX), runt-related transcription factor 2 (RUNX-2), and the Wnt and FGF signalling pathways, which are in charge of bone formation, aid in the differentiation of osteoblasts from mesenchymal progenitor cells. Additionally, osteoblasts have the capacity to develop into osteocytes, which have the ability to control osteoblastogenesis by producing specific inhibitors that inhibit Wnt signalling (14).

Osteoclast cells have a role in bone resorption, which is activated through the RANKL-RANK-OPG signalling pathway cross-talk. The RANKL-RANK-OPG signalling pathway is one of the main signalling routes that regulates bone remodelling. The interaction of these three proteins determines bone production and resorption at the specific location of bone defect. RANKL is a cytokine dispatched on the surface of osteocytes, chondrocytes, and osteoblasts. The function of RANKL at this stage is to activate Nf-κB and other signalling routes by binding to its receptor, namely RANK, which is appear at the osteoclast precursor cells. As a result, osteoclast differentiation—including osteoclast activation, production, and survival—is greatly influenced by RANKL-RANK activity. In contrast, osteoblasts and osteocytes express an inhibitor called osteoprotegerin (OPG) which has a high affinity for RANKL and can dissolve it, thereby blocking RANKL from interacting with RANK. Thus, the extent of osteoclast production and activity is regulated by the expression rate of RANKL-OPG.

Osteoclastogenesis

The process by which mature, multinucleated osteoclasts are created from haematological myeloid precursors that are generated in the bone marrow is known as osteoclastogenesis. Chemokines transport osteoclast precursors from the bone marrow into the bloodstream, where they remain until various substances generated in bone remodelling units (BRUs)—areas undergoing resorption—attract them back into the bone. These include cytokines and chemokines that are conveyed by cells in and around the BRU and necessary for the development and differentiation of osteoclast precursors in the cells, notably RANKL and macrophage-colony-stimulating factor (M-CSF) (15).

The first necessary cytokine, M-CSF, stimulates the production of osteoclasts by attaching itself to the c-fms receptor through the RANKL-RANK signalling cascade. The RANKL-RANK signalling route promotes osteoclast function and development while blocking osteoclast apoptosis, resulting in the induction of bone resorption. One of the cells that also play a role is osteocytes as a source of RANKL which induces osteoclastogenesis (16,17). Osteocytes are embedded in a matrix secreted

by osteoblasts during their differentiation. These cells make up 90% of the population of bone tissue and are located in the lacunae. Osteocytes have been shown to function as regulators the matrix around lacunae, and as mechanosensory cells.

The Interaction Between TNF-α, RANKL, and Osteoclast One of the most influential inflammatory cytokines is TNF-α. TNF-α performs a crucial part in the host response by inducing macrophage cell death. Apart from that, it has been demonstrated that TNF-α contributes to inflammation-related bone resorption. The process of bone resorption by osteoclasts is mostly mediated by inflammatory cytokines, such as TNF-α. This cytokine has the ability to regulate several cellular responses, including proliferation, inflammation, apoptosis, and antiviral activity. The elevation of RANKL and M-CSF expression in osteogenic cells, which enhances osteoclast function and differentiation, is one of the ways that TNF-α can affect osteoclastogenesis as well as bone resorption activity (8). Under the impact of RANKL, which interacts through the transcription factors Nf-κB and activator protein 1 (AP-1), differentiated cells progress along the trajectory toward the osteoclast phenotype. A variety of proinflammatory cytokines work cooperatively to control elevated osteoclastogenesis when RANKL is present. TNF-α signals via mitogen-activated protein kinase (MAPK) and Nf-κB and can be seen as in Figure 1 (18).

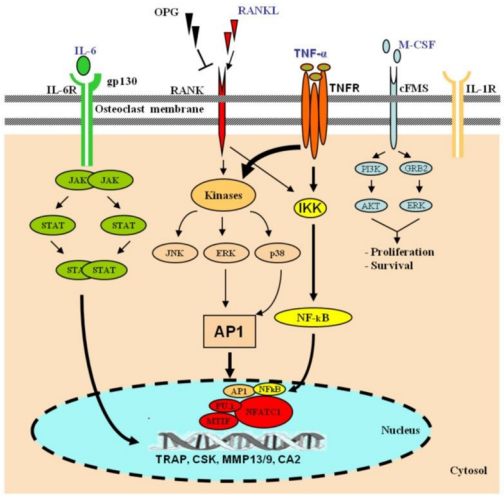

Figure 1: The role of TNF-α and RANKL in osteoclast development (Osta et al., 2014)

RANKL is a TNF superfamily member that functions via the RANK receptor. Preosteoblasts that exhibit RANKL-RANK interaction attract TNF receptor-associated factor proteins (TRAP), including TRAP 2/6, which can activate both Nf-κB and MAPK (19). This signalling pathway promotes transcription factors, including as AP-1, c-Fos, and nuclear factor of activated T cells-1 (NFATc1), that are necessary for the development of osteoclasts. The inflammatory patterns discussed above that activate osteoclasts result in excessive bone loss or resorption (8).

The study by Luo et al. (2018) demonstrated that TNF-α could not stimulate osteoclastogenesis independently, but it could increase osteoclastogenic activity when combined with RANKL, as demonstrated by a rise in the quantity of multinucleated osteoclasts that were positive for TRAP. In contrast to the studies above, some research showed that TNF-α can directly stimulates osteoclast development in vitro without RANKL (8). Despite the controversy surrounding this issue, the interaction between TNF-α, RANKL, and osteoclasts has been proven to have a close relationship with each other in the process of osteoclastogenesis.

Osteoblastogenesis

Osteoblastogenesis is the process of forming osteoblasts originating from mesenchymal stem cells (MSCs) that can differentiate into fibroblasts, chondrocytes, adipocytes, tendon and skeletal muscle cells. A wide variety of receptors and signalling cascades can be activated in osteoblasts under normal circumstances. The main pathways include BMP and its receptors, which work through SMAD proteins to activate RUNX-2 directly or transcriptionally, as well as the Wnt-frizzled pathway, which carries out additional action by means of β-catenin. Proinflammatory cytokines like IL-6 and TNF-α are released under inflammatory circumstances and impede osteogenic development through multiple pathways.

IL-6 inhibits MAPK activity by activating signal transducers and transcription activators called STATs, while TNF-α causes SMAD inhibition by activating SMAD ubiquitination regulatory factor-1 (SMURF1) and SMURF 2 (18).

Osteogenic differentiation by TNF-α can also be supported by activating Nf-κB and upregulating the level of OSX, BMP-2, RUNX-2, Wnt-signalling, and osteocalcin (OCN). The proliferation of osteoprogenitors, which results in the synthesis of proteins including fibronectin, histones, and type I collagen, is typically one of the initial steps of osteoblastogenesis. The expression of the genes that produce alkaline phosphatase (ALP) and bone sialoprotein will subsequently result in the formation of the osteogenic matrix, then by activating the genes responsible for the release of osteopontin, collagenase, and OCN, the extracellular matrix undergoes mineralisation. Numerous transcription factors, including RUNX-2 and RANKL surface expression, regulate this intricate cascade of gene expression and cellular development (18).

Demineralized Dentin Material Membrane as Guided Bone Regeneration

The clinical procedure called guided bone regeneration (GBR) produces sufficient bone volume to fill the area of the bone defect. The GBR treatment is based on the

phenomenon that the application of a barrier membrane generates space to facilitate the proliferation of basal angiogenic and osteogenic cells so that the bone defect area is not filled with fibrous tissue before bone maturation is complete (20–22). Thus far, GBR has become the gold standard for oral surgery, which requires the availability of space for bone growth. Barrier membrane properties that must be taken into account are biocompatibility, cell interactions, host incorporation, medical management, and acceptable mechanical and physical characteristics (23). The illustration of bioresorbable barrier membrane in GBR can be shown in Figure 2 (23).

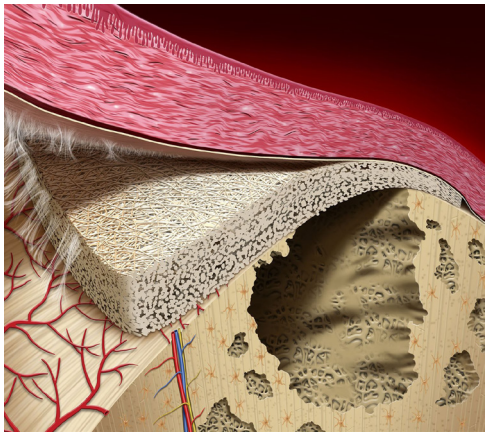

Figure 2: The GBR scheme uses bioresorbable barrier membrane (Elgali et al., 2017)

Demineralized dentin material membrane (DDMM) is a membrane that contains a large number of growth factors for bone growth, namely type I collagen and bone morphogenetic proteins (BMPs), which have excellent biological osteoinductive and osteoconductive effects so that they can help the wound healing process and induce bone regeneration (9). According to a number of pilot studies employing both autologous and xenogenous DDMM to repair craniofacial bone injuries and defects, DDMM can originate from either human or bovine dentin. It is believed that the dentin from bovine can be used to develop membranes and components for bone grafts that function similarly to autogenous bone (21,24,25). Figure 3 below depicts the possible mechanism of DDMM implantation as GBR when applied to craniofacial bone defect.

The role of DDMM as a passive barrier applied to craniofacial bone defects is expected to accelerate the inflammatory process by preventing invasion of soft tissue and non-osteogenic cellular components at the site of the defect before bone maturation is complete. The application of this material stimulates the attachment of tissue under the membrane and then the process of migration, proliferation, and differentiation of various cells including fibroblasts occurs which is also induced by TNF-α along with other growth factors such as BMP-2, ALP, and OSX. These growth factors directly play a role in osteoblastogenesis in osteoblast cell (shown on the right chart in Figure 3) which begins

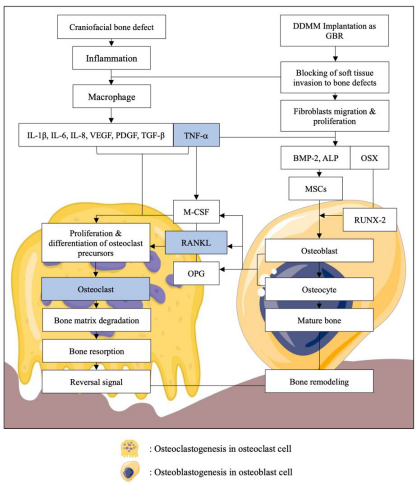

Figure 3: The mechanism of DDMM implantation to craniofacial bone defects

with the differentiation of MSCs into osteoblasts with the help of the RUNX-2 protein until mature bone is formed. The surface of osteocytes and osteoblasts as bone remodelling units will express RANKL, M-CSF, and OPG which have a major role for osteoclastogenesis in osteoclast cell as can be seen in the left chart of Figure 3. Mature osteoclasts then degrade the bone matrix through bone resorption, that will stop when there is a stop signal from the reversal cells. Signals from reversal cells stimulate the remodelling phase, characterized by the deposition of osteoblasts into the bone resorption area continuously until mature bone fills the bone defect area. The remodelling process consists of bone formation by osteoblasts and bone resorption by osteoclasts, which must coincide and balance to maintain bone homeostasis and produce adequate healing of craniofacial bone defects.

CONCLUSION

The primary mechanism of RANKL in osteoclastogenesis involves enhancing the differentiation of osteoclast precursors into bone-resorbing osteoclasts by binding itself to the RANK receptor on the surface of precursor cells. In contrast, TNF-α can influence bone resorption activity through various mechanisms that operate in conjunction with RANKL, delivering signals via the MAPK and Nf-κB pathways. Hence, TNF-α and RANKL perform a significant role in osteoclastogenesis. On the other hand, the use of demineralized dentin material membrane as guided bone regeneration is considered a viable treatment option for reconstructing craniofacial bone defect since the material has demonstrated its potency and possible mechanism in maintaining the equilibrium of bone remodelling.

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