Review Article

A Review Cascade of Molecular Signals in Bone during Orthodontic Mechanotherapy

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ABSTRACT

Aim: The aim of the present is to highlight the cascade of molecular events during bone remodeling induced by orthodontic forces.

Summary: The discovery of the receptor activator of nuclear factor-κB ligand (RANKL)/ receptor activator of nuclear factor (RANK) / osteoprotegerin (OPG) system and its application in the regulation of bone resorption demonstrate how both serendipity and a logic-based approach can identify factors that regulate cell function. Orthodontic tooth movement is mediated by interactions between PDL cells and those of the alveolus. One protein that is the receptor activator nuclear factor kappa B ligand (RANKL)—is critical for osteoclastogenesis, and osteoprotegerin (OPG) is a decoy ligand that competitively inhibits RANKL. A higher RANKL/OPG ratio is associated with areas of bone resorption, while a lower ratio occurs in areas of bone deposition and homeostasis.

Keywords: Molecular events, Bone remodeling, Osteoprotegerin (OPG), Receptor activator nuclear factor kappa B ligand (RANKL)

INTRODUCTION

Bone remodeling is a cyclic and continuous physiological process, which is responsible for bone homeostasis. Bone formation is a complex process in which both osteoblastic and osteoclastic activity takes place. The mismatch between the activities of osteoblasts and osteoclasts has immunopathologic implications associated with the change in bone mass mineral density. The balance of the trimolecular control factor complex composed of osteoprotegerin (OPG), receptor activator of NF-κB ligand (RANKL) and receptor activator of nuclear factor (RANK) maintains physiologic bone remodeling. This trimolecular complex functions as receptors and ligands and belongs to the superfamily of tumor necrosis factor (TNF).[1] Orthodontic tooth movement is induced by mechanical stimuli and facilitated by remodeling of the periodontal ligament (PDL) and alveolar bone. A precondition for these remodeling activities, and ultimately for tooth displacement, is the occurrence of an inflammatory process. Orthodontic tooth movement is achieved by the remodeling of periodontal ligament (PDL) and alveolar bone in response to mechanical loading and is believed to be mediated by several host mediators, such as cytokines.[2] The application of orthodontic forces to correct mandibular and maxillary teeth irregularities through alveolar bone remodeling involves a series of coordinated and regulated molecular and cellular events in the periodontium i.e. periodontal ligament (PL), alveolar bone (AB), cementum, and gingiva. The periodontal ligament and alveolar bone are the two important structures which actively participate in bone remodeling in response to mechanical forces. The major cell types which play an interactive role in the remodeling process are fibroblasts, osteoblasts, osteocytes, osteoclasts, odontoblasts, cementoblasts, chondrocytes and immune cells. Activation of these cells result in the production of several pro-inflammatory cytokines, growth factors, colony- stimulating factors, transcription factors and other regulatory molecules which modulate cell growth, proliferation, migration, differentiation, gene expression and cell function. Recently it has been shown that the role of SOX 9 gene transcriptase, parathyroid hormone related peptide (PTHrP), Indian hedgehog (IHH) protein is significant in understanding the molecular biology of orthodontic tooth movement (Fig-1).[3]

Tooth movement induced by orthodontic force application is characterized by remodeling changes in the dental and periodontal tissues. Two interrelated processes involved in orthodontic tooth movement are deflection, or bending, of

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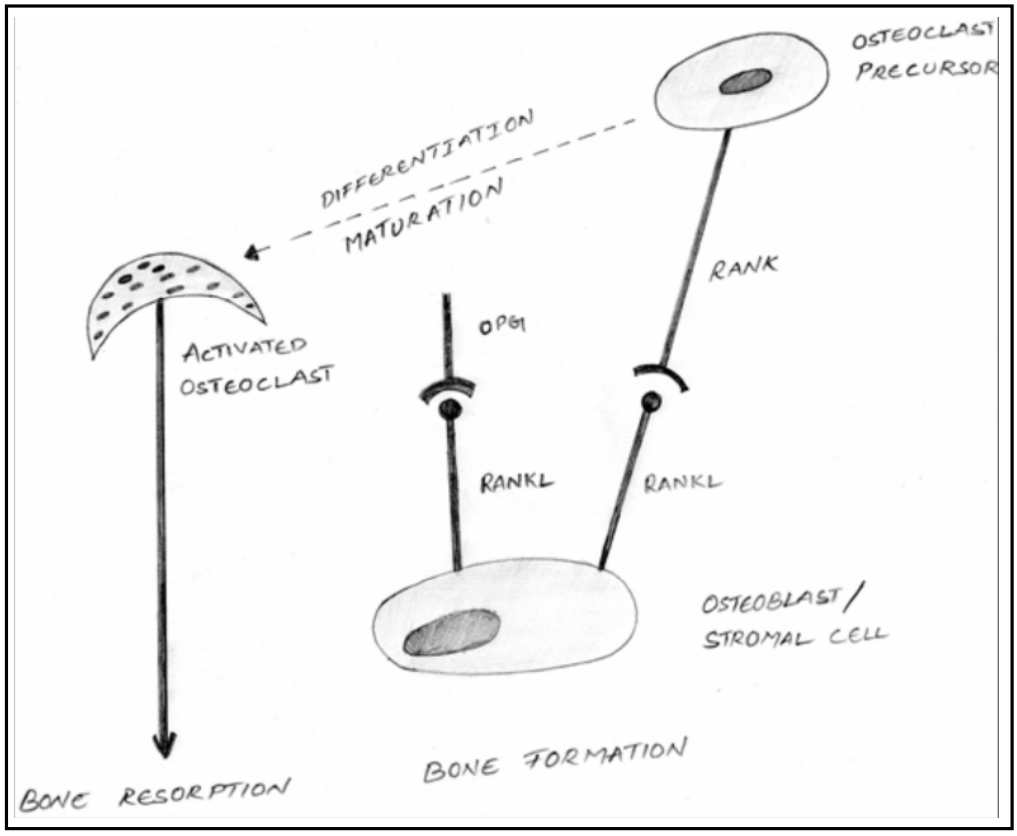

Figure 1: RANKL–RANK/osteoprotegerin molecular complex role in bone remodeling and its immunopathologic implications. (Redrawn from Kohli *et al.* Indian Journal of Endocrinology and Metabolism 2011;15:175-81)

the alveolar bone and remodeling of the periodontal tissues, including the dental pulp, periodontal ligament (PDL), alveolar bone, and gingiva.

Mechanical loading also alters periodontal tissue vascularity and blood flow, resulting in the local synthesis and release of various molecules such as neurotransmitters, cytokines, growth factors, colony-stimulating factors (cytokines that involved in maturing of various leucocyte, macrophage, and monocyte line), and arachidonic acid metabolites. The released molecules evoke cellular responses in the various cell types in and around teeth, providing a favorable microenvironment for tissue deposition or resorption.[4] Various cell-signaling pathways are activated, which ultimately stimulate PDL turnover, as well as localized bone resorption and bone deposition.[5]

Orthodontic tooth movement consists of three phases: the initial phase, the lag phase, and the post-lag phase. The initial phase is characterized by immediate and rapid movement and occurs 24 hours to 48 hours after the first application of force to the tooth. This rate is largely attributed to the displacement of the tooth in the PDL space. The lag phase lasts 20 to 30 days and shows relatively little to no tooth displacement. This phase is marked by PDL hyalinization in the region of compression. No subsequent tooth movement occurs until the cells complete the removal of all of the necrotic tissues. The post lag phase follows the lag phase, during which the rate of movement increases.[4]

Molecular events during Orthodontic Tooth Movement (OTM): The orthodontic tooth movement (OTM) exerts physical, biophysical and biochemical effects on the ECM and constituent cells of the periodontium and dental pulp shows the sequence of cellular and molecular events following the orthodontic tooth movement. The strain on the ECM causes fluid displacement in alveolar bone canaliculi, periodontal ligament vasodilatation, acute inflammation and inflammation-mediated nociceptive pain.[6] The fluid displacement leads to the physiological activation of osteocytes, osteoblasts, bone lining osteoblasts/osteoprogenitor cells as well as periodontal ligament fibroblasts.[7] The nociceptic pain causes the PL neurons to secrete neuropeptides such as Substance P, calcitonin gene related peptide (CGRP).[8-11] These peptides along with prostaglandin E-2 (PGE-2) and humoral factors cause the dilatation of periodontal ligament capillaries. This leads to the release of immune competent cells from the capillaries.[12] Migration of these cells is mediated by the chemotactic factors and vascular endothelial growth factor (VEGF) secreted by endothelial

cells and osteoblasts. VEGF is an essential mediator for bone angiogenesis and in bone development.[13] The ECM remodeling is followed by cytoskeletal re-organization in osteoblastic cells.[14] Cytoskeletal re-organization leads to phosphorylation of cellular proteins including extracellular signal-regulated kinases (ERK).[15] This triggers signal transduction via integrins/ fibronectin/kinase-pathway.[16-19] Intercellular communication occurs through gap junction proteins (connexions). Several matrix metalloproteinase (MMP) particularly 9, 3, 13, 1, 8 are increased on the pressure side and an active collagen remodeling occurs. Prostaglandin-2 (PGE 2) and COX 2 mRNA are also up regulated on the compression side.[20,21] M-CSF, a secretory product of osteoblasts regulates the differentiation of osteoclast precursors to mature osteoclasts.[22] Recent work from University of Hongkong on the cell biology of tooth movement and the role of transcription factor Sox-9 and parathyroid hormone related protein (PTHrp) and Indian Hedgehog protein are very significant in understanding the molecular biology of orthodontic tooth movement force transduction.[23] Recent evidence shows that SOX 9 directly regulates the Type II collagen gene.[22] It is a chondrogenic transcription factor and a target of signaling by the PTHrP in the growth plate of endochondral bone. SOX-9 also prevents the conversion of proliferating chondrocytes into hypertrophic chondrocytes.[24]

Indian Hedgehog (IHH) is protein involved in chondrocyte differentiation, proliferation and maturation especially during endochondral ossification. It regulates its effects by feedback control of PTHrP. PTHrP regulates extracellular matrix gene expression in cementoblasts and inhibits cementoblast-mediated mineralization in vitro,[25] all mechanisms of interest and importance in orthopaedic growth modification.

Application of Cytokines, Growth Factors and Transcription Factors: A number of messenger molecules are produced due to stretch and strain caused by orthodontic forces which induces the periodontal ligament fibroblast, osteocytes, osteoblast and osteoclast for production (Fig. 2). Periodontal ligament and PL immune cells produce pro-inflammatory cytokines (IL-1 beta, Il-6, IL-8, Il-12, IL-13 TNF alpha) and anti-inflammatory cytokine IL-10.[26-28] These molecules modulate cell growth, proliferation, cell migration, differentiation, gene expression and cell specific functions.[28,29] IL-1 β is considered an important cytokine in tooth movement due to its pleotropic effects. Tumour Necrosis Factor alpha (TNF α), is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation and is responsible for a diverse range of signaling events within cells including bone resorption by osteoclasts. RANKL is a member of the tumor necrosis factor (TNF) cytokine family which is a ligand for osteoprotegerin (OPG) and functions as a key factor for osteoclast differentiation and activation. The orthodontic tooth movement activates osteoblasts which in response, produce a number of key molecules including bone morphogenetic proteins (BMPs), macrophage colony stimulating factor (M-CSF), receptor activator of nuclear factor kappa-B ligand (RANKL), osteoprotegerin (OPG), transcription factors (osterix, Run X-2), heat shock protein (HSP), fibroblast growth factor (FGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF), transforming growth factor beta (TGF beta), insulin growth factor (IGF) and BMP-2 and BMP-7 are involved in osteoblast differentiation. Each molecule has a specific role to play in the complex signaling network. Osteoclast differentiation is induced by M-CSF stimulated by PTH. Osteoblast differentiation is also induced by hedgehogs and core binding transcription factor alpha-1(cbfa1). BMP-2 induces dental follicle cells to differentiate toward a cementoblast / osteoblast phenotype and regulates osterix through msx-2 and nunx-2,[30] during osteoblast differentiation. Ischemia and hypoxia occur on the pressure side as a result of reduced blood supply while Osteocytes produce sclerostin, phosphate regulating endopeptidase homolog, X-linked (PHEX), dentin, matrix phosphoprotein-1(DMP-1), c-fos, TGF beta, matrix extracellular phosphor-glycoprotein (MEPE), hypoxia induced factor (HIF-1), NO, PGE-2, IGF, c-fos. HIF-1 and c-fos are associated with hypoxia and angiogenesis,[31] and HIF-1also inhibits Wnt signaling which is responsible for osteoblast differentiation and thus inhibit the osteoblast differentiation. MEPE produced by osteoblasts are involved in integrin recognition[32] which play vital role in cell signaling mechanism. Integrins, are also produced by epithelial cells which also produces cytokines, vascular endothelial growth factor (VEGF).[33] PHEX and DMP 1 regulate fibroblast growth factor (FGF-23). Prostaglandin E-2 is produced by platelets, endothelium, and mast cells and also is liberated as breakdown products of membrane phospholipids during orthodontic tooth movement and is involved in inflammation, vasodilatation and pain. It stimulates osteoblasts that releases factor that stimulate bone resorption by osteoclasts. MMPs are secreted by fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes. They are responsible for the tissue remodeling and degradation of extracellular matrix substances including collagens, elastins, gelatin, matrix glycoproteins and proteoglycans. They are regulated by hormones, growth factors, and cytokines. Osteoclasts produce chemokines (CCR2, CCR5), and epidermal growth factor (EGFR).[3]

All cellular activities in the periodontium are regulated by multiple molecules and mechanisms. The key molecules are: cytokines, BMP, TIMPS, TGF beta, NO, sclerostin, noggin, PTH, integrins and DNA binding regulatory proteins.[3] The major signaling systems include Erk1/2, NFkβ, NO, RANK/ RANKL/OPG, P2X7 Wnt and Notch. The basic functions

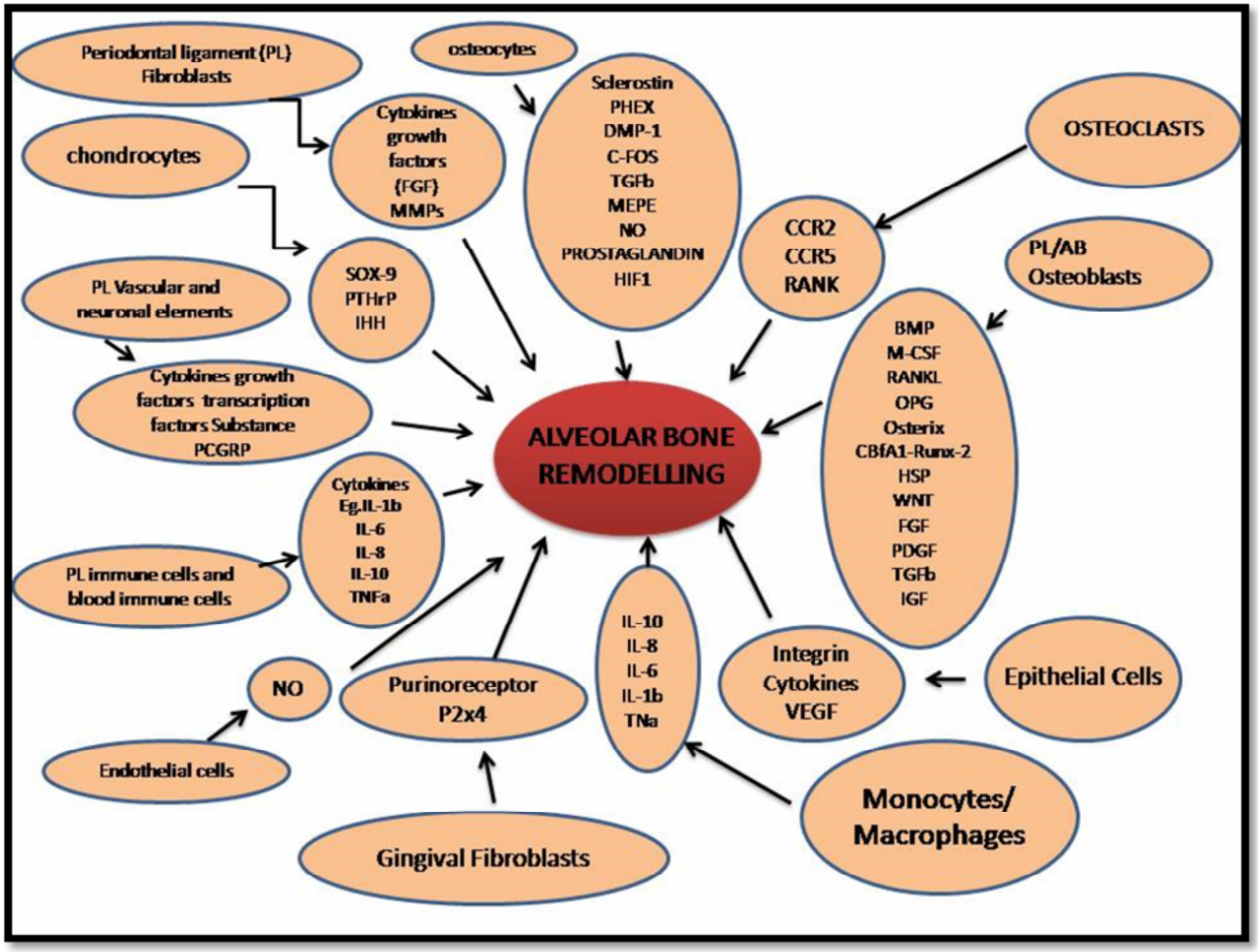

Figure 2: Molecular Biology of Orthodontic Tooth Movement (Nayak*et al.* J Dent Oral Health 2013; 1: 101)

of these molecules and pathways are to activate and regulate cell growth, proliferation, migration, differentiation, gene expression and cell functions and remodel ECM, PL, and alveolar bone.[3]

Application of growth factors:[3] Bone morphogenetic proteins are a group of growth factors with cytokine properties.[33] BMP 2 and BMP 7 are produced by osteoblasts and are involved in osteoblast differentiation.BMP 2 also plays role in cementoblast differentiation.[34] Periodontal ligament show an elevation in osteopontin (OPN) on the tension side of the PL.[35]Osteopontin(OPN) is a multifunctional protein,[36] biosynthesized by fibroblasts, osteoblasts, osteocytes, odontoblasts, bone marrow cells, and hypertrophic chondrocytes and contains an Arg-Gly-Asp (RGD) motif that is known to promote osteoclast attachment through integrins and CD4.[37-39]

Application of bone cells:[3] Osteoblasts are one of the active groups of cells in orthodontic tooth movement. They produce bone morphogenetic proteins (BMPs), macrophage-colony stimulating factors (M-CSF), receptor activator of nuclear factor kappa-B ligand (RANKL) RANKL, OPG, HSP, FGF, PDGF, TGF beta, IGF, IL-1 beta, IL-6, NFkB and transcription factors SOX 9, osterix, Cbfa1/ runx-2, Wnt. Runx-2 expression leads to enhanced production of OPN, Bone sialoprotein (BSP), Collagen 1, alkaline phosphatase (ALP). Osteocytes are mechanosensory cells. Osteoblasts and PL fibroblasts are mechano-responsive cells. These cells and their precursors play important role in PL and alveolar bone remodeling. They are multi-processed cells with relatively thin cytoplasm, connected to each other between lacunae and alveolar bone canaliculi and also in contact with bone lining osteoblasts and stem cells. They are the chief mechanosensory cells in the peridontium in response to orthodontic tooth movement.[3]

Osteocyte produces sclerostin, PHEX, DMP-1, c-fos, TGF beta, MEPE, NO, prostaglandins, HIF 1, IGF-52. PL fibroblasts produce a number of pro-inflammatory (IL-1 beta, IL-6, IL-8, TNF alpha) and anti-inflammatory (IL-10) and ECM proteins including Col 1. Osteoclasts

produce RANK, CCR2, CCR5. Osteoclasts differentiation is inhibited by IL-12, IL-18, IL-33, IFN. Osteoclasts are activated by TNF alpha, IL-1 and IL-17. Osteoclasts differentiation is regulated by PTH, calcitonin, IL-6, OPG and RANKL.[38-39]

CONCLUSION

Each external factor as orthodontic forces/orthopedic forces procedure is a cascade of molecular events witnessed by bone, periodontal membrane and tooth. The series includes bone remodeling factors as RANKL/RANK, osteoprotegerin, SOX9 gene transcriptase, parathyroid hormone related peptide, 1HH protein. Their actions are cell signaling pathways which work through basic inflammatory charges around the tooth and the related bone. Prime concern are the osteoclasts which are activated by TNF alpha, IL-1, IL-17 and regulated by PTH, calcitonin, IL-6, OPG and RANKL.

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