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Research Progress of Combined Application of All-trans Retinoic Acid and Bone Morphogenetic Protein in Bone Metabolism

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Abstract: All-trans retinoic acid (ATRA) can both promote and inhibit osteogenic differentiation of cells, which is closely related to the concentration, dose and cell type. Low dose of ATRA inhibited the function of osteoclasts and promoted the differentiation of osteoblasts, thus achieving bone remodeling: Bone formation is achieved through the coordination of osteoblasts and osteoclasts. ATRA can be combined with various factors to produce different effects, among which, when combined with bone morphogenetic protein (BMP), the bone formation effect is significant. Compared with homologous dimer, BMP heterodimer can repair the bone defect area more effectively with a smaller dose, and promote the formation of bone tissue. Reduce the probability of secondary lesions such as swelling in the operative area and excessive osteogenesis, and greatly reduce the economic burden of patients. In addition, in vitro experiments showed that ATRA and BMP2/7 combined with mouse embryonic osteoblast precursor cells (MC3T3-E1) had synergistic effect on osteogenesis. This article reviews the recent progress in the studies on the combined effects of ATRA and BMP in bone metabolism.

Keywords: Bone Morphogenetic Protein; All-trans Retinoic Acid; Ectopic Osteogenesis; Bone Defect.

1. Introduction

Bone defects can be caused by a variety of factors, such as trauma, tumor, osteoporosis caused by physiological or pathological factors, and bone loss caused by congenital dysplasia [1, 2]. Autologous bone transplantation is the gold standard for the treatment of bone defects, but the quality and bone mass of autologous bone transplantation will be seriously affected by the patient's age, physical health status and the site of bone extraction. In addition, secondary lesions in the donor area are often serious complications after bone transplantation. bone remodeling is a long and complex process, which is accomplished through the interaction of osteoblasts and osteoclasts [3]. Studies have found that [4] osteoblasts can significantly improve the expression of osteoblast-related genes, synthesize and mineralize extracellular matrix, and promote the formation of bone. Osteoclasts release calcitonin to promote bone absorption, so the dynamic balance of the two functions is the key to bone formation. With the increasing application of molecular therapies in clinical practice, it is possible to find a growth factor that can effectively promote bone repair and reduce the probability of postoperative side effects. This review focuses on the combined effects of ATRA and BMP in bone metabolism.

1.1. Background of ATRA Research

Retinoic acid was first discovered in 1913 [5]. Retinoic acid and all-trans retinoic acid are the most common forms of retinoic acid, which activate and inhibit gene expression mainly through genomic and non-genomic mechanisms; ATRA is A common acidic metabolite and derivative of vitamin A [6]. It plays an important role in the regulation of various cellular functions in the body. It is involved in the physiologic remodeling of bone and is one of the influencing factors of bone metabolism. Human epidemiological studies have found that [7] excessive intake of vitamin A can cause hyperproteinemia, and is associated with increased serum retinol level, decreased bone mass and increased risk of fracture; High doses of ATRA inhibit the differentiation of osteoblasts, promote the differentiation of bone marrow mesenchymal stem cells (BMSC) into adipocytes, activate osteoclast activity, promote bone resorption, inhibit new bone formation, and lead to osteoporosis and failure of bone defect repair [3]. It was found that ATRA significantly promoted the expression of alkaline phosphatase (ALP) and Runx2 genes at the early stage of osteogenesis. However, it inhibited the expression of osteocalcin (OCN), type I collagen (Col1) and other late-stage osteogenic genes [8]. ATRA can promote and inhibit bone repair for preparation, its effect on bone metabolism mainly depends on the dose, concentration and cell type of application. The effects of ATRA vary depending on the type of cell.

1.2. Effect and Mechanism of ATRA on Bone Formation

1.2.1. The Effect of ATRA on Bone Formation

ATRA is involved in the regulation of physiological activities of various cells in the body, for example [9] in the differentiation treatment of acute promyelocytic leukemia greatly improves the prognosis of the disease, and is widely used in the treatment of tumors in combination with various factors. Q. L et al. [10] found that ATRA induced myogenic differentiation of primary muscle cells in sheep and promoted the formation of muscle ducts. Other studies have shown that ATRA inhibits the expression of lipopolysaccharide (LPS) - induced tumor necrosis factor, interleukin, and nuclear factor-κB-ligand receptor activator (RANKL), and promotes osteogenic differentiation of BMSC on titanium [11]. Bone is the target of ATRA, and maintaining bone homeostasis is the

key to bone remodeling, which is a process of new bone formation and old bone resorption. Bone remodeling is achieved through the interaction of osteoblasts and osteoclasts to achieve a certain balance. Different concentrations of ATRA sometimes have opposite effects on cells in the body, Alkaline phosphatase (ALP) is one of the typical markers used to evaluate the activity and status of osteoblasts, and is a standard indicator for evaluating bone mineralization ability. In vivo and in vitro studies [12-14, 34], low concentrations of ATRA promoted the differentiation of osteoblasts, significantly inhibited the expression of osteoclast-related genes, decreased their reabsorption activities, and increased the expression of osteoblast-related genes such as osteocalcin (OCN), alkaline phosphatase (ALP), and vascular endothelial factor (VEGF). The area of cell bone nodules increased by 4 times after 10mM ATRA compared with the control group [15]. It can also transform the lipogenic effect of rosiglitazone into the osteogenic effect of BMSC and promote the formation of bone tissue [16]. The dosage and concentration of ATRA have obvious effects on tissues and cells. When used in bone tissue, ATRA at low concentration can effectively inhibit cell differentiation into osteoclasts and promote bone remodeling.

1.2.2. Mechanism of ATRA Promoting Bone Tissue Formation

ATRA acts mainly in combination with retinoic acid receptors (RAR) and retinoic acid "X" receptors (RXRs), both of which have α, β and γ subtypes and have different effects on different types of cell and tissue environments [17]. In vivo, RARβ can inhibit the promoting effect of colony stimulating factor (G-CSF) on osteoclast generation through negative regulatory effect [18]. ATRA has been shown to mobilize traditional cytoplasmic signaling molecules into the nucleus, where they drive differentiation. mRNA expression of relevant receptors was detected in all available stem cells by PCR, and Western blot showed that ATRA significantly up-regulated Runx-2 protein levels and promoted new bone formation [19]. It was also reported that ATRA increased the proliferation of human osteoclast progenitors, but inhibited the differentiation of mononuclear osteoclast progenitors into mature osteoclasts by inhibiting RANK, thereby preventing bone resorption and destruction [20]. ChenM et al. [21] treated mouse embryonic palatal mesenchymal stem cells with ATRA significantly inhibited their osteogenic ability and promoted their transformation into adipocytes. By binding with different receptors and tissue cells, it obviously enhances the remodeling of bone tissue and plays an increasingly important role in promoting the formation of bone tissue.

1.3. Effect and Mechanism of ATRA on Bone Formation

1.3.1. ATRA Inhibits Bone Formation

Bone tissue at developmental stages is less resistant to ATRA inhibition of bone formation. Studies have shown that childhood tumors treated with retinoids can lead to thinning or even premature closure of growth plates, affecting the development of long bones [22]. ATRA not only affects the changes of hormone levels, but also regulates the functions of various cells. ATRA can be used as a cell growth factor receptor activator for osteoclasts during bone tissue culture in vitro, promoting periosteal osteoclast generation and accelerating bone tissue absorption and destruction [7]. GreenAC et al. [18] found that the number of osteoclasts treated with RAR receptor antagonists did not change significantly, but the surface area increased, and sometimes even huge osteoclasts appeared. The effect of ATRA on osteoclasts was negatively correlated with its concentration and dose. Excess ATRA intake was associated with decreased bone density and an increased risk of fracture [23]. High dosage of ATRA inhibited the differentiation of osteoblasts, reduced the number of bone nodules and the degree of extracellular matrix mineralization, and thus inhibited the formation of bone tissue. Osteoclasts regulate bone mass through bone resorption and work with osteoblasts to maintain bone integrity.

1.3.2. Mechanism of ATRA Inhibiting Bone Tissue Formation

ATRA receptors are also present in osteoclasts and affect the transformation of chondrocytes and osteoblasts to varying degrees. it has been reported that [25] activated T cytokine C1 is one of the transcription factors of osteoclasts and can be activated after Rankl-activated RANK, and excess ATRA can increase the expression of receptor activator of nuclear factor RANKL, thus promoting osteoclast formation. Excess ATRA also inhibited the Ihh/PTHrP signaling pathway in chondrocytes, decreased the growth potential of growth plates, and inhibited chondrocyte proliferation and differentiation [22]. QinShen et al. [22] found that high dose of ATRA in 3-week-old rats for 10 days could lead to hyperproteinemia and overexpression of GH-IGF1-IGFBP3 gene. Increased the activity of osteoclasts and the amount of tartrate-resistant acid phosphatase 5b (TRAP5b), which was the marker of osteoclast activity in serum, and aggravated the injury of bone tissue.

1.4. The Role of BMP in Promoting Bone Tissue Formation

BMP was first discovered by Professor Urist in 1965 [26]. It is a group of dimer proteins belonging to the TGF-β superfamily and is one of the main cytokines in the field of bone regeneration research [27]. BMP2 homologous dimer and BMP7 homologous dimer are the most common, but BMP homologous dimer may cause side effects such as soft tissue swelling around the operative area and accidental osteogenesis due to its high dose in clinical effective application. Many studies have shown that [28-30] compared with BMP homologous dimer, low dose of BMP2/7 can promote the expression of osteogenic genes such as ALP, significantly induce bone tissue remodeling, reduce the applied dose of BMP in clinical treatment and the occurrence probability of related adverse reactions, and greatly reduce the economic burden of patients.

1.5. Combination of ATRA and BMP and its Possible Mechanism

ATRA can be used in combination with various factors in all clinical directions, and the combined application with BMP has remarkable effect on bone remodeling. When treated with 1uM ATRA alone, MC3T3-E1 could significantly inhibit the expression of osteogenic factors.BMP could antagonize the inhibition effect. The inhibitory effect of ATRA was completely inhibited by the concentration of BMP2/7 at 50ng/ml [9]. The combination of ATRA and BMP can sometimes produce opposite effects on different cells. 300ng/ml ATRA and BMP can significantly inhibit the proliferation of osteoblast progenitor cells (MC3T3-E1) and synergistic promote osteogenesis in the early stage when

combined with BMSC. BMPs signaling pathways include both Smad-dependent signal transduction pathways (SMad1/ 5/ 8) and non-Smad-dependent signal transduction pathways (ERK, p38, JNK, etc.). Smad1 is the main effector of BMP and is considered a central component of integrated communication between BMP and other pathways [31]. BMP binds to transmembrane Serine/threonine kinase receptors on the cell surface to trigger specific intracellular signaling pathways that activate and affect cellular transcription [3]. BMP is divided into RI receptors and RII receptors. The former can be divided into BMP-RIA receptors and BMP-RIB receptors after phosphorylation. The BMP-RIA receptor can promote lipogenesis, and the BMP-RIB receptor can regulate osteogenic differentiation. ATRA was found to inhibit the expression of BMP2/7-induced osteoclast precursor RANK, which is beneficial for bone formation [24]. However, retinoic acid did not interfere with the kinase activity of BMP receptors and ligands, and promoted Smad1 ubiquitination and proteomase degradation by enhancing the interaction between Smad1 and E3 ubiquitin ligase.BMP signaling activity was inhibited [32]. Other studies showed that [33] BMP genes (especially BMP2, BMP4 and BMP9) were also heavily expressed in mesenchymal cells (SMC). ATRA significantly up-regulated the expression of these genes by transcriptional regulation of BMP/Smad signaling pathway, thus enhancing the osteogenic differentiation ability of SMC. Combined application of the two can be used to treat large segmental bone defects, nonunion fractures and osteoporosis.

1.6. Outlook

The effect of ATRA on bone tissue repair and remodeling is closely related to the dose and concentration of ATRA and the cell type. Low concentration of ATRA can promote the transformation of BMSC into osteoblasts and increase the expression of osteogenic genes (such as ALP, Runx2, etc.), which is conducive to the formation of bone tissue. High concentration of ATRA can induce osteoclast activity and promote bone resorption. The combination of ATRA and BMP2/7 can effectively promote bone remodeling and reduce the dose and the risk of side effects. How to balance the effects of ATRA and BMP2/7 on osteoblasts and osteoclasts in vivo so that they can coordinate with each other to achieve the best osteogenic effect, as well as the synergistic or antagonistic effects of different factors on the formation of bone tissue and their mechanisms still need to be further explored. The optimal concentration ratio and dosage of the two combined effects on the bone defect area are the focus of future research.

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