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A Mini-Review of the Expression andMechanism of Osteoadherin/Osteomodulin in Biomineralization

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Abstract

Among the small leucine rich proteoglycans (SLRPs), osteoadherin (OSAD), also called osteomodulin (OMD), is one of the proteoglycans that only distribute in mineralized tissues. In recent years, the research on OSAD/OMD has focused on its expression characteristics in osteogenic differentiation, including its distribution in tissues, its expression in osteo/odontogenic differentiation and its possible mechanism involved. In this paper, we searched PubMed database for English scientific papers from 2000 to 2020 with the keywords of osteoadhesin, osteomodulin, osteogenic differentiation, SLRPs and BMP2. It is concluded that OSAD/OMD plays a positive role in the growth and differentiation of osteoblasts, and its mechanism involves a variety of signal pathways. It is believed that the study of the role of OMD in biomineralization can shed light on regulating osteogenesis in bone tissue engineering.

Keywords: Osteoadhesin; Osteomodulin; Osteoblastic differentiation; Odontoblastic differentiation; SLRPs

Introduction

Some oral diseases can cause local bone defects, such as periapical lesions, periodontitis, tumors and so on. In regenerative medicine, bone tissue engineering is one of the treatment methods which has been developed rapidly in recent years [1-3]. Among them, growth factors are considered to play a key role in promoting bone regeneration, and its activity can be precisely controlled by extracellular matrix (ECM) [4]. Proteoglycans in ECM of bone cellsplay a part in regulating matrix assembling and bone tissue formation by combining with various growth factors [5], and among which small leucine rich proteoglycans (SLRPs)is an important group of proteoglycans.

All SLRPs are proteins consisting of leucine-rich repeats (LRRs) and flanked by cysteine rich groups and at least with one glycosaminoglycan side chain [6]. According to sequence similarity, SLRPs family can be divided into five subfamilies. Among them, decorin and biglycan belong to first subfamily as having chondroitin/dermatan sulfate chains in their N-terminal domains. Fibromodulin, PRELP (proline arginine rich end leucine rich repeat protein) and lumican which contain sulfated tyrosine residues in the N-terminal domains are in the second subfamily category. Chondroadherin (Chad) belongs to the third subfamily, and its C-terminal has different types of disulfide ring structures [7]. Recent studies have found that SLRPs family members have some unique biological functions as working interaction with many cell surface receptors, cytokines, biogenic factors and other ECM components. SLRP scan regulate different signal pathways by affecting the phosphorylation of cell signal pathways, so as to play a role in cell proliferation, survival, adhesion, migration, ECM synthesis, biological factor activity regulation and so on. In addition, SLRPs also integrate with collagen for regulating its synthesis and degradation [6,8]. So far biglycan (class Ⅰ), decorin (class Ⅰ), lumican (class Ⅱ) proteins in SLRPs family have been widely studied. For example, mice with biglycan gene deficiency have abnormal postnatal development, and have osteoporosis in the early stage [9]. Other studies have shown that biglycan and decorin are also matrix components of dentin, which play an active part in the formation of dentin [10].

Osteoadherin (OSAD) was proved belonging to the second class of SLRPs family with 11 leucine-rich repeats which is the general pattern of SLRPs. It was isolated from the mineralized matrix of bovine long bone for the first time in 1998 which was expressed by mature bovine osteoblasts [11]. By comparing primary sequences to other members in SLRPs, OSAD is most similar to proteoglycans such as fibromodulin, lumican and PRELP with up to a 42% similarity of identical residues. Additionally the cysteines in the N-terminal region are separated by 3,1, and 9 residues which is the same alignment pattern of the other proteins. Thus, they all belong in the same category as class Ⅱ subfamily [12]. But still a few structural difference has been determined by testing primary sequences of OSAD. One characteristic feature is the size of its core protein (47 kD), which is larger than other proteoglycans in the family [13]. Another distinguishing structure is the relatively large and very acidic C-terminal peptide extension forming by 69 amino acids after the last cysteine [14]. This very highly negatively charged C- terminal region might tributes to its tight binding to hydroxyapatite through anchoring the protein to the mineral [11].

When OSAD was first discovered, it was named osteoadhesin for its high affinity for hydroxyapatite and its ability to bind to cells. Similarity searches of the EMBL data base showed protein sequences of rat OSAD (AF104362), human osteomodulin (OMD) (AB000114), and mouse OMD (AB007848) are with high similarities to bovine OSAD (U67279). The similarity between bovine OSAD and human OMD are 82% and 94%, 76% and 93% similarity between bovine OSAD and

mouse OMD, 79% and 92% similarity between human OMD and rat OSAD. The sequence of the typical leucine-rich repeats (LRRs) and the two specific cysteines of the bovine OSAD are highly conserved in rat OSAD and in human and mouse OMD. Consequently we consider OSAD and OMD as the same protein in different species [15]. In recent years, many studies have suggested that OMD is related to hard tissue mineralization [16,17]. Therefore, the study of the role of OMD in the process of biomineralization can provide us the inspiration for clinical application of growth factors in bone tissue engineering to achieve ideal osteogenic effect at physiological dose.

Materials and Methods

The English scientific papers from 2000 to 2020 were retrieved from PubMed search engine by using the following keywords: osteomodulin; osteoadhesin; osteoblastic differentiation; odontoblastic differentiation; SLRPs. The scientific papers of the search results as well as some references of these selected articles were then assessed for their relevance to the intended objectives.

109 articles were reviewed as well as some references of selected articles. There comes to a clear point of view about the expression of OSAD/OMD in biomineralization of hard tissue.

Discussion

Distribution and expression of OMD/OSAD

SLRPs are usually distributed in bone, teeth, skin, soft tissues, endometrium, tumor and other tissues, while OSAD/OMD is a member of SLRPs family only found in mineralized tissues, which is considered as a specific protein in mineralized tissue. When OSAD was first discovered, the researchers used Northern blot analysis to find that it was highly expressed only in bone cells. Besides, it was weakly expressed in trabeculae bone, and none in other tissues, such as articular cartilage, skin, kidney, liver, spleen and thymus. This expression seems toconfined to mature osteoblasts, because the OSAD mRNA shows the highest expression in osteoblasts of trabecular bone at a certain distance from the growth plate [18]. In the later experimental study about OSAD/OMD, scholars got the same discovery of its distribution pattern [19]. Ribonuclease protection assay (RPA) showed that OSAD mRNA was expressed in femur and skull, but not in cartilage, tendon or liver. The specific antiserum was prepared and used for immune histochemistry method to study the protein distribution during the development of femoral head. It was found that OSAD mainly existed in trabecular bone and no staining was found in cartilage. In situ hybridization showed that the strongest expression of OSAD was found in osteoblasts near the cartilage-bone interface around the growth plate. These results suggest that OSAD/OMD may hasa strong relevance to biomineralization of hard tissues.

OSAD/OMD maintains high affinity to hydroxyapatite in calcified tissue through acidic amino acid residues in its C-terminal structure [20]. The affinity has been demonstrated by chromatographic purification with hydroxyapatite columns [21]. More interesting finding about the distribution of OSAD/OMD during the development of hard tissue, was that a certain gradient of their expression was shown. The result of the experiment of Nikdin's et al. [22] show that with the maturation of odontoblast, the gradient of OSAD mRNA gradually built up, and the highest expression was found in secretory odontoblast which synthesizes predentin. Ultrastructural analysis (IEM) showed that during tooth mineralization, OSAD gathered in the front line of mineralization. The same experimental results appears in other scholars' experiments. The expression of OSAD during the development of three mineralized tissues (dentin, enamel and alveolar bone) was studied and found that there was a similar distribution gradient of OSAD in odontoblast layer and ameloblast layer. Positive signals were first detected in ameloblasts and odontoblasts and then spread occlusally in these two cell layers [15]. Also in a study of osteoadherin in murine tooth extracellular matrices, there came out a result as gold particles associated with collagen fibres in predentin appeared at the front of dentin mineralisation and also being detected near the secretory poles of ameloblasts in enamel matrix [23].

The high expression of OSAD/OMD in hard tissue may be related to its cell binding ability which is a classic function as a proteoglyca. In vitro studies have shown that OSAD works as effective as fibronectin in enhancing the attachment of osteoblasts [11]. This ability to increase cell adhesion may be related to integrin. This integrin has been found to bind a large number of extracellular matrix molecules including bone sialoprotein (BSP) [24] and osteopontin [25]. OSAD/OMD with ligands for integrin may act as an initial cell attachment factor for osteoblasts as well as an inducer of integrin-mediated signals. In another study, odontoblasts undergoing differentiation in vitro were stained with integrin antibodies and the staining area went from intercellular space to the whole cell membrane. This suggests that integrin may participate in the adhesion between odontoblasts and the binding of odontoblast to the surrounding predentin / dentin/dental pulp matrix through OSAD [26].

The influence of OMD expression on the biomineralization

It has been proved by certain studies that the expression of OSAD mRNA has been detected in mature osteoblasts [11,18,27]. Furthermore, the expression has been up-regulated in several mineralizing-induced cultures. In the induction of mineralizing rat dental pulp cultures, gene expression of a few members of SLRPs family was assessed after 3,7,14 and 21 days. By day 14 and day 21, a significant increase in all genes such as biglycan, decorin, fibromodulin (FMD), OSAD was observed up to 9-fold, specifically for OSAD and FMD. OSAD and FMD presented a similar increase and expression profile as decorin which was considered highly involved in biomineralization [22]. Another recent study was about osteo/odontogenic differentiation of hDPSCs which was cultured in human mesenchymal stem cell osteogenic differentiation medium (OM) for 3 weeks. The results showed OMD gene expression in hDPSCs which were incubated in OM up-regulated 35-fold which was analysed by real-time qPCR and RT-PCR. Some scholars [28,29] have studied the expression of osteogenic markers in the process of odontoblast differentiation of dental pulp cells (DPCs), and found that the transcription levels of DSPP and DMP1 continued to increase, and reached the peak 14 days after induction. However, we cannot conclude that OSAD has a direct effect on mineralization at the moment, because there is no obvious increase of OSAD markers when OSAD is over-expressed in the medium without osteogenic inducer [27].

Many studies have suggested that OSAD/OMD may be related to biomineralization, and osteoblasts and odontoblasts are the most likely targets of OSAD/OMD. To test the hypothesis that OSAD/OMD may be positively correlated with the osteo/odontogenic differentiation, many experiments have been done. Rehn et al. [27] studied the effects of OSAD on the differentiation behavior and maturation of MC3T3E1

osteoblasts. In this experiment, models of OSAD over-expressing and OSAD repressing by knocking down related genes with small-hairpin RNA (shRNA) were established. The results showed that in OSAD over-expression cells, the expression of differentiation characteristic indexes were significantly rised, such as alkaline phosphatase (ALP), osteocalcin (OC) and ossein (OGN), along with aincrease of in vitro mineralization. On the contrary, inhibition of OSAD expression resulted in inhibition of OGN expression, but not BSP and OC. It is concluded that OSAD can promote the maturation of osteoblasts and stimulate differentiation in vitro, thus increasing the number of downstream osteogenic markers. In another scholar's experiment [30], the models of over expression and knockdown of OMD gene in MC3T3-E1 osteoblasts were also established to the change of OMD expression level during osteoblast differentiation. In the results, although the expression levels of ALP and osteocalcin preprotein (BGLAP) mRNA were not significantly changed by OMD overexpression, the expression of CCN family 2 (CCN2) was significantly decreased. CCn2 is an ECM protein with rich cysteine working as a connective tissue growth factor. which regulates cell function as a synthetic metabolic growth factor [31,32]. The expression of CCN2 mRNA was approved to induce apoptosis of human aortic smooth muscle cells [33], breast cancer cells [34] and mouse bone cells [35], by down regulating anti-apoptotic genes. This mechanism might explain the over-expression of OMD increased the viability of MC3T3-E1 cells and decreased the activity of caspase 3/7, leading to the conclusion that OSAD plays a role in the apoptosis and growth of osteoblasts. In Lin's study OMD gene was knock down with short hairpin RNA (shRNA) [36]. Compared with the uninduced cells in control group, the expression of alkaline phosphatase (ALP), dentin matrix acidic phosphoprotein 1 (DMP1) and dentin sialophosphorprotein (DSPP) in odontoblasts were decreased by shRNA mediated OMD gene silencing, and the formation of mineralized nodules was confirmed by Alizarin Red S staining. Therefore, it is suggested that OMD may regulate the differentiation of osteo/odontoblasts.

Collagen is an important component of extracellular matrix and the thickness and shape of collagen fibers is crucial for tissue organization and maintenance. For example, in bone tissue fiber diameter is considered to be a key factor in bone mechanical strength. It has been found that OSAD/OMD is closely related to collagen fibers as it not only reduced the diameter of collagen fibers in bone tissue, but also inhibited the extensive intersection between collagen fibers [37,38]. Besides, there was no distorted collagen fibers formed in the presence of OMD under observation of scanning electron microscope, which resulting in changes in fiber morphology. This ability to regulate collagen fibers is mainly attributed to the negative charged residues of OMD. This weak electrostatic interaction makes the OMD combine and dissociate rapidly with type Ⅰ collagen fibers, and controls the growth of collagen fibers. These results suggest that OMD may adjust the mechanical strength of hard tissues in mineralized tissues.

Signaling pathways involved in the regulation of osteogenic differentiation by OSAD/OMD

SLRPs are located in the upstream of many signal cascades, which can affect intracellular phosphorylation events and interact with various growth factors and cell surface receptors so as to regulate downstream signaling pathways. By far a few studies have explored some possible signal cascades mechanisms involved in the regulation of osteogenic differentiation by OMD, and some have been proved to be involved in the regulation of osteogenic differentiation and organization. Among them, the most studied theory is the possible relationship between OMD and BMP/TGF-β signaling pathway.

There are many factors involved in the regulation mechanism of osteoblast differentiation and mineralization. One of the main factor family is transforming growth factor-β family (TGF-βs). This family is composed of a large group of members, but among them the most important factors for bone tissue are bone morphogenetic protein-2(BMP-2) and transforming growth factor β1(TGF-β1). The role of BMP-2 seems to be clear with conclusions from some previous studies which support that BMP promotes osteoblast differentiation in vitro and up-regulates several genes that encoding osteoblast phenotype related proteins [39,40].As for the role of BMP-2 in regulating the expression of OMD/OSAD, some scholars have confirmed that BMP-2 can increase the expression of OMD/OSAD in osteoblast differentiation. In the induced differentiation experiment of mouse osteoblast MC3T3 E1, the effect of BMP-2 on OSAD was confirmed at both RNA and promoter levels and BMP-2 also supports osteoblast differentiation by directly inducing other osteoblast markers [41]. In the study of Hamaya et al. BMP2 also enhanced the expression of OMD in 2C12 myoblast cells [42]. Regarding to the effect of TGF-β1, there are two different views on the regulation of OSAD/OMD gene by TGF-β1. One supports that the expression of OSAD is inhibited by TGF-β1. Although TGF-β1 can promote the expansion of pre-osteoblast population in the early stage of cell differentiation, while the expression of OSAD decreases in the final stage of osteoblast differentiation [41,43,44]. However, some scholars have different opinions on the role of TGF-β1. Lucchini et al. [45] studied the effect of TGF-β1 on the expression of OSAD in human dental pulp cells. After stimulated by TGF-β1signal, the synthesis of OSAD in mature odontoblasts increased. It is believed that TGF-β1stimulates the expression of OSAD in dental pulp fibroblasts, early secretory cells and mature odontoblast cells. The result from this experiment as OSAD is up-regulated under the influence of TGF-β1, which is different from the previous results, may be a cause of different experiment duration for osteogenic differentiation. The former experiment lasted for 24 hours, and the latter was 72 hours. Therefore, the increased OSAD protein signal in the latter experiment may be caused by the increase of cell numbers, rather than the result of increased transcriptional activity.

In the research so far, the interaction between BMP/Smad and calcium calmodulin signal, Wnt/calcium signal, ERK MAPK and STAT pathway has been confirmed. As for the mechanism of BMP/TGF-β signaling pathway on OMD, we have been able to prove that the signal is mediated by Smad response element in promoter which is sensitive to TGF-β signal [41]. However, the activation mechanism of BMP-2 is more complex. Recent studies have shown that BMP-2 enhances the expression of OMD gene and activates the OMD gene promoter. Because a series of recognition sites on promoter are affected by signal, such as AP-1, Runx2 and Smads. Our electronic study of OSAD promoter showed that there was a conserved region in the first 360 bp of the transcriptional initiation site. At this site, we found the recognition sites of SMAD-3 and Smad-4 which are effectors of TGF-β and BMP-2 signal molecule respectively. In addition, several recognition sites of Runx2 and AP-1 are also located in this region. Runx2 is a powerful and necessary factor for inducing osteoblast phenotype [46]. Therefore, BMP-2 affects the promoter through a series stimulation of pathways involving Smad, MAPK and Runx2 signals [47].

In addition, there are some other mechanisms has been studied, such as the transcription factor osterix (Sp7) may also be involved in the expression of OMD/OSAD in osteogenic differentiation. Sp7 is a tissue-specific transcription factor, which is only specifically expressed in bone tissue, and plays an important role in the process of tissue differentiation and formation. In Zhu's experiment, osterix knockout group decreased the expression of OMD gene, reduced bone mineralization and cell proliferation [48]. Some scholars found that Sp7 is a downstream molecule of Runx2. In the experiment, Sp7 -/- mice did not show the expression of osteoblast markers, and with no osteoblast and bone formation, but there was normal expression of Runx2 gene. However, in the Runx -/- mice group, osterix gene could not be expressed normally [49]. Another theory is that the mechanism of OMD promoting osteogenic differentiation may be related to apoptosis. The overexpression of OMD increased the number of living cells and decreased the activity of caspase 3/7 in MC3T3-E1 cells. On the contrary, in OMD knockout group, the number of living cells decreased and the activity of caspase 3/7 increased [42]. However, the mechanism of OMD regulating caspase 3/7 activity is still unclear, and further research is needed to determine the relevant molecular mechanism.

Conclusion

According to the literature review OSAD/OMD may play a positive role in regulating osteoblast differentiation via a variety of signaling pathways and cytokines. After osteogenic induction, the expression of OSAD/OMD protein was increased, and overexpression or inhibition of OSAD/OMD expression had a significant effect on the expression of osteogenic markers and the formation of mineralized nodules in vitro. However, the further mechanism remains to be studied, and the application of OSAD/OMD in bone regeneration therapy has a certain research prospect.

Conflict of Interests

There is no conflict of interest between authors.

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