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Mini review

Bone sialoprotein and its transcriptional regulatory mechanism

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Background and Objective: Bone sialoprotein is a mineralized tissue-specific noncollagenous protein that is glycosylated, phosphorylated and sulfated. The temporo-spatial deposition of bone sialoprotein into the extracellular matrix of bone, and the ability of bone sialoprotein to nucleate hydroxyapatite crystal formation, indicates a potential role for bone sialoprotein in the initial mineralization of bone, dentin and cementum. Bone sialoprotein is also expressed in breast, lung, thyroid and prostate cancers.

Material and Methods: We used osteobast-like cells (rat osteosarcoma cell lines ROS17/2.8 and UMR106, rat stromal bone marrow RBMC-D8 cells and human osteosarcoma Saos2 cells), and breast and prostate cancer cells to investigate the transcriptional regulation of bone sialoprotein. To determine the molecular basis of the transcriptional regulation of the bone sialoprotein gene, we conducted northern hybridization, transient transfection analyses with chimeric constructs of the bone sialoprotein gene promoter linked to a luciferase reporter gene and gel mobility shift assays.

Results: Bone sialoprotein transcription is regulated by hormones, growth factors and cytokines through tyrosine kinase, mitogen-activated protein kinase and cAMP-dependent pathways. Microcalcifications are often associated with human mammary lesions, particularly with breast carcinomas. Expression of bone sialoprotein by cancer cells could play a major role in the mineral deposition and in preferred bone homing of breast cancer cells.

Conclusion: Bone sialoprotein protects cells from complement-mediated cellular lysis, activates matrix metalloproteinase 2 and has an angiogenic capacity. Therefore, regulation of the bone sialoprotein gene is potentially important in the differentiation of osteoblasts, bone matrix mineralization and tumor metastasis. This review highlights the function and transcriptional regulation of bone sialoprotein.

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The formation of bone by osteoblastic cells requires the deposition of an extracellular matrix consisting of type I collagen and a variety of noncollagenous proteins, which subsequently mineralizes by the formation of hydroxyapatite crystals. Type I collagen fibers are the most abundant organic constituent and they may be involved in aligning the mineral crystals (1–3). Noncollagenous proteins, comprising $\approx 10\%$ of the organic matrix of bone, could serve important functions in the regulation of mineralization. Those proteins appear to be bound to the hydroxyapatite crystals, and include proteoglycans, osteopontin, bone sialoprotein, dentin matrix protein-1, secreted protein which is acidic and rich in cysteine/osteonectin and osteocalcin (2–7). The major phosphoproteins in

bone are osteopontin and bone sialoprotein, which contain an Arg-Gly-Asp (RGD) cell-attachment sequence (6–8). The phosphorylation sites in osteopontin and bone sialoprotein are in the amino-terminal half of the molecule and are catalyzed by casein kinase II (9). The RGD sequence is essential for the cell-binding properties of fibronection, vitronectin, fibrinogen and small integrin-binding ligand, N-linked glycoproteins (SIBLING) (8,10). The SIBLING family of proteins (bone sialoprotein, osteopontin, dentin matrix protein-1, dentin sialophosphoprotein and matrix extracellular phosphoglycoprotein) have a noncoding exon 1, a leader sequence and the first two amino acids in exon 2, casein kinase II phosphorylation consensus sequences in exons 3 and 5, a proline-rich and often basic exon 4, and the integrin-binding RGD sequence within one of the last two exons (10–12) (Fig. 1). The RGD sequence in bone sialoprotein is recognized by an $\alpha_V\beta_3$ vitronectin receptor ($\alpha_V\beta_3$ integrin) (8). The RGD motif in bone sialoprotein induces intracellular concentration of free Ca^{2+} in osteoclasts through $\alpha_V\beta_3$ integrin (13). However, RGD-independent Ca^{2+} signaling occurs in bone sialoprotein-stimulated osteoclasts (14). SIBLING genes were found to be clustered within a 375,000 base pair region in human chromosome 4 (mouse chromosome 5, rat chromosome 14) and they could be derived from a single ancient gene (4,15). Flanking the RGD sequence at the C-terminus of bone sialoprotein are 7–12 sulfated tyrosine residues (Fig. 2). Osteopontin contains 5% sialic acid and a stretch of nine consecutive aspartic acid residues, and is rich in phosphate groups. Bone sialoprotein contains ≈15% sialic acid, tyrosine sulfates and clusters of up to 10 consecutive glutamic acid residues. These negatively charged domains are presumably responsible for the strong binding of bone sialoprotein to hydroxyapatite (6,7). Bone sialoprotein expression is restricted in mineralized connective tissues and bone sialoprotein is first expressed at the onset of bone, cementum and dentin formation (16). Bone sialoprotein has been shown to induce the formation of hydroxyapatite in a steady-state agarose-gel system (17). Therefore, bone sialoprotein might be involved in the nucleation of hydroxyapatite at the mineralization front of bone. The formation of bone and cementum is an integral part of tooth and periodontal tissue development. Bone sialoprotein, osteopontin and osteocalcin are prominent constituents of cementum and mantle dentin. The temporal appearance and spatial distribution of these noncollagenous proteins in the tooth are similar to those seen during bone formation (16,18).

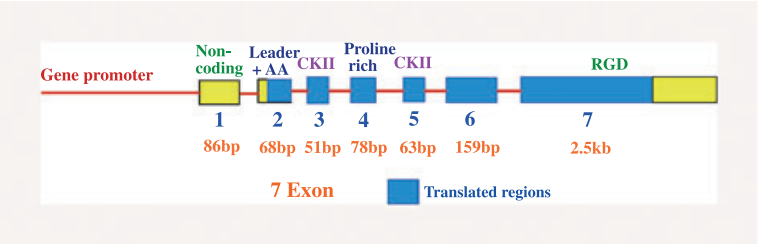

Fig. 1. Gene structure of bone sialoprotein. Exons are shown as boxes and introns as connecting lines. Exon 1 is noncoding, a leader sequence and the first two amino acids are present in exon 2, casein kinase II phosphorylation consensus sequences are present in exons 3 and 5, exon 4 is proline-rich and often basic, and the integrin-binding RGD (Arg-Gly-Asp) sequence is present in exon 7. AA, amino acid; CKII, casein kinase II.

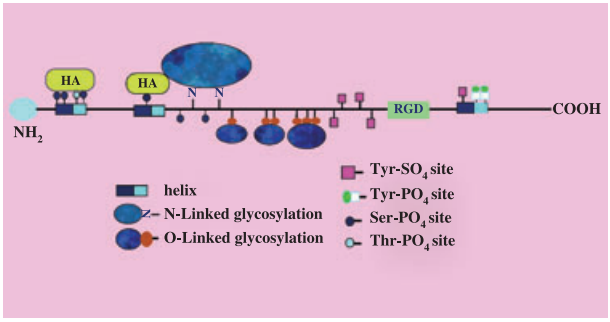

Fig. 2. The schematic protein structure of bone sialoprotein. The N-terminal region contains polyglutamic acid stretches that interact with hydroxyapatite. The RGD (Arg-Gly-Asp) cell-attachment sequence at the C-terminus of bone sialoprotein is a tyrosine-rich region. Several sulfated tyrosine sites, and serine and threonine phosphorylation sites and glycosylation sites are present, as shown in this figure. HA, hydroxyapatite; Ser, serine; Thr, threonine; Tyr, tyrosine.

Bone sialoprotein expression in cancer cells

Bone sialoprotein is expressed in breast, lung, thyroid and prostate cancers that metastasize to bone (19–22). Breast cancer metastasizes to bone more frequently than to any other organ, and over 80% of advanced breast cancer patients develop bone metastases. High bone sialoprotein expression in breast cancer cells is usually associated with the presence of microcalcifications within the lesion, suggesting that bone sialoprotein could participate with hydroxyapatite deposition in malignant breast lesions. Moreover, expression of bone sialoprotein in primary human breast cancer is associated with poor prognosis, independent of lymph node status, and bone sialoprotein is expressed on the cell surface of estrogen receptor-positive cells that metastasize to bone with high frequency (23,24). Serum from patients with breast, lung, colon and prostate cancer was found to have significantly elevated levels of bone sialoprotein and osteopontin (25). Bone sialoprotein binds to factor H and protects cells from complement-mediated

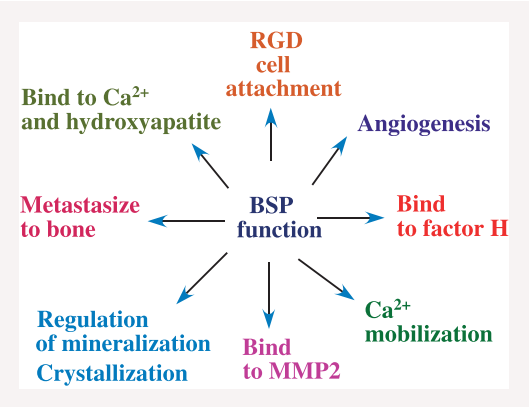

Fig. 3. Bone sialoprotein is a multifunctional protein. BSP, bone sialoprotein; MMP2, matrix metalloproteinase 2; RGD, Arg-Gly-Asp.

cellular lysis (26), and bone sialoprotein also binds to matrix metalloproteinase 2 (MMP-2) and has related roles in the local activation of specific MMPs in mineralizing and nonmineralizing tissues (27). Bone sialoprotein mediates human umbilical vein endothelial cell attachment and migration (28), thereby demonstrating an angiogenic capacity (Fig. 3). Thus, bone sialoprotein expression in metastatic cancers may provide enhanced angiogenic potential to metastasizing cancer cells, in addition to the ability to evade complement-mediated lysis and to become more invasive when bone sialoprotein forms a cell-surface trimolecular complex by linking MMP-2 to integrin $\alpha_V\beta_3$ (26–28).

Effects of hormones on bone sialoprotein expression

Bone marrow cells plated and grown in the presence of 10^{-8} M dexamethasone and 10 mM β-glycerophosphate produce mineralized bone nodules. However, in the absence of dexamethasone, few mineralized nodules are observed. The formation of mineralized nodules was found to be reflected by the uptake of Ca^{2+} and revealed an increase in type I collagen, alkaline phosphatase, osteopontin, bone sialoprotein and osteocalcin mRNA expression (29,30). When these cells were grown in the presence of serum-free medium, bone sialoprotein mRNA was not induced by dexamethasone and bone nodules were not formed, indicating that the induction of the bone sialoprotein gene in pre-osteoblastic cells may require cell replication (31). 1,25-$(OH)_2D_3$ (vitamin D_3) inhibits osteoprogenitor cell differentiation at an early stage and at a time during cell growth is stimulated (32). 1,25-$(OH)_2D_3$ suppresses bone sialoprotein expression and glucocorticoid-induced bone sialoprotein expression (33,34), and type I collagen synthesis (35). Bone sialoprotein transcription was increased by androgen receptor overexpression in the rat osteosarcoma cell line ROS17/2.8 (36). However, the endogenous and overexpressed bone sialoprotein mRNA levels were not affected by 5α-dihydrotestosterone (10^{-8} M, 24 h). Androgen receptor overexpression increased bone sialoprotein gene transcription in a ligand-independent manner by targeting the cAMP response element and activator protein 1/glucocorticoid response element in the promoter of the rat bone sialoprotein gene (36).

Parathyroid hormone (human 1–34 parathyroid hormone, 10 nM) increases the expression of bone sialoprotein in ROS17/2.8 cells (37). Parathyroid hormone acts through a protein kinase A pathway, involving cAMP, to stimulate bone sialoprotein transcription by blocking the action of a pituitary-specific transcription factor-1 (that suppresses bone sialoprotein transcription) by binding a pituitary-specific transcription factor-1 suppressor element (37). On the other hand, parathyroid hormone-related protein (100 nM, 48 h) down-regulates bone sialoprotein gene expression in cementoblasts (38).

Estrogen receptor binding affinity and estrogenic activity have been reported for the isoflavone derivatives genistein and daidzein (39). Genistein and daidzein (50 μM, 12 h) increased bone sialoprotein transcription in ROS17/2.8 cells (40). Quercetin is a typical flavonol-type flavonoid and is present in a variety of vegetables. Quercetin and its conjugated metabolite, quercetin 3-glucuronide (5 μM, 12 h), increased the bone sialoprotein mRNA expression in ROS 17/2.8 cells (41).

Effects of growth factors and cytokines on bone sialoprotein expression

Epidermal growth factor (50 ng/mL) down-regulates osteoblastic cell differentiation and inhibits the expression of alkaline phosphatase, bone sialoprotein and osteocalin in MC3T3-E1 osteoblast-like cells (42). Whereas fibroblast growth factor 2 decreased bone sialoprotein expression in MC3T3-E1 cells, it (10 ng/mL, 6 h) increased bone sialoprotein transcription in ROS17/2.8 cells via the fibroblast growth factor 2 response element in the proximal promoter of the bone sialoprotein gene that mediates both constitutive and fibroblast growth factor 2-induced bone sialoprotein transcription (43). Fibroblast growth factor 2 (10 ng/mL) and cyclic AMP (forskolin; 1 μM) synergistically up-regulate bone sialoprotein gene expression in ROS17/2.8 cells (44). Fibroblast growth factor 2 [tyrosine and mitogen-activated protein kinases (MAPKs)] and cAMP-dependent signaling pathways converge at the CCAAT box, the cAMP response element, the fibroblast growth factor 2 response element and the pituitary-specific transcription factor-1 element in the proximal promoter of the bone sialoprotein gene to stimulate bone sialoprotein gene expression in a synergistic manner, and nuclear factor-Y, cAMP response element binding protein, runt homeodomain protein 2 (Runx2), Smad1, distalless 5 (Dlx5) and pituitary-specific transcription factor-1-like proteins may regulate bone sialoprotein transcription

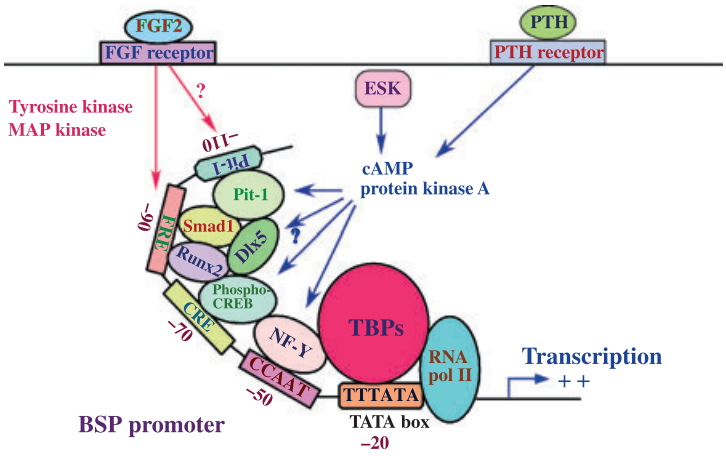

Fig. 4. Model of bone sialoprotein promoter regulation in osteoblasts by fibroblast growth factor 2 and cAMP. Fibroblast growth factor 2 and forskolin synergistically regulated bone sialoprotein gene expression supported by protein–DNA interactions at the inverted CCAAT box, cAMP response element, fibroblast growth factor 2 response element and pituitary-specific transcription factor-1, respectively. CRE, cAMP response element; FGF2, fibroblast growth factor 2; FRE, fibroblast growth factor 2 response element; FSK, forskolin; MAP kinase; mitogen-activated protein kinase; NF-Y, nuclear factor-Y; Pit-1, pituitary-specific transcription factor-1; PTH, parathyroid hormone; TBP, TATA binding protein.

(37,44,45) (Fig. 4). Fibroblast growth factor 2 induced the expression of dentin sialoprotein and bone sialoprotein mRNAs in rat dental pulp cells in collagen type I gel cultures, but not in plate-coated collage type I, fibronectin, laminin or collagen type IV cultures (46). These results suggest that the differentiation stage of the cells and culture conditions (three-dimensional scaffold) are crucial for the fibroblast growth factor 2 effects on bone sialoprotein expression in osteoblasts and odontoblasts.

Insulin-like growth factor 1 promotes bone formation by stimulating the proliferation and differentiation of osteoblasts. Insulin-like growth factor 1 (50 ng/mL, 12 h) increased bone sialoprotein mRNA levels in human osteoblast-like Saos2 cells and rat stromal bone marrow (RBMC-D8) cells. Insulin-like growth factor 1 stimulates bone sialoprotein transcription by targeting the fibroblast growth factor 2 response element and homeodomain protein-binding site in the proximal promoter of the bone sialoprotein gene through tyrosine kinase, Ras/MAPK and phosphatidylinositol 3-kinase/Akt pathways (45).

Tumor necrosis factor-α and lipopolysaccharide are major mediators of inflammatory responses in periodontal disease and they inhibit bone formation and stimulate bone resorption. Tumor necrosis factor-α (10 ng/mL, 24 h) suppresses bone sialoprotein gene transcription through a tyrosine kinase-dependent pathway that generates reactive oxygen species, and the tumor necrosis factor-α effects are mediated by a cAMP response element in the proximal bone sialoprotein gene promoter (47). Lipopolysaccharide (1 μg/mL, 12 h) suppresses bone sialoprotein gene transcription through protein kinase A and tyrosine kinase-dependent pathways and the lipopolysaccharide effects are mediated through the cAMP response element and the fibroblast growth factor 2 response element in the proximal bone sialoprotein gene promoter (48).

Prostaglandin E_2 has anabolic effects on the proliferation and differentiation of osteoblasts via diverse signal transduction systems. Treatment of rat osteosarcoma UMR 106 cells with prostaglandin E_2 (3 μM, 12 h) increased the steady-state levels of bone sialoprotein mRNA. Prostaglandin E_2 induces bone sialoprotein transcription in UMR 106 cells through the juxtaposed cAMP response element and the fibroblast growth factor 2 response element in the proximal promoter of the bone sialoprotein gene (49).

Effects of enamel matrix proteins on bone sialoprotein expression

Enamel matrix derivative (EMD), the acid extract of the porcine cheese-like enamel matrix, has been developed as a clinical treatment to promote periodontal regeneration. A total of 90% of the protein in the EMD is amelogenin and the remaining 10% is made up of nonamelogenin enamel matrix proteins such as enamelin, sheathlin (ameloblastin) and growth factors (50–54). The results from a controlled clinical trial and a case report study have also demonstrated that the treatment of intrabony periodontal defects with EMD leads to significant gain in clinical attachment level and bone regeneration, as observed on radiographs (55,56). EMD (50 μg/mL, 12 h) induces bone sialoprotein expression in ROS17/2.8 cells through the homeodomain protein-binding site and the transforming growth factor-β activation element in the rat bone sialoprotein gene promoter (57,58). EMD (50 μg/mL) increases core binding factor a1 (Cbfa1) and bone sialoprotein transcription, and suppresses myoblas determination mRNA expression, in C2C12 myoblasts. This result indicates that EMD can convert the differentiation pathway of C2C12 myoblasts into that of osteoblast lineage cells (59).

Amelogenin-deficient mice display an amelogenesis imperfecta phenotype and also show root resportion and reduced expression of bone sialoprotein (60,61). These results indicate that amelogenin may regulate bone sialoprotein expression. Recombinant amelogenin (1 μg/mL, 12 h) induced bone sialoprotein gene expression in ROS 17/2.8 cells through fibroblast growth factor 2 response elements and transforming growth factor-β activation elements in the rat bone sialoprotein promoter (62,63).

Effects of mechanical stress on bone sialoprotein expression

Physical forces (mechanical strain) may play a fundamental role in the regulation of cell function in bone (64). The absence of physical stress on the

skeleton, as seen in immobilization or during space flight, can lead to an osteoporotic condition (65,66). Magnetic fields of sufficient magnitude have been shown to affect various biologic systems at the organ, tissue, and cellular and subcellular levels. There are two major types of magnetic field: pulsing electromagnetic fields and static magnetic fields (67,68). The mechanisms of pulsing electromagnetic field effects are different from those of static magnetic fields. Pulsing electromagnetic fields generate an electric current in the tissue, whereas static magnetic fields create only a magnetic field (69,70). Application of 300 and 800 gauss static magnetic fields increased bone sialoprotein mRNA levels, after 24 h of stimulation, in UMR106 cells. Static magnetic fields increase bone sialoprotein transcription through a tyrosine kinase-dependent pathway, and static magnetic field effects are mediated through juxtaposed fibroblast growth factor 2 response element and pituitary-specific transcription factor-1 motifs in the proximal promoter of the bone sialoprotein gene (71).

Chlorpromazine, a tranquilizing agent for treating psychiatric disorders, mimics hypotonic stress and causes membrane deformation (72) and mechanical stress to cardiac myocytes (73). Application of 10 μg/mL of chlorpromazine suppressed bone sialoprotein mRNA levels after 12 and 24 h in osteoblast-like ROS17/2.8 cells and rat stromal bone marrow cells. Chlorpromazine suppresses bone sialoprotein gene transcription through tyrosine- and MAPK-dependent pathways, and chlorpromazine effects are mediated by the cAMP response element and the fibroblast growth factor 2 response element in the proximal promoter of the rat bone sialoprotein gene (74).

Bone sialoprotein gene promoter

A comparison of the promoter sequences of the rat (75), mouse (76) and human (77,78) bone sialoprotein genes revealed that 75% of the nucleotides were conserved in the first 370-bp region of the proximal promoter (79). Relatively poor conservation

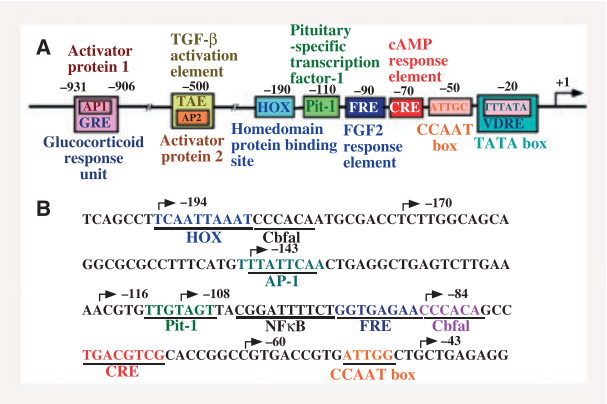

Fig. 5. Regulatory elements in the proximal rat bone sialoprotein promoter. (A) The position of the inverted TATA and CCAAT boxes, a cAMP response element, a fibroblast growth factor 2 response element, a pituitary-specific transcription factor-1, a homeodomain protein-binding site, a transforming growth factor-β activation element overlapping with activator protein 2, glucocorticoid response elements overlapping with activator protein 1, and a vitamin D response element that overlaps the inverted TATA box are shown in the proximal promoter region of the rat bone sialoprotein gene. The numbering of nucleotides is relative to the transcription start site (+). (B) The nucleotide sequence of the rat bone sialoprotein gene proximal promoter, encompassing an inverted CCAAT box, and cAMP response element, core binding factor a1, fibroblast growth factor 2 response element, nuclear factor-κB, activator protein 1 and homeodomain protein-binding site elements, is shown from base pairs −201 to −35. AP1, activator protein 1; AP2, activator protein 2; CRE, cAMP response element; FGF2, fibroblast growth factor 2; FRE, fibroblast growth factor 2 response element; GRE, glucocorticoid response element; HOX, homeodomain protein-binding site; NF-κB, nuclear factor-κB; Pit-1, pituitary-specific transcription factor-1; TAE, transforming growth factor-β activation element; TGF-β, transforming growth factor-β; VDRE, vitamin D response element.

of the nucleotide sequence in the proximal promoter of the avian bone sialoprotein gene was observed when compared with the mammalian genes (80). Studies on the transcriptional regulation of bone sialoprotein have identified a highly conserved proximal promoter region in which an inverted CCAAT element (base pairs −50 to −46) and TATA box (base pairs −24 to −19) are separated by 21 nucleotides (81–83) (Fig. 5). In addition to its critical role in basal transcription, the inverted CCAAT element is a target of *src* regulation through nuclear factor-Y (40,83,84). c-Jun and E1A regulate basal transcription of bone sialoprotein in osteosarcoma cells by recruiting the nuclear factor-Y transcriptional complex to the inverted CCAAT element (83). An inverted TATA box overlaps with a vitamin D_3 response element (34). In addition, a cAMP response element (base pairs −75 to −68) (44,47,49), two putative Cbfa1/Runx2 binding sites (base pairs −84 to −79, and −184 to −179), a fibroblast growth factor 2 response element (base pairs −92 to −85) (43–45,49,63,71), a pituitary-specific transcription factor-1 motif (base pairs −111 to −105), which mediates the stimulatory effects of parathyroid hormone (37,71), a homeodomain protein-binding site (base pairs −199 to −192) (45,58,85) and a transforming growth factor-β activation element (base pairs −499 to −485) (57,58,63), have been characterized. Potential sites of glucocorticoid regulation have been identified further upstream at similar positions in the rat and human promoters. However, whereas the putative human glucocorticoid response element (base pairs −1038 to −1022) forms a glucocorticoid response unit with a retinoic acid response element (77), the rat glucocorticoid response element (−921 to −906) is associated with an activator protein 1 site (glucocorticoid response element/activator protein 1) (31,86). The ability of bone

sialoprotein-expressing ROS 17/2.8 cells and nonexpressing NRK 49F cells to mediate the transcription of rat bone sialoprotein promoter constructs spanning ≈3 kb of the bone sialoprotein promoter linked to a luciferase reporter gene was analyzed in transient transfection assays. However, cell-specific expression was not clearly evident (87). Previous studies using luciferase reporter constructs ligated to 2.5-kb mouse and 2.7-kb rat bone sialoprotein promoter constructs failed to show absolute tissue-specific expression in transgenic mice (88,89). Tissue- and bone cell-specific expression of bone sialoprotein is directed by a 9.0-kb mouse promoter in transgenic mice, which includes four Cbfa1/Runx2 sites (90). However, a recent study of the mouse bone sialoprotein promoter has identified a Dlx5 transcription factor and a homeodomain protein-binding site (base pairs −199 to −192) as a potential tissue specific regulator (85). The same group reported that cooperative interactions between Runx2 and homeodomain protein-binding sites are critical for the osteoblast-specific expression of the bone sialoprotein gene (91). While they showed that Runx2 could bind to two putative Cbfa1/Runx2 binding sites (base pairs −84 to −79, and −184 to −179), our recent results show that Runx2 can bind to only one Cbfa1/Runx2 binding site (base pairs −84 to −79), which juxtaposed the fibroblast growth factor 2 response element (45). Although the putative homeodomain binding element in the bone sialoprotein promoter acts as a tissue-specific enhancer of bone sialoprotein expression in both osteoblasts and hypertrophic chondrocytes, this site is unlikely to bind homeodomain factors, including Dlx5 (92). In contrast to the rat and mouse bone sialoprotein promoters, which show a conserved sequence extending several kilobases upstream, the human promoter showed no sequence conservation beyond ≈0.99 kb. The human bone sialoprotein promoter contains a 3.48-kb insert (−4.47 to −0.99 kb) coding for an L1 retrotransposon element. The retrotransposon element suppresses transcription by < 80% (93).

Possible role of bone sialoprotein in periodontal regeneration

A mini bone sialoprotein peptide, Glu7-Pro-Arg-Gly-Asp-Thr, which contains a putative hydroxyapatite-binding site (poly Glu) and an RGD cell-attachment site, has affinity to hydroxyapatite. The peptide affected *in vitro* mineralization in a gel system, indicating interaction between this peptide and calcium phosphate. The results suggest that the peptide containing the poly Glu and RGD sequence could induce mineralization *in vivo* (94). Type I collagen matrix gel induced the expression of osteoblastic phenotypes of bone marrow cells, and monoclonal anti-bone sialoprotein suppressed the expression of these phenotypes. Furthermore, bone sialoprotein enhanced alkaline phosphatase activity and osteocalcin synthesis of bone marrow cells. Therefore, bone sialoprotein is a crucial factor in osteoblastic differentiation (95). However, bone sialoprotein gene transfer to periodontal ligament cells may not be sufficient to promote mineralization *in vitro* or *in vivo* (96). These results suggest that additional factors, such as growth factors or cytokines, are necessary for mineralization. When purified bovine bone sialoprotein with type I collagen or collagen alone were implanted into rat calvarial defects and thoracic subcutaneous pouches, bone sialoprotein-collagen, but not collagen alone, elicited mineral deposition on days 4–5 followed by osteoblast differentiation and synthesis of new bone in the calvarial defects. In contrast, implantation of bone sialoprotein-collagen into subcutaneous pouches did not induce calcification (97). These results demonstrate that bone sialoprotein stimulates site-specific osteogenesis and that the local environment and the specificities of responding cells may be crucial for the function of bone sialoprotein.

Conclusion

Bone sialoprotein is a multifunctional protein that has a critical role in the early onset of mineralization, attachment to hydroxyapatite and tumor metastasis. Also, bone sialoprotein expression could be very important for periodontal regeneration. Transcription of bone sialoprotein is regulated mainly by tyrosine kinase, MAPK and cAMP. Therefore, control of these signaling pathways may have positive effects on periodontal regeneration, such as formation of new connective tissue attachments and new alveolar bone.

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