Effect of basic fibroblast growth factor on osteopontin gene expression

Won-Su Bae, Hyun-Jung Kim, Hyun-Mo Ryoo*, Young-Jin Kim, Soon-Hyeun Nam

*Department of Pediatric Dentistry and Oral Biochemistry**,
Collage of Dentistry, Kyungpook National University

Abstract

The Fibroblast growth factors(FGFs) plays an important role in the control of osteogenesis during skeletal development. Especially, FGF-2 is a potent mesodermal inducer during embryogenesis and FGF receptors (FGFRs) messages are strongly expressed in developing bones.

In this study, we investigated the effect of bFGF on osteopontin(OPN) gene expression in ST-2 cells and tried to elucidate the mechanism of its stimulatory effects.

The obtain results were as follows:

The treatment of bFGF(1ng/*ml*) upregulates OPN, fibronectin mRNA levels and downregulates type I collagen mRNA levels. But, there was no remarkable difference in alkaline phosphatase mRNA levels between two groups. The OPN gene expression increased in a dose-dependent manner up to 10ng/*ml* and OPN gene began to occur at around 3h with continuous increase up to 24h then decreased to basal level at 48h. 30 minutues pretreatment with cycloheximide (500ng/*ml*), a protein synthesis inhibitor, prior to addition bFGF resulted in blocking bFGF induced OPN expression. These results suggest that bFGF increased the level of OPN mRNA in a dose and time-dependent manner via the synthesis of certain transcriptional regulatory proteins.

Key Words : Basic Fibroblast growth factor, Osteopontin, Fibronectin, Alkaline phosphatase

I. Introduction

Fibroblast growth factors (FGFs) are a family of at least fifteen genes which are involved in the control of a variety of processes such as proliferation, migration, differentiation, and cell survival[1,2]. The biological functions of the prototypic members of this family, FGF-1 and FGF-2, are mediated by binding to high-affinity receptors. Prior association of FGFs with low-affinity heparan sulfate proteoglycans is thought to facilitate their binding to the high-affinity receptors which subsequently dimerize, leading to activation of intrinsic tyrosine phosphorylation and initiation of downstream kinase cascades which culminate in altered gene transcription[3,4]. Another mechanism has been described whereby cytoplasmic FGF-2 can be translocated into the nucleus where it activates gene transcription[5,6]. In many cells, including osteoblasts, the synthesis of the nuclear proto-oncogene products such as c-Fos and c-Jun and related proteins is an immediate early response to bFGF. c-Fos can act as a transcriptional regulator and has been shown to interfere with normal bone development[7].

Several observations indicate that FGFs may play an important role in the control of osteogenesis during skeletal development. FGF-2 is a potent mesodermal inducer during embryogenesis and FGF receptors (FGFRs) messages are strongly expressed in developing bones[8,9]. Studies in bovine calvaria cells showed that FGF-2 is produced by osteoblasts and accumulated in the bone matrix[10]. In bovine and rodent calvaria-derived cells, FGF-1 and FGF-2 stimulate cell proliferation but inhibit alkaline phosphatase (ALP) activity and reduce collagen type I (Col I) and osteocalcin (OC) expression[11-13], indicating that FGF-2 has independent effects on calvaria

cell replication and differentiation. The effects of FGFs on osteoblastic cell differentiation and bone matrix formation in long-term culture are conflicting since positive[14] and negative[15] effects have been reported depending on the cell culture system. In humans, FGF-2 was found to reduce osteoblasts markers in marrow stromal cells[15]. In contrast, FGF-2 was found to promote osteoblast differentiation from precursor cells in the rat bone marrow stroma and to stimulate of endosteal rat bone formation[16-19] and fracture repair[20].

Craniosynostosis, the premature fusion of cranial sutures, presumably involves disturbance of the interactions between different tissues within the cranial sutures. Point mutations in the genes encoding for the fibroblast growth factor receptors cause various types of human craniosynostosis syndromes. There are four fibroblast growth factor receptors, FGFR1-4, three of which(FGFRs1-3) are known to play important roles in skeletal differentiation and growth, as revealed by the identification of specific mutations in previously recognized clinical syndromes[21,22]. They are transmembrane receptors with three immunoglobuline-like domains (Ig Ⅰ, Ig Ⅱ, Ig Ⅲ) in the extracellular region and a split tyrosine kinase domain in the intracellular region of the molecule[23]. Among these, the FGFR2 gene is of particular interest in the context of craniofacial development. Dominantly acting missense mutations located mainly in the Ig Ⅲa/Ig Ⅲc domain or in the Ig Ⅱ/Ig Ⅲ linker region are associated with a variety of craniosynostosis syndromes (Crouzon, Pfeiffer, Jackson-Weiss, Apert and Beare-Stevenson syndromes)[24]. There are two FGFR2 variants. One, known as KGFR, including the Ⅲa/Ⅲb domain, the other, known as BEK, including Ⅲa/Ⅲc domain[25]. The bFGF, having affinity to BEK, is localized in a pattern that is largely reciprocal of FGFR2 transcripts, being high in areas in which bone differentiation has been initiated and low within the suture mesenchyme and FGFR2 expression is correlated with cell proliferation adjacent to and mutually exclusive with areas, in which bone differentiation is been initiated[26]. These observations suggest that FGF/FGFR2 signal transduction is indispensible for the osteoblastic differentiation.

Recently, in the middle of the process elucidating the effect of bFGF signaling on the rate of suture closure in fetal mouse calvaria organ culture, we have shown that implantation of bFGF soaked bead onto both the osteogenic fronts and mid-mesenchyme of sagittal suture of mouth calvarial explants induces OPN expression. But, positive regulation by bFGF of OPN transcriptional activity in osteoblastic cells has not been demonstrated in detail. Therefore, in this report, we investigated the effect of bFGF on osteopontin gene expression and tried to elucidate the mechanism of its stimulatory effects.

Ⅱ. Material and Method

1. Materials

RPMI1640, fetal bovine serum, were purchased form GibcoBRL (Grand Island, NY, U.S.A.). The bFGF was from Promega (Madison, WI, U.S.A.), Cycloheximide, demethylformamide, formamide, and MOPS were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Tissue culture plastic wares were from Corning (NY, U.S.A.) or Falcon (NJ, U.S.A.). Express Hyb solution was from Clontech (Palo Alto, CA, U.S.A.). [α-32P]dCTP, Hybond-N+membrane, and Megaprime DNA labelling system kit were from Amersham (Arilington, IL, U.S.A.).

2. Cell culture

Murine bone marrow-derived stromal cells(ST-2 cells) were maintained in RPMI1640 containing 10% heat-inactivated fetal bovine serum (FBS:Gibco BRL) and 100 U/*ml* penicillin /streptomycin at 37℃ in a humidified atmosphere containing 5% of CO_2. Cells were seeded at 6.5×10^5/100mm culture plate and grown for 3 days. After that, cells were treated with bFGF at given concentrations for 24h: controls were treated with equivalent amount of bovine serum albumin (BSA) for 24h. To test time course of bFGF on OPN expression, cells were grown for 3 days. After that, cells were treated with bFGF (1 ng/*ml*) for given times. Controls were treated with equivalent amount of BSA. To assess the effect of cycloheximide, cells were pretreated for 30min with 500ng/*ml* of cycloheximide dissolved in phosphate buffered saline.

3. RNA extraction and Northern blot analysis

Total RNA was isolated as previously described (Chomczynski and Sacchi, 1987) and quantified by spectrophotometer. 10 ㎍ of total RNAs were heated to 65℃ for 15 min in 50% formamide, 40 mM MOPS(3-[-N-

morpholino]propanesulfonic acid), 10mM sodium acetate, 1mM EDTA, 0.1㎎/㎖ ethodium bromide prior to gel electrophoresis on 1% agarose, 55% formaldehyde, 40 mM MOPS, 10 mM sodium acetate, 1mM EDTA. The RNA was blotted onto a Hybond-N+ membrane in 20×SSPE. RNA was cross-linked by exposure to ultraviolet light and then air-dried. The probes were labelled with [α-32P]dCTP by Megaprime DNA labelling system kit(Amersham pharmacia biotech). Prehybridization and hybridization were performed by using the Express Hyb solution. After hybridization, the membrane was washed in 2×SSC-0.1%SDS at room temperature and then in 0.1×SSC-0.1%SDS at 55℃ and exposed to Agfa X-ray film at -70℃ with intensifying screens.

Ⅲ. Result

1. Treatment of ST-2 cells with bFGF upregulates OPN, fibronectin(FN) mRNA levels and downregulates type Ⅰ collagen mRNA levels.

The effects of bFGF on OPN, FN, Col Ⅰ, ALP were studied in ST-2 cells. Northern blot analysis revealed that bFGF upregulates OPN, FN mRNA levels and downregulates type Ⅰ collagen mRNA levels. But, there was no remarkable difference in ALP mRNA levels between two groups.

2. The effect of bFGF on OPN mRNA levels increase in a dose-dependent manner

To test the dose response of OPN mRNA induction by bFGF, confluent ST-2 cells were treated with at a given concentration of bFGF (0, 0.1, 1, 10, 20, 30ng/㎖) for 24h. Total RNAs were isolated and analyzed. Northern blot analysis revealed the increase in OPN gene in a dose-dependent manner. But, there was no remarkable difference between 10ng/㎖ and higher concentrations.

3. bFGF requires hours of incubation to increase osteopontin gene expression

To test the time response of OPN mRNA induction by bFGF, confluent ST-2 cells were treated with 1 ng/㎖ bFGF and at various times total RNAs were isolated and analyzed. Northern blot analysis revealed the increase in OPN gene began to occur at around 3h with continuous increase up to 24h, and then decreased to the basal level at 48h. Although, untreated cells expressed the same patterns those of treated groups, the amount of RNAs were lower. This observation indicates that bFGF seems to require at least 3 hours to induce OPN transcription.

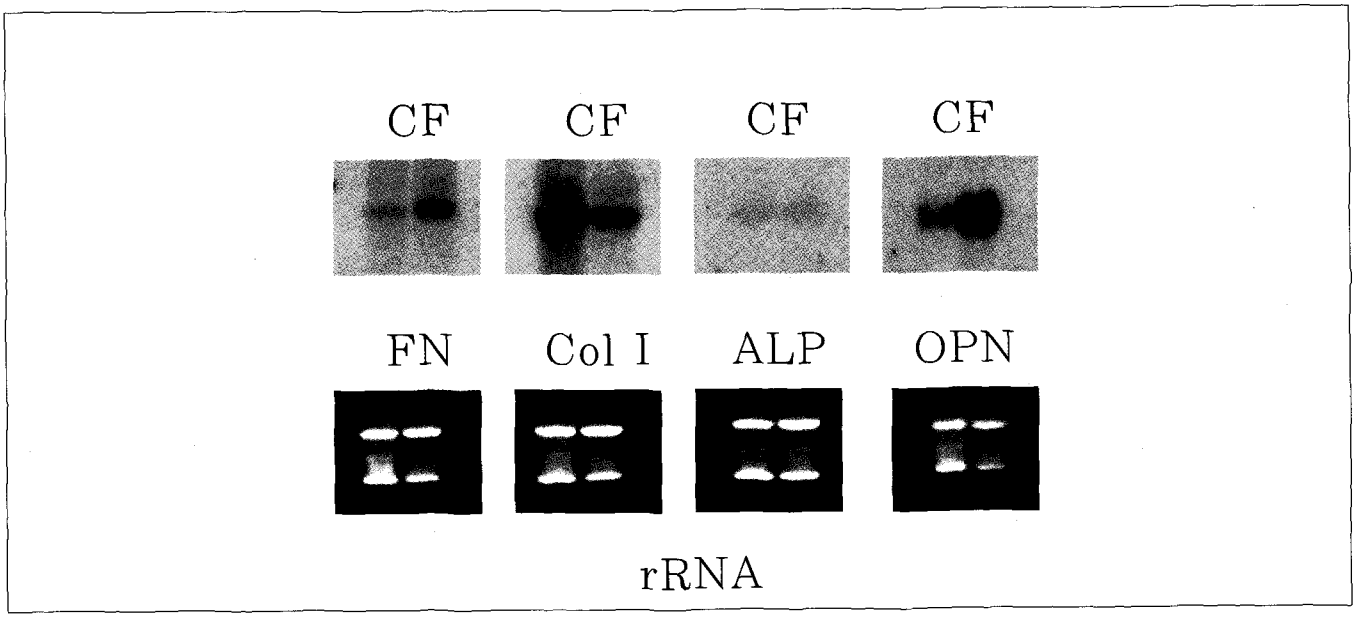

Fig. 1. Effects of bFGF on OPN, ALP, Col Ⅰ, FN mRNA
ST-2 cells were plated at 6.5×10⁵cells/100mm culture plate in 10% FBS. Three days later, media were changed and cells were cultured for 24h in the abscence (C) or presence (F) of 1 ng/㎖ bFGF. Total RNA (10 ㎍) were analyzed by Northern blot hybridization with cDNA probe for osteopontin. The amount of rRNA in the same samples was visualized by ethidium bromide staining. OPN,osteopontin; ALP,alkaline phosphatase; Col Ⅰ,type Ⅰ collagen; FN,fibronectin.

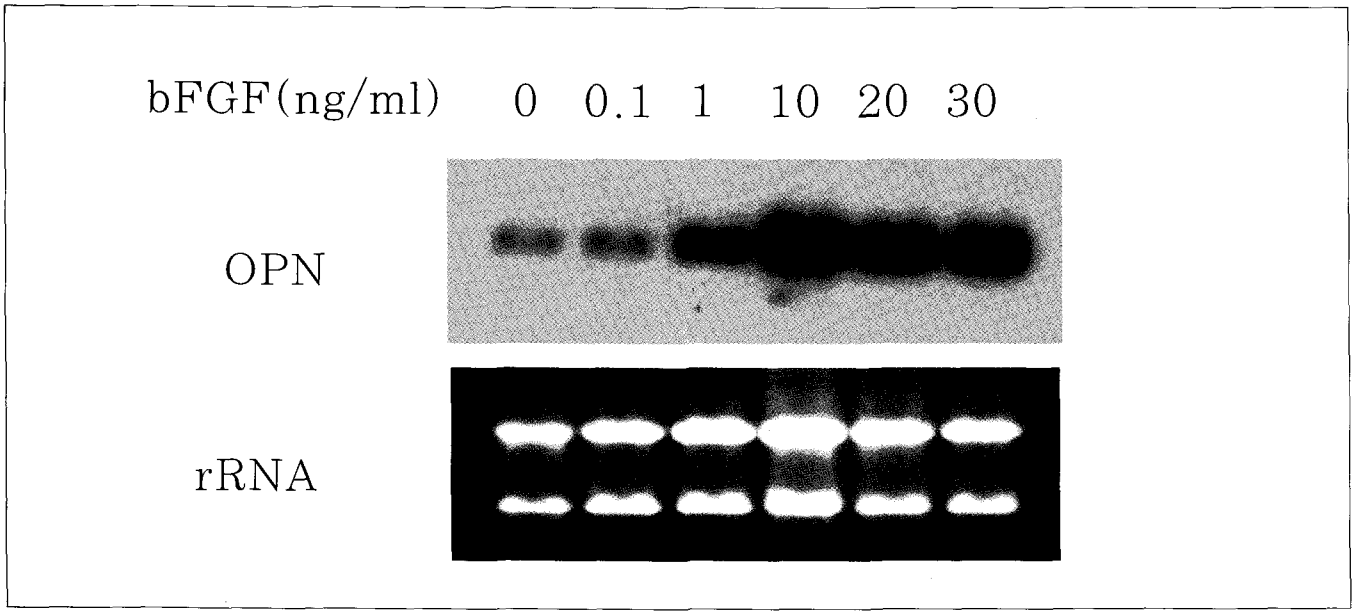

Fig. 2. Dose response experiments for the effect of bFGF on OPN mRNA levels.
ST-2 cells were plated at 6,5×10^{5}cells/100mm culture plate in 10% FBS. Three days later media were changed and cells were cultured at a given concentration of bFGF (0, 0.1, 1, 10, 20, 30 ng/*ml*). Controls were added equivalent amount of BSA. Total RNA (10 *μg*) were analyzed by Northern blot hybridization with cDNA probe for osteopontin. The amount of rRNA in the same samples was visualized by ethidium bromide staining. Lane 1: control, lane 2, 3, 4, 5, and 6 are bFGF at 0.1, 1, 10, 20, 30 nM respectively.

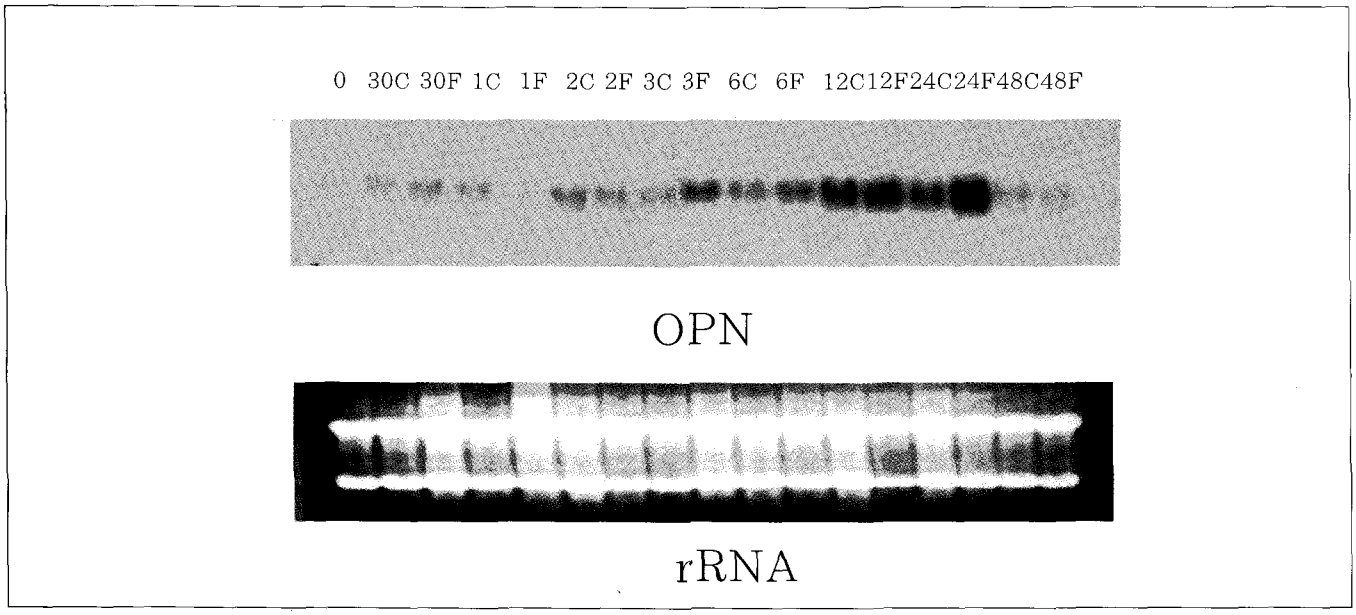

Fig. 3. Time course for the effect of 1nM bFGF on OPN mRNA levels in ST-2 cells.
ST-2 cells were plated at 6,5×10^{5}cells/100mm culture plate in 10% FBS. Three days later media were changed and cells were cultured for 30 min, 1, 2, 3, 6, 12, 24 and 48h in the abscence (C) or presence (F) of 1 ng/*ml* bFGF. Total RNA (10 *μg*) were analyzed by Northern blot hybridization with cDNA probe for osteopontin. The amount of rRNA in the same samples was visualized by ethidium bromide staining.

4. bFGF-induced osteopontin gene expression requires de novo protein synthesis

In order to determine whether bFGF-stimulated increase in steady-state OPN mRNA requires the synthesis of new protein. ST-2 cells were treated with bFGF (1 ng/*ml*) alone, or in combination with cycloheximide (500 ng/*ml*), a protein synthesis inhibitor. Cells were cultured

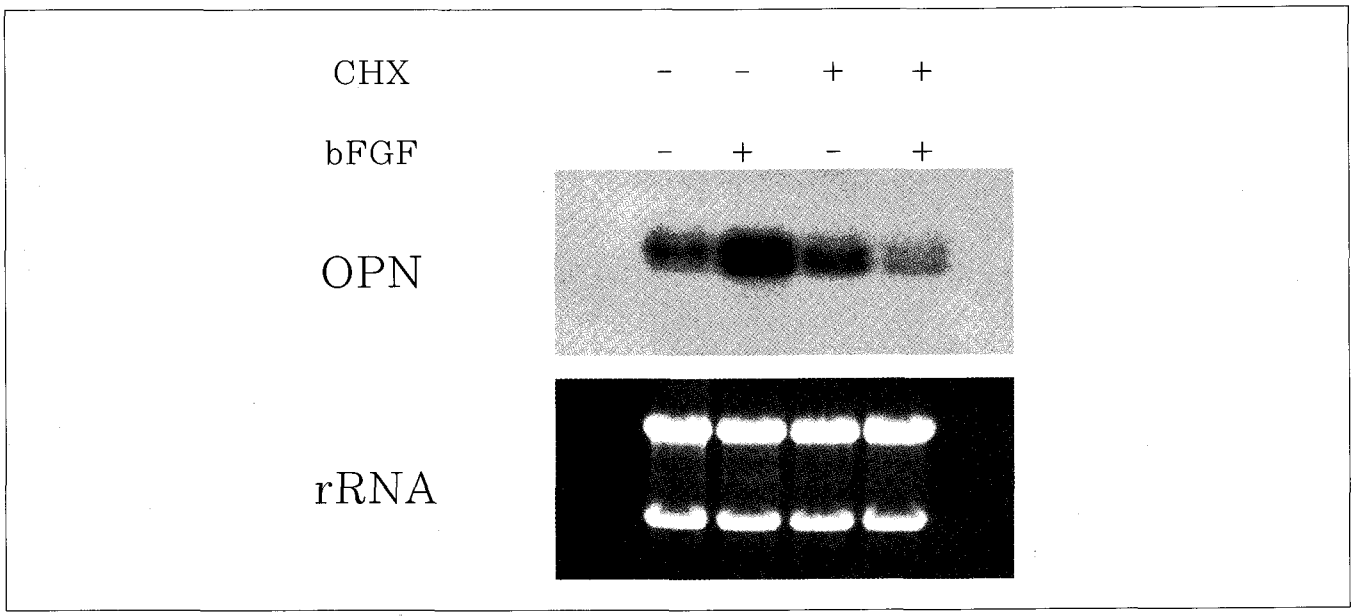

Fig. 4. The effect of bFGF on osteopontin gene expression in combination with cycloheximide.
ST-2 cells were plated at 6,5×10^{5}cells/100mm culture plate in 10% FBS. ST-2 cells were cultured for 3 days and treated for final 24h with or without bFGF (1 ng/*ml*) alone or in combination with cycloheximide (500 ng/*ml*). Total RNA (10 μg) were analyzed by Northern blot hybridization with cDNA probe for osteopontin. The amount of rRNA in the same samples was visualized by ethidium bromide staining. OPN, osteopontin; bFGF, basic fibroblast growth factor; CHX, cycloheximide.

for 3 days and prior to the addition of bFGF, cells were pretreated for 30 minutes with 500 ng/*ml* of cycloheximide in case of the treatment of both bFGF and cycloheximide.

Although, ST-2 cells slightly expressed OPN mRNA in the presence of cycloheximide, treatment with cycloheximide at 500 ng/*ml* effectively diminished the increase in OPN mRNA that was induced by bFGF in the absence of cycloheximide. This result implicates that bFGF induced OPN expression takes place at least in two steps. the direct induction of the transcription of a transcriptional regulatory protein, in turn, activates other genes and produce a delayed, secondary response.

Ⅳ. Discussion

OPN, a secreted glycosylated phosphoprotein of Mr-66,000, is rich in acidic amino acids and sialic acid, and it is expressed in various tissues, including bone[27], cartilage[27], kidney[28], decidua and placenta[29], smooth muscle cells of arteries[30] and cells of the immune system[31]. OPN is also expressed by carcinomas and sarcomas[32] and by mouse

epidermal cells treated with 12-O-tetradecanoylphorbol-13-acetate[33]. The primary sequence of OPN from various mammalian species exhibits several motifs, such as a cell surface-attachment sequence(Arg-Gly-Asp), numerous phosphorylation sites for various protein kinases, a polyaspartate motif, an N- and several O-glycosylation sites, three thrombin cleavage sites, and two heparin binding domains[34]. The tissue localization, primary structure, and theoretical considerations have led to the notion that osteopontin is various extracellular functions. These functions include the initiation and localization of apatite crystals in bone[27], the regulation of calcium and phosphate[35], the attachment of normal osteoclasts to the bone surface during the process of bone remodeling[36] and of neoplastic cells to the extracellular matrix[32], and the immune response[31].

In rat calvaria osteoblast, Thomas et al. showed that the expression pattern of OPN gene exhibits a biphasic pattern during the osteoblast development sequence, with mRNA levels during the period of cell proliferation that are about 25% those observed during the period of mineralization[37]. At the transcriptional level, OPN has been shown to be regulated by a number of hormones and growth factors associated with bone formation and bone remodeling, such as TGF-β1, TGF-β2, retinoic acid, endothelin and bone morphogenetic protein (BMP). Here, we investigated the effect of bFGF on OPN gene expression in rat bone marrow derived stromal cells.

In the present study, we showed that in ST-2 cells,

bFGF upregulates OPN, FN mRNA levels and downregulates type I collagen mRNA levels. But, there was no remarkable difference in ALP mRNA levels between two groups (Fig.1). Our studies are consistent with the previous studies having shown that bFGF inhibits type I collagen gene[13] and increases OPN mRNA[38].

OPN and FN contain RGD(Arg-Gly-Asp) sequence in their genes and are expressed during the period of proliferation in rat calvaria osteoblast. The GRGDS (RGD) sequence has been shown in numerous studies to be involved in cell attachment and spreading, intracellular signaling, and cell migration, mediated by its interaction with cell surface integrins[39]. Somerman et al.(1987)[40] showed that OPN was as effective as fibronectin in promoting the attachment and spreading of fibroblast. The attachment activity of OPN was long-lived, relative to that of fibronectin: OPN continued to enhance cell attachments for more than 24h, while the activity of fibronectin was diminished after 90 minutes. Considering these results, we could postulate that bFGF may involve in osteogenesis through the regulation of genes involved in cell attachment and spreading.

Our studies showed that bFGF upregulated OPN gene expression in a dose and time- dependent manner. bFGF increased OPN mRNA levels up to the concentration of 10ng/*ml* bFGF (Fig. 2). In the time course, OPN gene began to occur at around 3h with continuous increase up to 24h, then decreased to the basal level at 48h. Although, untreated cells expressed the same patterns those of treated groups, the amount of RNAs were lower (Fig. 3). In recent experiments, implantation of bFGF soaked beads onto the fetal coronal suture resulted in ectopic OPN expression, encircled by FGFR2 expression, and increased FGF/FGFR signaling resulted in shifting the cell proliferation/differentiation balance towards differentiation by enhancing the normal paracrine downregulation of FGFR2[26] and Sue[41] reported that FGF-mediated FGFR signaling has a important role in regulating the cranial bone growth and maintenance of cranial suture, and suggested that FGF-mediated FGFR signaling is involved in regulating the balance between the cell proliferation and differentiation through inducing the expression of OPN and Msx1 genes.

Increase in OPN gene expression by bFGF seems to lead new protein synthesis since northern blot analysis (Fig. 4) showed that bFGF didn't increase basal steady-state OPN mRNA level in the case of the pretreatment of cycloheximide (500ng/*ml*), whereas OPN mRNA level was markedly upregulated in ST-cells treated with bFGF alone. Cycloheximide, an inhibitor of protein synthesis, suppress of the transcription of secondary-response genes. The direct induction of the transcription of a small number of specific genes within about 30 minutes is known as 'the primary response', the products of these genes ,in turn, activate other genes and produce a delayed, secondary response. In our studies, we have shown that it requires at least 3 hours to induce OPN gene transcription by bFGF. This result suggests that induction of OPN gene transcription by bFGF requires more than two transcription steps. The promoter region of OPN contains several sites to which transcription factors are expected to bind. The major candidates for the transcriptional factors are steroid hormone receptors including vitamin D receptors, AP-1 (fos/jun), bHLHS, ets gene family and runt gene family etc[42]. Sato et al.(1998)[43] reported that in polyomar enhancer binding protein (PEBP) 2αA knockout mice, the marked decrease of OPN mRNA expression. This result suggests that the transcription of OPN gene is controlled by PEBP2αA.

In conclusion, the present study provides evidences that bFGF increased the level of OPN mRNA in a dose and time-dependent manner via the synthesis of certain transcriptional regulatory proteins in rat bone marrow-derived stromal cells.

Ⅴ. Summary

The treatment of bFGF(1ng/*ml*) upregulates OPN, FN mRNA levels and downregulates type I collagen mRNA levels. But, there was no remarkable difference in ALP mRNA levels between two groups. OPN gene expression increased in a dose-dependent manner up to 10ng/*ml* and OPN gene began to occur at around 3h with continuous increase up to 24h then decreased to basal level at 48h. 30 minutues pretreatment with cycloheximide (500 ng/*ml*), a protein synthesis inhibitor, prior to addition bFGF resulted in blocking bFGF induced OPN expression. These results suggest that bFGF increased the level of OPN mRNA in a dose and time-dependent manner via the synthesis of certain transcriptional regulatory proteins.

References

1. Burgess WH, Maciag T: The heparin-binding(fibroblast) growth factor family of proteins. Ann Rev Biochem 58:575-606, 1989.
2. Bikfalvi A, Klein S, Pintucci G et al.: Biological roles of fibroblast growth factor-2. Endocr Rev 18:26-45, 1997.
3. Klagsbrun M, Baird A: A dual receptor system is required for basic fibroblast growth factor activity. Cell 67:229-231, 1991.
4. Jaye M, Schlessinger J, Dionne CA: Fibroblast growth factor receptor kinases: Molecular analysis and signal transduction. Biochem biophys Acta 1135:229-231, 1992.
5. Baldin V, Roman A-M, Bosc-Bierne I et al.: Translocation of bFGF to the nucleus is G1 phase cell cycle specific in bovine aortic endothelial cells. EMBO J 9:1511-1517, 1990.
6. Nakanishi Y, Kihara K, Mizuno K et al.: Direct effect of basic fibroblast growth factor on gene transcription in a cell-free system. Oroc Natl Acad Sci USA 89:5216-5220, 1992.
7. Genz R, Rauscher FJ, Abate C et al. Science 243:1695-1699, 1991.
8. Kimelman D and Kirschner M: Synergistic induction of mesoderm by FGF and TGF-β and the identification of an mRNA coding for FGF in the early xenopus embryo. Cell 51:869-877, 1987.
9. Orr-Urtreger A, Givol D, Yayon A et al.: Developmental expression of two murine fibroblast growth factor receptors, flg and bek. Development 113:1419-1434, 1991.
10. Globus PK, Plouet J, Gospodarowicz D: Cultured bovine bone cells synthesize basic fibroblast growth factor and store it in their extracellular matrix. Endocrinology 124:1539-1547, 1989.
11. Rodan SB, Wesolowski G, Thomas KA et al.: Effects of acidic and basic fibroblast growth factors on osteoblastic cells. Connect Tissue Res 20:283-288, 1989.
12. Shen V, Kohler G, Huang J et al.: An acidic fibroblast growth factors stimulates DNA synthesis, inhibits collagen and alkaline phosphatase synthesis and induces resorption in bone. Bone Miner 7:205-219, 1989.
13. Hurley MM, Abreu C, Harrison JR et al.: Basic fibroblast growth factor inhibits type I collagen gene expression in osteoblastic MC3T3-E1 cells. J Biol Chem 268:5588-5593, 1993.
14. Pitaru S, Kotev-Emeth S, Noff D et al.: Effect of basic fibroblast growth factor on the growth and differentiation of adult stromal bone marrow cells: Enhanced development of mineralized bone-like tissue in culture. J Bone Miner Res 8:919-929, 1993.
15. Rifas L, McDermott MJ, Zhang SF et al.: Basic fibroblast growth factor inhibits growth and differentiation of human bone marrow osteoprogenitor cells. J Bone Miner Res 10(Suppl 1):S411, 1995.
16. Aspenberg P, Thorngren K, Lohmander LS: Dose-dependent stimulation of bone induction by basic growth factor in rats. Acta Orthop Scand 62:481-484, 1991.
17. Mayahara H, Ito T, Nagal H et al.: In vivo stimulation of endosteal bone formation by basic fibroblast growth factor in rats. Growth Factors 9:73-80, 1993.
18. Nagai H, Tsukuda R, Mayahara H: Effects of basic fibroblast growth factor(bFGF) on bone formation in growing rats. Bone 16:367-373, 1995.
19. Nakamura T, Hanada K, Tamura M et al.: Stimulation of endosteal bone formation by systemic injections of recombinant basic fibroblast growth factor in rats. Endocrinology 136:1276-1284, 1995.
20. Kawaguchi H, Kurokawa T, Hanada K et al.: Stimulation of fracture repair by recombinant human basic fibroblast growth factor in normal and streptozotocin-diabetic rat. Endocrinology 135:774-781, 1994.
21. Muenke M, Schell U: Fibroblast-growth-factor receptor mutations in human skeletal disorders. Trends Genet 11:308-313, 1994.
22. Wilkie AOM, Wall SA: Craniosynostosis: novel insights into pathogenesis and treatment. Curr Opin Neurobiol 9:146-152, 1996.
23. Wilkie AOM, Morriss-Kay GM, Jones EY et al.: Functions of fibroblast growth factors and their receptors. Curr Biol 5:500-507, 1995.
24. Cohen M Jr: Transforming growth factor βs and fibroblast growth factors and their receptors: Role in sutural biology and craniosynostosis. J Bone Miner Res 12:322-331, 1997.
25. Orr-Urtreger A, Bedford MT, Burakova T et al.: Developmental localization of the splicing alternatives of fibroblast growth factor receptor-2(FGFR2). Dev Biol 158:475-486, 1993.

26. Iseki S, Wilkie AOM, Heath JK et al.: Fgfr2 and osteopontin domains in the developing skull vault are mutually exclusive and can be altered by locally applied FGF2. Development 124:3375-3384, 1997.
27. Mckee MD, Glimcher MJ, Nanci A: High-resolution immunolocalization of osteopontin and osteocalcin in bone and cartilage during endochondral ossification in the chicken tibiae. Anac Rec 234:479-492, 1992.
28. Yoon K, Buenaga R, Rodan GA: Tissue specificity and developmental expression of rat osteopontin. Biochem Biophys Res 148:1129-1136, 1987.
29. Mark MP, Butler W, Prince CW et al.: Developmental expression of 44-kDa bone phosphoprotein (osteopontin) and bone γ-carboxyglutamic acid (Gla)-containing protein (osteocalcin) in calcifying tissue of rat. Differentiation 37:123-136, 1988.
30. Giachelli CM, Bae N, Almeida M et al.: Osteopontin is elevated during neointima formation in rat arteries and in a novel component of human atherosclerotic plaques. J Clin Invest 92:1686-1696, 1993.
31. Patarca R, Freeman GJ, Singh RP et al.: Structural and functional studies of the early T-lymphocyte activation 1 (Eta-1) gene: definition of a novel T-cell dependent response associated with nucleotide sequence. J Biol Chem 265:14432-14438, 1990.
32. Senger DR, Perruzzi CA, Papadoploulos A: Elevated expression of secreted phosphoprotein I (osteopontin, 2ar) as a consequence of neoplastic transformation. Anticancer Res 9:1291-1300, 1989.
33. Smith JH, Denhardt DT: Molecular cloning of a tumor promoter-inducible mRNA found in JB6 mouse epidermal cells: induction is stable at high, but not at low cell densities. J Cell Biochem 34:13-22, 1987.
34. Denhardt DT, Guo X: Osteopontin: a protein with diverse functions. FASEB J 7:1475-1482, 1993.
35. Glimcher MJ: Mechanism of calcification: role of collagen fibrils and collagen-phosphoprotein complexes in vitro and in vivo. Anat Rec 224:139-153, 1989.
36. Reinholt FP, Hultenby K, Oldberg A et al.: osteopontin: a possible anchor of osteoblasts to bone. Proc Natl Acad Sci USA 87:4473-4475, 1990.
37. Owen TA, Aronow M, Shalhoub V et al.: Progressive development of the rat osteoblast phenotype in vitro: Reciprocal relationships in expression of genes associated with osteoblast proliferation and differentiation during formation of the bone extracellular matrix. J Cell Physiol 143:420-430, 1990.
38. Rodan SB, Wesolowski G, Yoon K et al. : Opposing effects of fibroblast growth factor and pertussis toxin on alkaline phosphatase, osteopontin, osteocalcin, and type I collagen mRNA levels in ROS 17/2.8 cells. J Biol Chem 264:19934-19941, 1989.
39. Oldberg , Franz n A, Heineg rd D: Cloning and sequence analysis of rat bone sialoprotein(osteopontin) cDNA reveals an Arg-Gly-Asp cell binding sequence. Proc Natl Acad Sci USA 83:8819-8823, 1986.
40. Somerman MJ, Prince CW, Sauk JJ et al.: Mechanism of fibroblast attachment to bone extracellular matrix: Role of 44 kilodalton bone phosphoprotein. J Boen Miner Res 2:259-265, 1987.
41. 서경환: FGF signaling이 두개봉합부의 초기형태발생 및 유지기전에 미치는 영향. 경북대학교 치의학박사 학위논문 1999.
42. Guo X, Zhang YP, Mitchell DA et al.: Identification of a ras-activated enhancer in the mouse osteopontin promoter and its interaction with a putative ETS-related transcription factor whose activity correlates with the metastatic potential of the cell, Mol Cell Biol 15:476-487, 1995.
43. Sato M et al.: Transcriptional regulation of osteopontin gene in vivo by PEBP2αA/CBFA1 and ETS1 in the skeletal tissues. Oncogene 17:1517-1525, 1998.

국문초록

Basic fibroblast growth factor가 osteopontin 유전자 발현에 미치는 영향

배원수 · 김현정 · 류현모* · 김영진 · 남순현

경북대학교 치과대학 소아치과학교실, 구강생화학교실*

Fibroblast growth factor(FGF)는 세포의 성장과 이동, 분화와 생존과 관련된 여러가지 과정을 조절하는 것으로 알려져 있다. 이들의 prototype인 FGF-1과 FGF-2는 FGF receptors (FGFRs)를 통해서 세포내로 신호를 전달하는데, 두개봉합부의 조기융합을 보이는 craniosynostosis는 FGFRs중, 특히 FGFR-2의 point mutation에 의해서 야기된다. 최근 여러 보고에서 FGF/FGFR 신호전달은 골아세포의 분화에 있어 필수적인 역할을 한다고 하였으며, bFGF soaked bead를 쥐 두개골의 시상봉합부의 mid-mesenchyme과 osteogenic front부위에 적용하였을 때 osteopontin(OPN) 유전자의 발현을 유도한다고 하였다. 이에 본 연구에서는 ST-2 cell line를 이용한 in vitro 실험에서 bFGF가 OPN 유전자 발현에 미치는 영향과 그 기전을 Northern blot analysis를 통해서 연구하고자 하였다. 1 ng/ml bFGF의 투여는 OPN, fibronectin 유전자 발현을 증가시켰으며, type I collagen 유전자 발현은 감소시켰으나 alkaline phosphatase 유전자 발현에는 영향을 미치지 않았다. OPN은 그 발현양상이 bFGF의 농도 증가에 따라 증가하는 양상으로 나타났으며, 시간경과에 따른 발현양상도 bFGF 투여 3시간째부터 bFGF를 투여한 군에서 대조군에 비해 높게 나타났으며 이는 24시간까지 시간의 경과에 따라 증가하는 양상을 보였다. 단백질 합성 억제제인 cycloheximide를 처리한 군에서는 OPN의 증가 양상을 보이지 않았는데 이는 bFGF에 의한 OPN 유전자 발현이 새로운 전사조절 단백질 합성 등의 여러 단계를 거쳐서 일어남을 의미한다. 결론적으로 bFGF는 새로운 전사조절 단백질의 합성을 통해서 OPN 유전자 발현을 농도 및 시간 의존적으로 증가시킨다.

주요어: Basic Fibroblast growth factor, Osteopontin, Craniosynostosis, Fibroblast growth factor receptor