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ARTICLES

# A TGF-β-Inducible Cell Adhesion Molecule, βig-h3, Is Downregulated in Melorheostosis and Involved in Osteogenesis

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**Abstract** Melorheostosis is a rare bone disease characterized by linear hyperostosis and associated soft tissue abnormalities. The skin overlying the involved bone lesion is often tense, shiny, erythematous, and scleodermatous. In order to look for genes differentially expressed between the normal and involved skin, we cultured skin fibroblasts from the skin lesions of several afflicted patients, and identified differentially expressed genes by reverse dot-blot hybridization. We found that the genes human TGF-β-induced gene product (βig-h3), osteoblast-specific factor 2, osteonectin, fibronectin, and type I collagen were all downregulated in the affected skin fibroblasts, with βig-h3 the most significantly affected. The expression of βig-h3 was induced by TGF-β in both affected and normal fibroblasts. In an effort to determine the mechanism of bone and skin abnormalities in melorheostosis, we made recombinant βig-h3. Both immobilized and soluble recombinant βig-h3 proteins with or without an RGD motif inhibited bone nodule formation of osteoblasts in vitro. Taken together, our results suggest that altered expression of several adhesion proteins may contribute to the development of hyperostosis and concomitant soft tissue abnormalities of melorheostosis, with βig-h3 in particular playing an important role in osteogenesis. J. Cell. Biochem. 77:169–178, 2000. 

**Key words:** melorheostosis; TGF-β-induced gene product (βig-h3); osteoblast-specific factor 2; fibronectin; osteoblast differentiation

Melorheostosis is a rare disease characterized by a "flowing" hyperostosis of the cortex, resembling wax dripping down one side of a candle. The condition may affect only one bone, one limb, or multiple bones. In rare instances, the disease has been reported to involve more than one limb and the trunk, as well as the spine, skull, and facial bones [Greenspan, 1991]. A number of other disorders have been found in association with melorheostosis, including scleroderma, osteopoikilosis, osteopathia striata, neurofibromatosis, tuberous sclerosis, and hemangioma [Iglesias et al., 1994; Kawabata et al., 1984; Roger et al., 1994; Wagers et al., 1972]. Histologic studies demonstrated that the skeletal lesion is characterized

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by endosteal thickening during infancy and childhood and then by periosteal new bone formation during adulthood [Greenspan, 1991]. Bony lesions are sclerotic with thickened irregular lamellae that may occlude the haversian systems. Marrow fibrosis is also observed. Unlike the case in true scleroderma, the collagen of the sclerodermatous skin lesions of melorheostosis appears normal [Wagers et al., 1972]. Thus, this dermatosis has been called linear melorheostotic scleroderma. Of unknown etiology, it was suggested that an early postzygotic mutation of the mesenchyme may result in asymmetric involvement of skeletal structures, with concomitant vascular and hamartomatous changes in the overlying soft tissues [Fryns, 1995]. To understand the mechanisms responsible for the localized hyperostosis and the soft tissue abnormalities observed in melorheostosis, we have undertaken studies to determine which genes are deregulated in melorheostosis. We cultured fibroblasts from both affected skin and normal skin and have screened differentially expressed genes by reverse dot-blot hybridization. We found that several genes coding for adhesion proteins were downregulated, with transforming growth factor-β (TGF-β)-induced gene product (βig-h3) the most significantly affected. In addition, we demonstrated that βig-h3 was able to regulate osteoblast differentiation.

## MATERIALS AND METHODS

### Specimens

Normal and affected skin tissues were obtained from two patients with melorheostosis during an orthopedic surgical operation in Kyungpook National University Hospital. One patient was a 5-year-old girl, who had an equinovarus deformity in one foot and simultaneous hyperostosis in the affected clubfoot and overlying sclerodermatous skin. The other sample was from a 21-year-old woman who displayed "flowing" hyperostosis of the left ulna and overlying sclerodermatous skin. Two samples were isolated from this patient, with a 6-month interval.

### Primary Cell Culture

The tissue specimens were washed two to three times in phosphate-buffered saline (PBS) and finely minced, and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Gibco-BRL) at 37°C in 5% $CO_2$. After 15 days, the fibroblasts were grown out from the tissues; the tissues were removed and cells were trypsinized and replated in fresh medium.

### Preparation of RNA

Skin fibroblasts from normal and affected skin were lysed with 600 μl of GIT (guanidinium isothiocyanate) solution [Chomzynski and Sacchi, 1987]. Total RNA was extracted from each culture by adding 60 μl of 2 M sodium acetate (pH 4.0), 600 μl of water-saturated phenol, and 120 μl of chloroform-isoamyl alcohol (49:1) and vortexed briefly. After incubation on ice for 15 min, the mixture was centrifuged at 12,000 rpm for 20 min at 4°C, and the aqueous phase was transferred to a new tube. The aqueous phase was mixed with an equal volume of isopropanol and placed at −20°C for at least 1 h to precipitate RNA. RNA was recovered by centrifugation at 12,000 rpm for 20 min at 4°C. The RNA pellet was rinsed with 1 ml of 75% cold ethanol in diethylpyrocarbonate (DEPC)-treated water, dried in air, and dissolved in DEPC-treated water. The quality and quantity of RNA were determined by the absorbance at 260 nm and 280 nm with an ultraviolet (UV)/visible spectrophotometer.

### Preparation of Probe

A total of 20 μg of total RNA was incubated with 1 μg oligo(dT) primer at 70°C for 10 min and then placed on ice. A total of 5 μl of cDNA synthesis buffer composed of 100 mM Tris-HCl (pH 8.3), 100 mM KCl, 8 mM $MgCl_2$, and 10 mM dithiothreitol, 1 μl of 100 mM dNTP, 2.5 μl (200 U/μl) of Molony murine leukemia virus reverse transcriptase (Gibco-BRL), 1 μl (40 units) of human placental RNase inhibitor (Gibco BRL) and 2 μl of $^{32}$P-dCTP were added and the first-strand cDNA synthesis was carried out at 42°C for 50 min. The DNA probe was made by random priming of mRNA by reverse transcription. Specifically, 10 μl of random primers was added to the first-strand cDNA; the mixture was incubated at 95°C for 5 min and then placed at room temperature for 10 min. A total of 20 μl of Klenow buffer, 12 μl of $^{32}$P-dCTP, and 4 μl of Klenow (1 U/μl) was added to the first-strand cDNA reaction mixture. The random primer reaction was allowed to proceed at 37°C for 30 min and then stopped

by the addition of 10 μl of 0.5 M EDTA. A "nick column" (Amersham, Arlington, IL) was used to separate incorporated from unincorporated radioactive nucleotides.

### Reverse Dot-Blot Hybridization

A Gene Screen Plus™ (Du Pont, Boston, MA) membrane was used for blotting. Specifically, cDNA clones randomly selected from a unidirectional human dermal papilla cell cDNA library were fixed to the membrane and hybridized [Mou et al., 1994; Liew et al., 1994; Chalifour et al., 1994]. Briefly, the membranes were cut into the size of the slot-blot manifold (Bio-Rad, Hercules, CA) and wet with distilled water for 5 min. The membrane was placed into the manifold and clamped. The plasmid DNA harboring EST genes were denatured for 10 min at room temperature in 0.25 N NaOH/0.5 M NaCl and diluted in 0.1× saline-sodium citrate (SSC)/0.125 N NaOH; 200 ng DNA was then fixed per slot. The membranes were removed from the manifold, neutralized by rinsing in 0.5 M NaCl/0.5 M Tris, pH 7.5, dried in air, and then immobilized by UV cross-linking at 120 mJ, using an UV Stratalinker™ (Stratagene). The blots were hybridized with the radiolabeled probe in ExpressHyb™ hybridization solution (Clontech, Palo Alto, CA) according to the manufacturer's instructions. Briefly, the membranes were wet in 2× SSC for 1 min and prehybridized in 5 ml of ExpressHyb hybridization solution at 68°C for 30 min. The prehybridization buffer was removed and replaced with 5 ml of fresh hybridization solution containing radioactive DNA probe ($2\times 10^6$ cpm/ml) from normal or affected skin fibroblasts and the membranes were hybridized for 1 h. The membranes were then washed with 2× SSC/0.05% SDS (sodium dodecyl sulfate) at room temperature for 40 min and with 0.1× SSC/0.1% SDS at 50°C for 40 min to remove nonspecific bound probe. After brief air drying, the membranes were exposed to X-OMAT× (Kodak) film for 3–7 days before development. pBluescript $SK^+$ vector plasmid served as the negative control for spurious cross-hybridization to vector DNA.

### Northern Blot Hybridization

A total of 10 μg of total RNA from normal and affected skin fibroblasts was denatured in loading buffer containing 50% formamide/18% formaldehyde/40 mM MOPS for 20 min at 65 °C and then fractionated on 0.7% agarose gel containing 18% formaldehyde and 40 mM MOPS. The gel was denatured with 0.05 M NaOH/1.5 M NaCl for 30 min, neutralized with 0.5 M Tris-Cl (pH 7.4)/1.5 M NaCl for 20 min, and rinsed with 20× SSC for 45 min. The nylon membrane (Amersham) was wet in DEPC-treated water and then soaked in 20× SSC for 10 min. The transfer was allowed to proceed overnight, the membrane was rinsed briefly in 2× SSC, dried in air, baked for 1 h at 80°C under vacuum, and then cross-linked by exposure to UV light. Hybridization was performed as described in the previous section.

### Collagen Production

Confluent skin fibroblasts cultured in six-well culture plates were washed with PBS and replaced with serum-free media supplemented with 1% bovine serum albumin (BSA). After 24 h of incubation, TGF-β (0, 1, and 5 ng/ml) was added and incubated for 24 h. Cells were labeled with $^3$H-proline (4 μCi/ml) in the presence of ascorbic acid (50 μg/ml) and β-aminopropionitrile (BAPN) (50 μg/ml) for the last 6 h. After 0.1 M phenylmethylsulfonyl fluoride (PMSF) was added, the medium was collected and centrifuged at 12,000 rpm for 10 min at 4°C. The supernatant was collected into fresh tubes. The radioactivity of the medium was determined by trichloroacetic acid (TCA) precipitation [Grover and Adamaon, 1985]. Samples with equal amounts of radioactivity were used for pepsin digestion. Pepsin digestion was performed by adding 0.5 M glacial acetic acid, 25 mM EDTA, 1 mM PMSF, and 0.3 mg/ml pepsin for 3 h at 15°C. The precipitated proteins were washed with 70% EtOH and denaturated in sodium dodecyl sulfate (SDS) sample buffer with heating at 100°C for 2–3 min. Samples were analyzed by SDS-polyacrylamide gel electrophoresis (PAGE), using a 9% gel, and subjected to fluorography using EN$^3$HANCE™ (NEN) as a fluorography enhancer according to the manufacturer's instructions.

### Production of Recombinant βig-h3

A *Nde*I/*Bgl*II fragment of βig-h3 cDNA cloned in pBluescript was inserted into the *Eco*RV and *Eco*RI sites of pET-29b$^+$ (Novagen), known as pHisβ-a. This fragment corresponds

amino acids 175–653 of βig-h3. A clone in the correct orientation was selected and sequenced. βig-h3 protein expression was induced by 1 mM IPTG for 3 h and inclusion bodies dissolved in 8 M urea in 20 mM Tris-HCl (pH 7.8) containing 0.5 M NaCl and 5 mM imidazole. The truncated and his-tagged βig-h3 protein was purified using Ni-NTA resin (Qiagen) according to the manufacturer's recommendations. The purified βig-h3 was dialyzed sequentially from high to low urea in 20 mM Tris-HCl buffer containing 50 mM NaCl. Two more βig-h3 plasmids, pHisβ-c and pHisβ-d, were constructed in pET-29b$^+$ vector to make recombinant βig-h3 proteins with and without an RGD sequence. To make the pHisβ-c plasmid encoding amino acids 1653, 1.35-kb *Nco*I fragment of βig-h3 cDNA in pBluescript was fused to the *Nco*I site of pHisβ-a. pHisβ-d encoding amino acids 1–640 was derived from pHisβ-c by cutting out a 3'-fragment of pHisβ-c plasmid with *Aoc*I and *Not*I, followed by blunting and self-ligation. These two plasmids were sequenced to confirm their integrity. Proteins were induced and purified as described above.

### Bone Nodule Formation

An osteoblastic cell line, KS483, was subcloned from mouse osteoblastic KS-4 cells previously described by Dr. Yamashita [1996], and grown in α-minimal essential medium (MEM) supplemented with 10% FBS at 37°C in a humidified atmosphere of 95% air-5% $CO_2$. Cells were plated at density of $3.6 \times 10^4$ cells/well in a 48-well tissue culture plate and cultured for 3 days. The media were replaced and supplemented with ascorbic acid (50 μg/ml), β-glycerophosphate (5 mM), and dexamethasone (10 nM) to stimulate differentiation in the presence or absence of soluble proteins. To stain bone nodules, cells were rinsed twice with the ice-cold PBS and incubated for 10 min in 40 mM Alizarin red solution at room temperature. Finally, cells were washed twice with distilled water followed by washing with PBS.

### Treatment of βig-h3 and Fibronectin

The 48-well culture plates were coated with 5, 10, 50, and 100 μg/ml of each Hisβ-c, Hisβ-d and FN overnight at 4°C and then washed twice with PBS. In order to block unbound sites, each well was incubated with 20 mg/ml of heat-denatured BSA for 90 min at room temperature. Soluble Hisβ-a and FN were added at concentrations of 5, 10, 50 and 100 μg/ml when cells reached to the confluency.

## RESULTS

### Screening and Isolation of Differentially Expressed Genes

Total RNA free of genomic DNA was isolated from normal and affected skin fibroblasts as described under Materials and Methods and reverse transcribed using an oligo(dT) primer. Labeling of first-strand cDNA by random-primed reverse transcription usually resulted in specific activities of $10^5$ cpm/μg DNA. The DNA library panels from human dermal papilla cells were hybridized with the labeled cDNA probes. Most dots exhibited the same degree of intensity with cDNA probes generated from mRNA of both normal and affected skin fibroblasts. However, several dots showed significantly higher or lower expression levels in affected skin fibroblasts than in normal skin fibroblasts (Fig. 1, arrows). Dots of higher expression level and lower expression level were 57 and 23 out of 1,500, respectively. Spots that showed the most dramatic alteration of expression were selected for further analysis.

### Northern Blot Analysis

To confirm differential expression of clones selected in reverse dot hybridization, we performed Northern blot hybridization. In our hands, several genes showing higher expression in affected skin fibroblasts in reverse dot-blot hybridization were not significantly different by Northern blot hybridization (data not shown). By contrast, other genes clearly showed lower expression in affected skin fibroblasts by Northern blot hybridization. Passage 1 skin fibroblasts from patients with melorheostosis showed considerably decreased levels of βig-h3 mRNA in affected skin fibroblasts as compared with normal skin fibroblasts (Fig. 2). Other differentially expressed genes—OSF-2, osteonectin, fibronectin, and type I collagen—selected by reverse dot-blot hybridization, were also tested with the same membranes showing decreased expression in affected skin fibroblasts (Fig. 2). Among them, βig-h3 appeared to be the most dramatically affected.

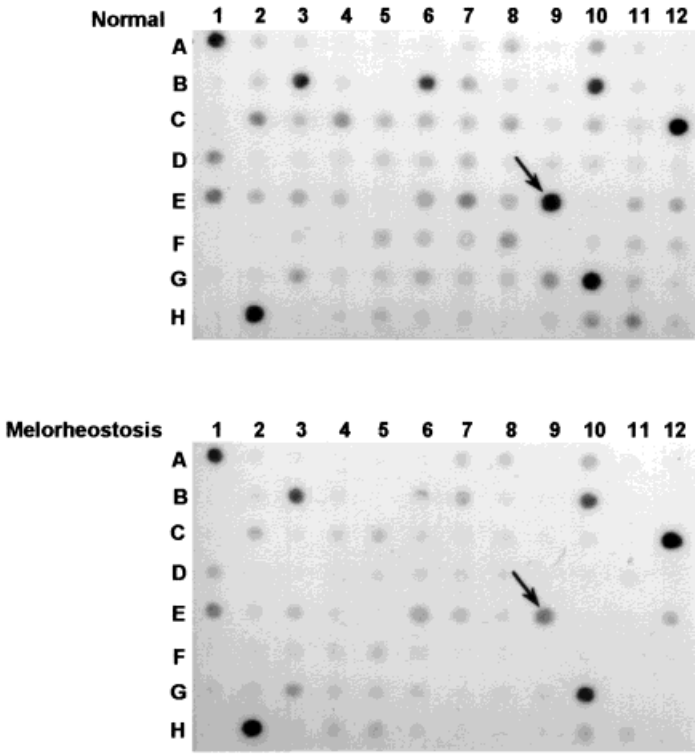


**Fig. 1.** Reverse dot-blot hybridization of panels of human dermal papilla cell cDNA library with first-strand cDNA of mRNA isolated from normal and affected skin fibroblasts of melorheostosis patient. Arrows, position of differentially expressed gene, βig-h3. A1 dot is β-actin as a positive control; A2 dot is pBluescript $SK^+$ vector plasmid as a negative control.

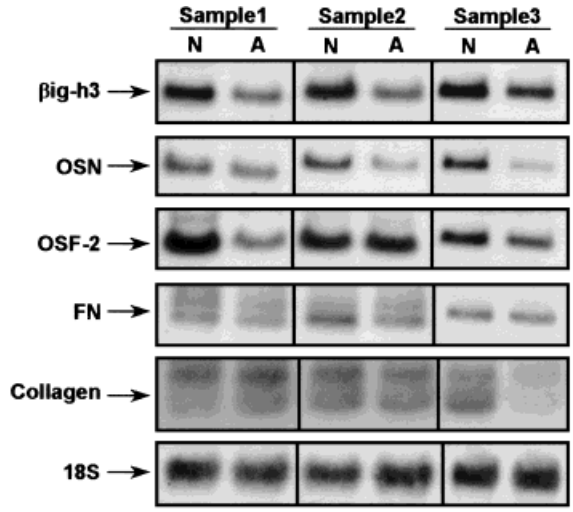


**Fig. 2.** Northern blot hybridization of differentially expressed genes in normal (N) and affected (A) skin fibroblasts cultured from three samples taken from melorheostosis patients. βig-h3, human transforming growth factor-β (TGF-β)-induced gene product; OSN, osteonectin; OSF-2, osteoblast specific factor 2; FN, fibronectin; collagen, α1(I) procollagen; 18S, 18S rRNA.

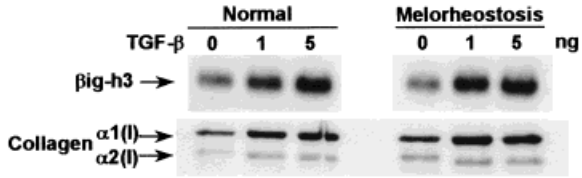


**Fig. 3.** Effect of transforming growth factor-β (TGF-β) on βig-h3 mRNA levels, collagen synthesis, and fibronectin (FN) synthesis in normal and affected skin fibroblasts. Cells were treated with indicated concentrations of TGF-β for 24 h. Collagen synthesis was measured after labeling with $^3$H-proline for last 6 h. Fibronectin (FN) synthesis was measured by immunoprecipitation after labeling with $^{35}$S-methionine for last 2 h.

## βig-h3 mRNA Levels Were Induced by TGF-β in Both Normal and Affected Skin Fibroblasts

We examined the effect of TGF-β on βig-h3 mRNA levels in normal and affected skin fibroblasts. Confluent cells were treated with different concentrations of TGF-β (0, 1, and 5 ng/ml). After incubation for 24 h, RNA was extracted from cells, and Northern blot hybridization was performed with probe DNA of βig-h3. The expression of βig-h3 was induced by TGF-β in both affected and normal cells, indicating that there was no difference in responsiveness to TGF-β between affected and normal skin fibroblasts (Fig. 3). We also examined the effect of TGF-β on collagen (Fig. 3) and fibronectin synthesis (data not shown). As in βig-h3, both collagen and fibronectin synthesis were also in-

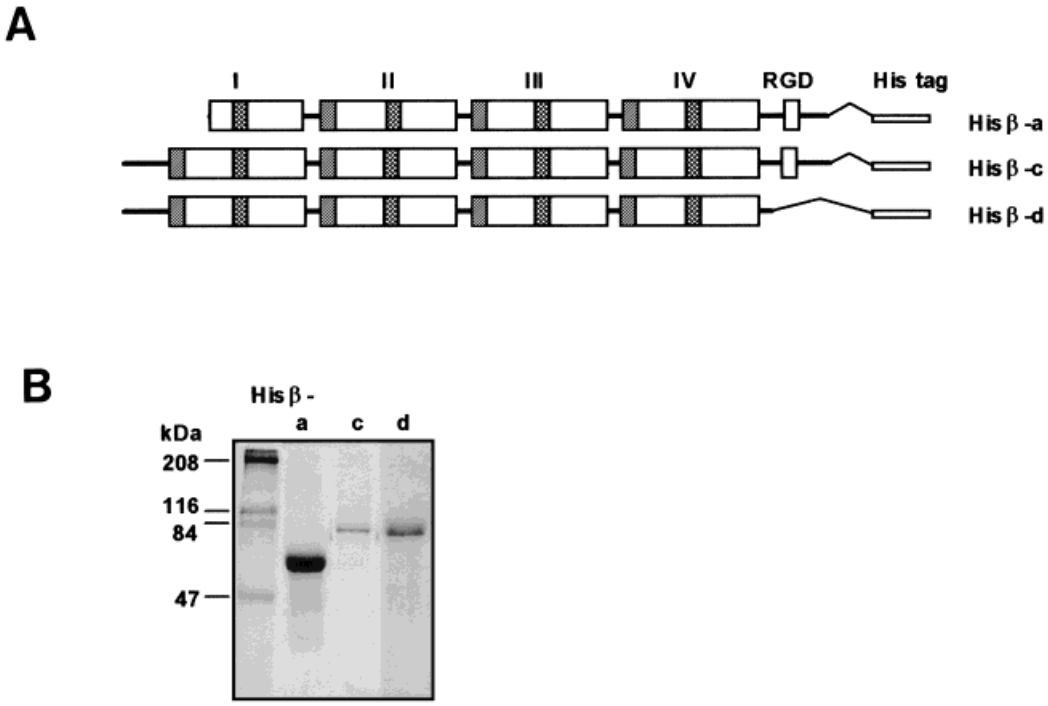


**Fig. 4.** Diagrammatic illustration of the recombinant human βig-h3 constructs (**A**) and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of purified recombinant proteins (**B**). **A**: Four repeated domains and RGD sequence are shown schematically. The two highly homologous regions of each domain are indicated as hatched and cross-hatched boxes. All recombinant proteins were produced as fusion proteins with histidine tag. **B**: Samples were analyzed using 10% polyacrylamide gels stained with Coomassie blue dye. Lanes were loaded with the purified proteins Hisβ-a (**lane 2**), Hisβ-c (**lane 3**), and Hisβ-d (**lane 4**). Molecular markers (**lane 1**) were run together and indicated as dashes. Numbers indicate the molecular masses (kDa) of known standards.

duced equally by TGF-β in both affected and normal skin fibroblasts.

### Immobilized and Soluble Recombinant βig-h3 Proteins Inhibited Bone Nodule Formation of Osteoblasts

We made three recombinant βig-h3 proteins in *Escherichia coli*, using a pET vector and purified using Ni-NTA resin. As shown diagrammatically in Figure 4A, two have an RGD sequence at the C-terminus, and the other does not. All βig-h3 proteins appeared to be highly pure on SDS-PAGE (Fig. 4B). To study the effect of βig-h3 on osteoblast differentiation, we used a mouse osteoblast cell line, KS483. Bone nodule formation was examined at day 21 in βig-h3- or FN-coated wells. As shown in Figure 5, βig-h3-coated wells showed progressively less Alizarin-positive bone nodules as the concentration of βig-h3 increased. At 100 μg/ml of βig-h3, no Alizarin-stained bone nodules were observed. There was no difference between RGD-containing βig-h3 and RGD-lacking βig-h3 in inhibitory activity of bone nodule formation indicating sequences other than RGD are critical [Yamada, 1991]. At lower concentrations, plasma fibronectin did not affect bone nodule formation but at higher concentrations, it slightly inhibited nodule formation. βig-h3-mediated inhibition of bone nodule formation was also observed when it was added as a soluble form in a dose-dependent manner. As known previously, soluble fibronectin significantly inhibited bone nodule formation (Fig. 6).

## DISCUSSION

In order to find genes that are deregulated in melorheostosis, we isolated fibroblasts from the normal skin and the affected skin of same patient, and performed a reverse dot-blot hybridization against cDNA panels containing about 1,500 different cDNA. We found the expression of genes for several extracellular matrix proteins to be altered, with βig-h3 the most dramatically downregulated in fibroblasts cultured from the affected skin. βig-h3 was first

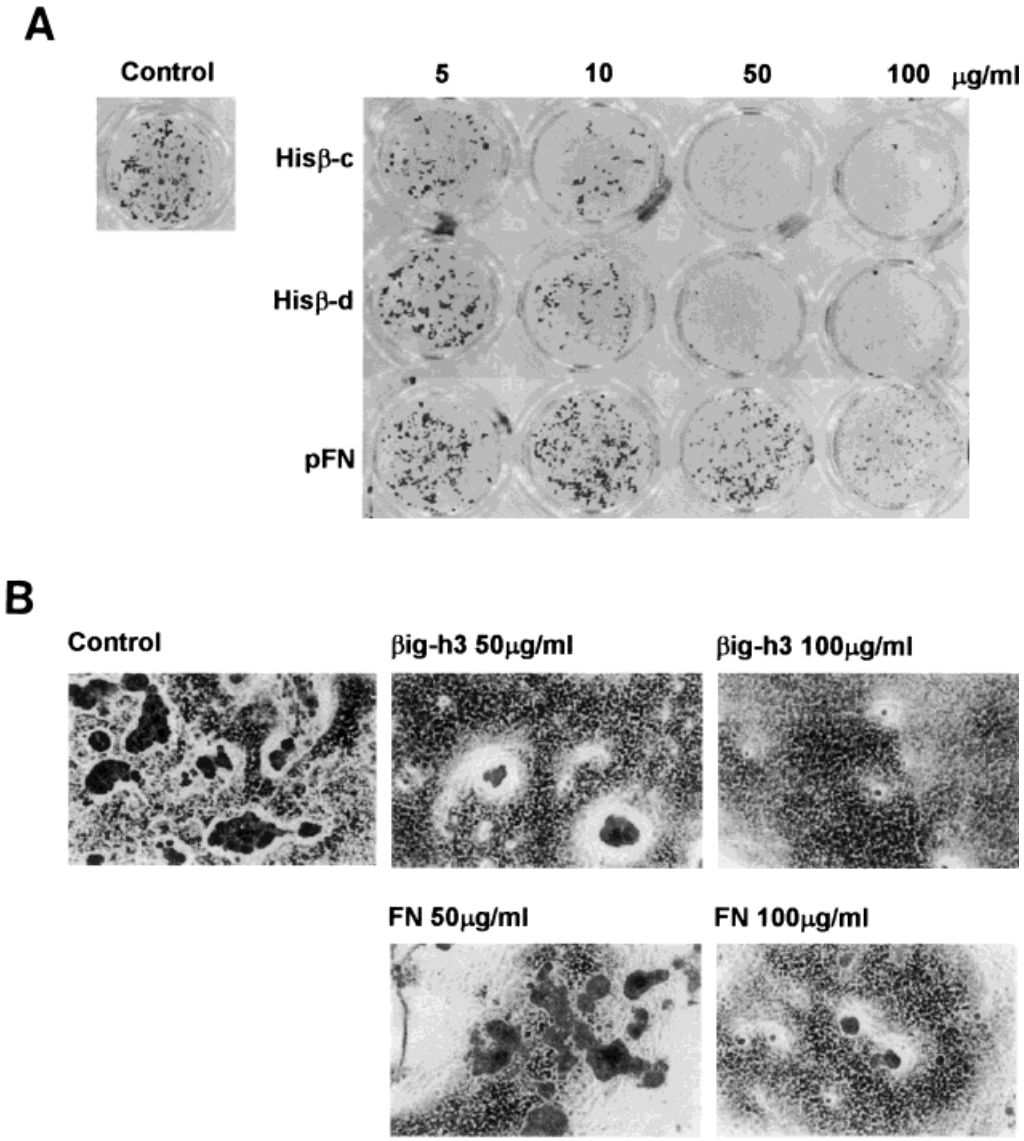


**Fig. 5.** Coated βig-h3 proteins inhibited bone nodule formations. Confluent KS483 cells cultured on different concentrations of βig-h3 or fibronectin (FN)-coated wells were initiated to form bone nodules and were stained with Alizarin red on days 21. Macroscopic (**A**) and microscopic (40×) (**B**) pictures are shown. The control wells were not coated. Numbers of Alizarin red-positive bone nodules were decreased by the treatment of two recombinant βig-h3 proteins, Hisβ-c and and Hisβ-d.

identified by Skonier et al. [1992], who isolated it by screening a cDNA library made from a human lung adenocarcinoma cell line (A549) treated with TGF-β. This novel protein consists of 683 amino acids containing an N-terminal secretory sequence and a C-terminal Arg-Gly-Asp (RGD) sequence that can serve as a ligand-recognition site for several integrins. βig-h3 also contains short amino acid regions homologous to similar motifs in *Drosophila* fasciclin-I and four homologous internal domains, which can be folded into a potential bivalent structure and could act as a bridge between cells expressing the appropriate ligand.

It has been suggested that βig-h3 might be involved in mediating some of the signals of TGF-β, since this gene was induced in several cell lines whose proliferation was affected by TGF-β [Skonier et al. 1992]. Transfection of βig-h3 expression plasmids into Chinese hamster ovary (CHO) cells led to a marked decrease in the ability of these cells to form tumors in nude mice and also demonstrated that the purified βig-h3 protein inhibited the attachment

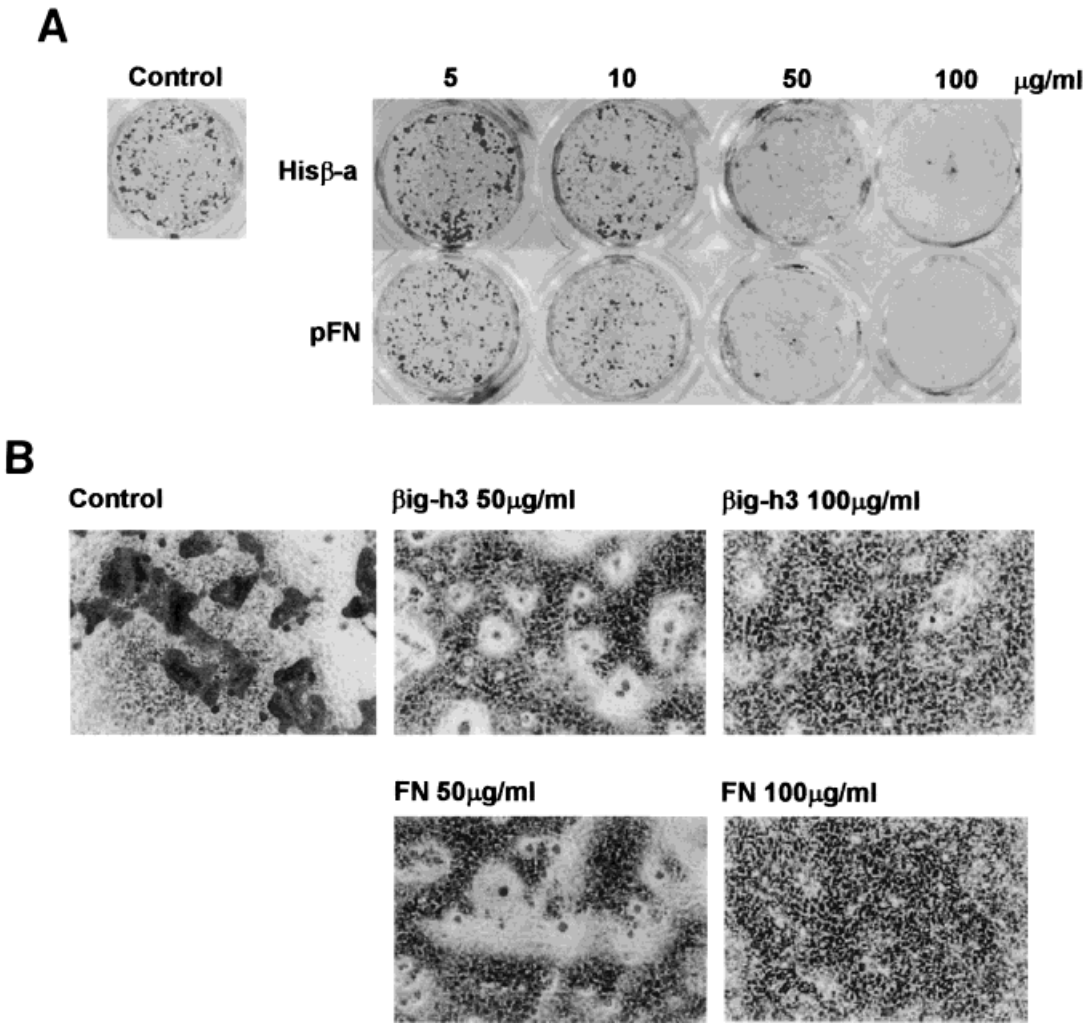


**Fig. 6.** Soluble βig-h3 inhibited bone nodule formation. Confluent KS483 cells cultured were initiated to form bone nodules in the presence or absence of different concentrations of recombinant βig-h3 (Hisβ-a) or fibronectin (FN) and then stained with Alizarin red on days 21. Media were changed every 3 days supplemented with Hisβ-a or FN. Macroscopic (**A**) and microscopic (40×) (**B**) pictures were shown. Both Hisβ-a and FN inhibited bone nodule formation.

of A549, HeLa, and WI-38 cells to plastic in serum-free media [Skonier et al. 1994].

Recently, two other groups [Rawe et al., 1997; Hashimoto et al., 1997] independently cloned βig-h3 gene from the cornea and the cartilage. They found that βig-h3 was a collagen fiber-associated protein. Taken together, it appears that βig-h3 functions as an important factor in morphogenesis and in the interactions between cells and the extracellular matrix of various tissues. However, nothing has been reported about the role of βig-h3 in bone metabolism in normal and diseased states. In this regard, it is interesting to note that the expression of βig-h3 was dramatically downregulated in human bone marrow stromal cells in response to dexamethasone, which is known to modulate their differentiation into bone cells [Dieudonne et al., 2000]. Indeed, our results indicated both soluble and coated βig-h3 clearly inhibited bone nodule formation.

Although it is unclear how βig-h3 regulates osteoblast differentiation, βig-h3 seems to act on osteoblast differentiation somewhat in a similar manner with FN; FN is known to act in the early stages of osteogenesis and is detected in the area of bone tissue where recruitment and commitment of osteoblast precursors occur [Gronowicz et al., 1991; Weiss and Reddi, 1980]. Further, in situ hybridization showed

that βig-h3 was expressed in preosteoblasts in the primary spongiosa and in both periosteum and perichondrium but not in osteoblasts and osteocytes [Dieudonne et al., 2000]. In addition, we and others [Moursi et al., 1996] demonstrated that soluble FN inhibited osteoblast differentiation in a fashion similar to that shown by βig-h3. FN, however, is required not only for osteoblast differentiation [Moursi et al., 1997] but also as a survival factor for differentiated osteoblasts [Globus et al., 1998]. βig-h3 is known to support cell adhesion and spreading possibly through interaction with integrins [LeBaron et al., 1995]. Thus, immobilized and soluble βig-h3 added to osteoblasts may compete with FN or other adhesion molecules for integrin interaction, which is prerequisite for osteoblast differentiation. Taken together, these findings suggest that βig-h3 is involved in the early stage of bone formation and plays a negative role as a regulator of osteoblast differentiation.

Interestingly, two recent reports using a subtractive hybridization method showd that βig-h3 gene was distinctly downregulated in an embryonal rhabdomyosarcoma cell line [Genini et al., 1996] and in mesenchymal tumor cells [Schenker and Trueb, 1998]. These studies also showed that fibronectin and OSF-2 genes were downregulated in the tumor cell lines. Although it is not entirely clear how the downregulation of these genes is related to tumor cell function, it was suggested that these genes might have an important role in the determination or maintenance of the normal phenotype, and thus their loss is possibly involved in the progression of malignancy. The fact that βig-h3 decreases the cell growth rate when it is overexpressed and leads to a marked decrease in the ability of CHO cells to form tumors in nude mouse [Skonier et al., 1994] further supports this hypothesis.

βig-h3 shares homology with OSF-2 in the fourfold repeated domain that also shows homology to the insect protein fasciclin I [Skonier et al., 1992]. OSF-2 was cloned by a combined subtraction hybridization/differential screening approach using MC3T3-E1 osteoblasts [Takeshita et al., 1993]. The gene is strongly expressed in bone, weakly in lung, and not in other tissues. It is negatively regulated by the osteotrophic factors, EGF and 1,25-$(OH)_2D_3$ but is upregulated by TGF-β. In our experiments, OSF-2 was also downregulated in fibroblasts from affected skin, although it was not decreased as much as βig-h3. The role of OSF-2 in bone metabolism is unknown, but it may function as homophilic adhesion molecule in bone formation on the basis of the amino acid homology with fasciclin I. We found that other adhesion molecules such as fibronectin and osteonectin were also downregulated in the affected skin fibroblasts but not as significantly as βig-h3.

Melorheostosis is a disease caused by an early postzygotic mutation of the mesenchyme resulting in asymmetric involvement of skeletal structures, with concomitant vascular and hamartomatous changes in the overlying soft tissues [Fryns, 1995]. When we characterized gene expression patterns from patients, we found numerous genes downregulated that are implicated in cell adhesion. Cell adhesion is a critical process in normal cell development and homeostasis. Hence, a mechanism by which gene expression of several adhesion proteins is altered may contribute to the development of hyperostosis and concomitant soft tissue abnormalities occurred in melorheostosis. Here, we provide the first evidence for a functional role of a TGF-β-inducible cell adhesion molecule, βig-h3 as it relates to bone tissue formation in normal and pathological situations.

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