Expression and regulation of amphiregulin in Gsα-mutated human bone marrow stromal cells of fibrous dysplasia of mandible

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Objectives: Fibrous dysplasia (FD) is a focal bone lesion composed primarily of immature bone marrow stromal cells along with spicules of immature woven bone. However, cellular differentiation and proliferation in mutant human bone marrow stromal cells (hBMSCs) of FD have not been fully elucidated. Therefore, the aim of this study was to investigate the occurrence of Gsα mutation at the Arg201 codon in hBMSCs and human trabecular bone cells (hTBCs, osteoblast-like cells). In addition, we evaluated the gene expression and protein secretion of amphiregulin from hBMSCs and hTBCs and assessed the biologic activity and possible mechanism of amphiregulin in our system.

Study design. Mutant hBMSCs from FD patients and hTBCs from disease-free bone specimens of the same patient were successfully cultured. We studied the Gsα mutations at the Arg201 codon by means of polymerase chain reaction (PCR)–restriction fragment length polymorphism. Gene expression and protein secretion of amphiregulin in hBMSCs and hTBCs was confirmed by reverse-transcription (RT) PCR and Western blotting analysis. The modulation proliferation and possible mechanism of the exogenous addition of amphiregulin and epidermal growth factor receptor tyrosine kinase inhibitor (AG-1478) were assessed by using Wst-1 assays.

Results. The Gsα mutations in clonal adherent mutant hBMSCs and hTBCs were successfully identified. We identified amphiregulin to be highly expressed in hBMSCs compared with hTBCs. The growth of hBMSCs was stimulated by the exogenous addition of amphiregulin and inhibited by AG-1478.

Conclusions. The Gsα-mutant hBMSCs were successfully identified, and amphiregulin may play a significant role in the proliferation of hBMSCs.

Fibrous dysplasia (FD) is a focal bone lesion composed primarily of immature bone marrow stromal cells along with spicules of immature woven bone and, in some cases, islands of hyaline cartilage. These lesions generally expand from the marrow cavity into the surrounding cortical bone and contain osteoclasts at the periphery. The most frequently involved sites are the craniofacial bones and the proximal femur. FD can occur as an isolated monostotic or polyostotic disease or as a part of the McCune-Albright syndrome (MAS), which also includes skin hyperpigmentation and one or more endocrinopathies. Histologically, FD is composed of slender and curved trabeculae of bone and a cellular proliferation of fibroblast-like cells, which are characteristically associated with long bones. Moreover, some cases of FD also show sclerotic patterns, particularly in craniofacial bones.

Mutation of the subunit of signal-transducing G proteins (Gsα) at the Arg201 codon (replacement of the arginine by either cysteine or histidine), stimulating cyclic adenosine monophosphate (cAMP) formation, has been identified in various tissues in MAS and is also thought to underlie the development of FD associated with a cellular retraction and deposition of abnormal bone matrix led by increased cAMP formation. These activating mutations occur postzygotically such that the affected individuals are somatic mosaics and represent a heterogeneous patient population depending on the pattern of distribution of mutated cells throughout the body. The causative mutations inhibit the intrinsic guanosine triphosphatase activity of Gsα such that it remains active in stimulating adenylyl cyclase and leads to the overproduction of cAMP. Therefore, the mutated cells constitutively generate high levels of cAMP and have a high rate of proliferation. Activation of the Gsα/protein kinase A (PKA)/cAMP response...
element–binding (CREB) pathway also induces c-Fos overexpression in FD lesions.9

Thus, FD is due to abnormal proliferation and differentiation of human bone marrow stromal cells (hBMSCs) due to GNAS (G protein alpha stimulating activity polypeptide) mutations. The histologic hallmark of this condition is extensive proliferation of fibrous tissue within the bone marrow, produced by these abnormally differentiated preosteoblastic cells. Consequently, it is thought that increased mutant cell proliferation plays an important role in the establishment and growth of some FD lesions. Therefore, the investigation of inhibition of mutant cell proliferation may be a potential therapy for FD/MAS patients.

Amphiregulin belongs to the epidermal growth factor (EGF) family which includes EGF, transforming growth factor α, heparin-binding EGF, betacellulin, and various heregulins. These factors mediate biologic functions of epithelial and mesenchymal cells through the EGF receptors (EGFRs).10 Several studies have demonstrated that the EGF family and the EGFR signaling pathway play a crucial role in the osteoblast lineage, fibrous cell, and cells associated with microvascular walls in FD tissues with mosaic-activated mutation of GNAS.11,12 Amphiregulin is a 252–amino acid transmembrane glycoprotein, and was originally isolated from human breast carcinoma cell line MCF-7.13 The mRNA expression for amphiregulin can be detected in a variety of carcinoma cell lines and in nontransformed epithelial and mesenchymal cells from the colon, stomach, lung, breast, ovary, and kidney.14,15 Also, amphiregulin stimulates the proliferation of keratinocytes, fibroblasts, and epithelial cells.14 Recently, Shigeishi et al. demonstrated that amphiregulin stimulates mesenchymal cell proliferation of human osseous dysplasia.16 Moreover, amphiregulin-null mice displayed significantly less tibial trabecular bone than wild-type mice.17

Taken together, mutant hBMSC differentiation and proliferation of FD have not been fully elucidated. On the other hand, amphiregulin can stimulate mesenchymal cell proliferation of human osseous dysplasia. Therefore, in the present study, we investigated the occurrence of GNAS mutation at the Arg201 codon in hBMSCs derived from FD patient and human trabecular bone cells (hTBCs, osteoblast-like cells) derived from disease-free bone specimens of the same patient. In addition, we evaluated the gene expression and protein secretion of amphiregulin from hBMSCs and hTBCs. To assess the biologic activity and possible mechanism of amphiregulin in our system, we tested how amphiregulin modulates proliferation of hBMSCs by means of Wst-1 assays.

MATERIAL AND METHODS

Patient

A 16-year-old girl was referred to the Department of Oral and Maxillofacial Surgery at the Provincial Hospital, Affiliated Shandong University, for investigation of a painless swelling of the right side of the jaw of 2 years’ duration. Oral examination showed a hard, painless enlargement of the mandible posterior area. The patient did not have any previous surgical interventions in the area. Diagnosis of FD was confirmed based on clinical history, radiographic findings (Fig. 1, A and B), and histopathologic examination (Fig. 1, C). Also, the patient did not exhibit endocrine hyperfunction or macular pigmented skin lesions. Under the approval of the Ethical Committee, Provincial Hospital, Affiliated Shandong University, fresh surgical fragments were obtained from the patient after informed consent was obtained.

Isolation of bone marrow stromal cells

hBMSC cultures were established as previously described.18,19 Briefly, cells were released by scraping bone marrow of fresh specimens of the right inferior border of the mandibular angle lesions into nutrient medium consisting of alpha-modified minimum essen-

Fig. 1. Representative panoramic radiography, computerized tomography, and photomicrography of hematoxylin-eosin–stained fibrous dysplasia. A, B, fibrous dysplasia is homogeneously radiopaque with a ground glass appearance and poorly defined margins indicated by the arrows. C, Histologic section of FD lesion showing the typical pattern of spicules of woven bone interspersed within whorls of fibrous tissue composed of immature osteoprogenitor cells.
tial medium (α-MEM; Biofluids, Rockville, MD, USA) plus 20% fetal bovine serum (Sijiqing Life Technologies, Hangzhou, China), glutamine, streptomycin, and penicillin (Biofluids). Single-cell suspensions prepared by serial passage through needles of decreasing diameter and a cell sieve (70 μm; Falcon; Becton Dickinson, Lincoln Park, NJ, USA), were plated into 75-mL flasks and incubated at 37°C in an atmosphere of 100% humidity and 5% CO2 to generate single colony–derived strains. The medium used was the same as above, which supports proliferation rather than differentiation of stromal progenitors, as described previously.20 Cell strains at the third passage were used for analysis of \(G_{\alpha}\) mutation and amphiregulin expression.

Isolation of human trabecular bone cells

hTBCs derived from disease-free bone specimens (left inferior border of the mandibular angle, confirmed by histopathologic examination) of the same patient were established as described previously.21 Briefly, fragments of trabecular bone were immediately washed, minced in phosphate-buffered saline solution, and subsequently plated in low-calcium (0.02 mmol/L) nutrient medium containing a 50:50 mixture of DMEM/Ham F-12K (Biofluids) with 10% fetal bovine serum, glutamine, penicillin, and streptomycin (Biofluids) under 5% CO2 in air at 37°C. The medium was changed every 3-4 days.

Genomic DNA extraction

Genomic DNA was extracted from hBMSCs and hTBCs by using the Puregene DNA isolation kit according to the manufacturer’s instruction (Gentra, Minneapolis, MN, USA).

Polymerase chain reaction–restriction fragment length polymorphism

We used the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) procedure to detect \(G_{\alpha}\) mutations at the Arg\(^{201}\) codon (CGT) with strategy primers as reported previously,22,23 with minor modifications. The primer sequences are presented in Table I. Approximately 50 ng genomic DNA was amplified by PCR using primers P1 and P2, and 1 μL of this amplification reaction was used as template for subsequent nested PCR-RFLP assays to detect GNAS Agr201 mutations (95°C for 30 seconds, 55°C for 30 seconds, and 75°C for 45 seconds). Then, 1 μL of the amplified product was diluted 20-fold and used as a template for a PCR reaction with the primer P2 and the primer P3 which flanks the EagI restriction site, because the primer P3 creates a new restriction site for EagI (CGGCCG) through the change to G in the first position of codon 200 in the normal allele, thus enabling the detection of a mutation at the first and the second positions of the Arg 201 codon. The thermocycling conditions were as follows: predenaturation at 95°C for 4 minutes, amplification (denaturation at 95°C for 20 seconds, annealing at 55°C for 30 seconds, and extension at 68°C for 30 seconds) for a duration of 30 cycles, with a final extension at 68°C for 10 minutes. As a result, EagI cleaves amplicons from the normal GNAS alleles into 79bp and 23-bp fragments. In contrast, the presence of any mutation at the Arg201 codon eliminates this EagI site, and, therefore, amplification products (102-bp fragment) from mutant alleles are resistant to EagI digestion. Fragments (134 bp) of p53 gene exon 8 were amplified for 30 cycles (94°C for 45 seconds, 55°C for 1 minute, and 72°C for 30 seconds) as a positive control to test for the suitability of the respective material for PCR amplification. For each of the digested products, 6 μL was electrophoresed on a 3% agarose gel in the presence of ethidium bromide and visualized by the gel documentation system.

Reverse-transcription PCR analysis of amphiregulin expression

The mRNA expression for amphiregulin in hBMSCs and hTBCs was assessed by reverse-transcription (RT) PCR analyses. Briefly, PCR reaction with 2 μL cDNA

Table I. Description of the designed primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Strand</th>
<th>Primer sequences</th>
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<tbody>
<tr>
<td>(G_{\alpha})</td>
<td>P1</td>
<td>Sense</td>
<td>5’-CCATTGACCTCAATTTTGTTTCAG-3’</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>Antisense</td>
<td>5’-GGTAAACAGTGGCTTACTGGAAGTTG-3’</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>Sense</td>
<td>5’-TTTTGTTTTCAGGACTGTTCGCGCCG-3’</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>Antisense</td>
<td>5’-ACTTGTCCACTGGAACCTGGTCTC-3’</td>
</tr>
<tr>
<td>p53 Exon 8</td>
<td>P5</td>
<td>Sense</td>
<td>5’-TGGTAACTAAGGAGCAAGGA-3’</td>
</tr>
<tr>
<td></td>
<td>P6</td>
<td>Antisense</td>
<td>5’-GCCTAGCTGCTCCTGGGGGC-3’</td>
</tr>
<tr>
<td>Amphiregulin</td>
<td>P7</td>
<td>Sense</td>
<td>5’-ACGGTTCGACACACTGCGTGC-3’</td>
</tr>
<tr>
<td></td>
<td>P8</td>
<td>Antisense</td>
<td>5’-AGCAGCGGGGCTCTCATGG-3’</td>
</tr>
<tr>
<td>GAPDH</td>
<td>P9</td>
<td>Sense</td>
<td>5’-AGTTGGTGACGGACCTGGCG-3’</td>
</tr>
<tr>
<td></td>
<td>P10</td>
<td>Antisense</td>
<td>5’-ATTCCAGTTGCTGGGGGACCTGAG-3’</td>
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was performed for 30 cycles of 1 minute at 94°C (denaturation), 30 seconds at 59°C (annealing), and 45 seconds at 72°C (extension) in a Superscript First Strand Synthesis System (Life Technologies, Gaithersburg, MD, USA) using the amphiregulin-specific primer (Table I). After PCR amplification, the product was separated on a 2% (w/v) agarose gel in TAE buffer [Tris, 40 mmol/L; Na₂EDTA, 1 mmol/L; and Na acetate, 40 mmol/L (pH 8.0)], stained with 0.5 μg/mL ethidium bromide, and photographed under ultraviolet light. GAPDH transcripts were used to control for the RNA that was used to amplify the genes by RT-PCR.

**Western blotting**

Samples of hBMSCs and hTBCs were homogenized on ice-cold Tris lysis buffer (pH 7.6) containing protease inhibitors. Lysates were then centrifuged for 10 minutes at 20,000g and the supernatant collected. Protein samples were applied to 10% sodium dodecyl-sulfate–polyacrylamide gel electrophoresis. After gel electrophoresis, proteins were transferred to a nitrocellulose membrane (Schleicher and Schuell, Keene, NH, USA). The membranes were blocked with 5% nonfat milk in phosphate-buffered saline solution (PBS) containing 0.05% Tween 20 for 1 hour at room temperature and incubated with primary antibodies overnight at 4°C. Anti–human amphiregulin rabbit polyclonal antibody (Sigma, St. Louis, MO, USA), anti–human EGF receptor monoclonal antibody (Sigma), and β-actin monoclonal antibody (Sigma) were applied at dilutions of 1:500. Membranes were washed 3 times for 10 minutes in PBS with Tween before 1 hour of room-temperature incubation with horseradish peroxidase and conjugated secondary antibodies (goat anti-rabbit IgG, 1:1,000; Santa Cruz Biotechnology, Santa Cruz, CA, USA). After 6 × 5 minutes washes in PBS with Tween and 1 × 20 minutes wash in PBS, the membranes were incubated with electrochemiluminescence (ECL) for 1 minute before exposure to Kodak X-Omit AR film (Eastman Kodak, Rochester, NY, USA). Membranes were reprobed with β-actin as a loading control.

**Cell proliferation in the presence of different concentration of amphiregulin**

The cell proliferation after cultivation was determined quantitatively using Wst-1 colorimetric assay, which measures the cellular mitochondrial dehydrogenase activity. The hBMSCs and hTBCs were seeded at a density of 3 × 10⁴ cells per well in 96-well cell culture plates. The cells were then exposed to medium containing recombinant amphiregulin (0.1, 1, 10, 100, 500, and 1,000 ng/mL) or no amphiregulin. To ascertain the effect of amphiregulin, the cells were exposed to the medium with 1% FBS. Cells were incubated for 6 days, and then 50 μL Tyrode HEPES buffer and 5 μL Wst-1 solution (5 mmol/L Wst-1 and 0.2 mmol/L 1-methoxy-5-methylphenazinium methylsulfate in water) were added to each well and incubated at 37°C for 2 hours. Then, 20 μL of each reaction mixture was transferred to a new 96-well plate. The absorbance at 450 nm was measured with a microplate reader (Infinite F200; Tecan, Salzburg, Austria). The cell number was expressed as a percentage relative to the cell number of control samples incubated with medium alone.

**Cell proliferation in the presence of amphiregulin and EGF receptor tyrosine kinase inhibitor at different times**

The hBMSCs and hTBCs were seeded at a density of 3 × 10⁴ cells per well in 96-well cell culture plates. The cells were then exposed to medium containing recombinant amphiregulin (100 ng/mL) or no amphiregulin for 2, 4, 6, 8, 10, and 12 days. To ascertain the effect of amphiregulin, we exposed the cells to the medium with 1% FBS. The cells were also exposed to medium containing 5 μmol/L EGF receptor tyrosine kinase inhibitor (AG-1478; Calbiochem, San Diego, CA, USA). After 2, 4, 6, 8, 10, and 12 days of culture, the cell numbers were counted as described previously.

**RESULTS**

**Cytology of hBMSCs and hTBCs**

Using tissue culture techniques that have been established as previously described, we successfully cultured mutant hBMSCs from FD patients and hTBCs from disease-free bone specimens of the same patients.¹⁸,¹⁹,²¹ The hBMSCs derived from the FD tissue showed stable growth and a spindle-shaped appearance. The population doubling time of hBMSCs was 72 hours (data not shown).

**Mutation analysis**

PCR-RFLP analyses of genomic DNA from the mutant hBMSCs demonstrated the presence of an R201H mutation in GNAS gene, thereby validating our assays. Digestion of the genomic PCR products with the appropriate restriction enzyme showed the presence of both normal and mutant alleles (Fig. 2), consistent with the frequency of the mutant allele in this DNA sample, as determined previously.²⁴ On the other hand, hTBCs used as a control showed no mutation in the GNAS gene at the Arg⁰⁰⁰ codon (Fig. 2). Further PCR amplification of EagI-digested products showed amplification only in the mutant hBMSCs and not in any of the hTBCs (Fig. 2), confirming the absence of mutant alleles.

**Expression of amphiregulin in hBMSCs**

Gene expression of amphiregulin in hBMSCs and hTBCs was confirmed by RT-PCR and Western blot-
ting analysis. The results showed that amphiregulin mRNA was expressed at higher levels in hBMSCs compared with hTBCs (Fig. 3, A). We also confirmed the protein expressions of amphiregulin and EGFR in hBMSCs (Fig. 3, B).

### Effects of amphiregulin on hBMSC and hTBC proliferation

Amphiregulin stimulated an increase in the cell number in a dose-dependent manner (Fig. 4). Maximal stimulation of growth in hBMSCs was observed at a concentration of 100 ng/mL (Fig. 4). Then, hBMSCs were exposed to medium containing recombinant amphiregulin (100 ng/mL) or no amphiregulin (control) for 2-12 days. The hBMSCs had higher rates of growth with the addition of amphiregulin compared with no amphiregulin (Fig. 5, A). However, hTBCs did not show higher rates of growth in the addition of each concentration of amphiregulin compared with no amphiregulin (Fig. 5, B). These studies indicate that exogenous amphiregulin can affect the proliferation of hBMSCs.

### Inhibition of cell proliferation in hBMSCs by AG-1478

If the amphiregulin-induced cell proliferation involves an interaction between amphiregulin and EGFR,
it should be possible to block this mitogenesis with an inhibitor directed against the extracellular domain of EGFR. Therefore, in the next experiment, hBMSCs were treated with amphiregulin (100 ng/mL) in the presence or absence of AG-1478 (5 μmol/L) for 6 days. Total viable cell numbers were assessed by Wst-1 assays. As shown in Fig. 6, these effects were significantly inhibited by the addition of AG-1478, a specific inhibitor of EGFR tyrosine kinase, suggesting that biologic effects of amphiregulin are mediated through EGFR-mediated signaling pathways. To further confirm the possible mediation of EGFR-mediated signaling pathways, amphiregulin mRNA expression and protein secretion were analyzed by RT-PCR and Western blotting. As shown in Fig. 7, AG-1478 almost completely blocked amphiregulin-induced amphiregulin mRNA and protein expression.

DISCUSSION

Clinically, FD is a benign fibrous-osseous lesion of the bone. The spectrum of skeletal involvement varies from asymptomatic monostotic lesions to polyostotic involvement resulting in progressive functional deficits and esthetic problems. The polyostotic variants may be accompanied by skin pigmentation and a variety of endocrine disturbances, including sexual precocity, pituitary adenoma, and hyperparathyroidism (MAS). Radiographically, the lesions present as unicocular or multiloc-
ular radiolucent lesions or as a mottled radiolucent/radiopaque pattern or dense radiopaque mass. The most typical radiographic feature is that of a ground glass opacification with ill-defined margins, blending imperceptibly into the surrounding bone. Histologically, fibrous dysplasia shows irregularly shaped trabeculae of immature woven bone in a cellular fibroblastic stroma. In addition, a lamellar maturation can be present, especially in older patients.26,29

Etiologically, activating missense mutations of the GNAS gene, encoding the α-subunit of Gs, underlie all forms of FD of bone and are thought to occur postzygotically, leading to a somatic mosaic state.30,31 Somatic mosaicism has been invoked as the mechanism allowing for survival of mutant cells during development.31 The observation that the disease is never transmitted through the germ line is consistent with this view. However, cells with both the normal and the disease-associated genotype are found within an individual FD lesion, even at the level of individual clonalogenic progenitors of bone-forming cells (colony-forming unit–fibroblasts).18 Transplantation of clonal populations of normal hBMSCs into the subcutis of immunocompromised mice resulted in normal ossicle formation. In contrast, transplantation of clonal populations of mutant hBMSCs always led to the loss of transplanted cells from the transplantation site and no ossicle formation.18 This makes each FD lesion a somatic mosaic itself, rather than simply the “wrong” piece of a macroscopic patchwork of phenotypically abnormal tissue and normal tissue. However, the mutant stromal cell type, ranging from local mutational load to stimuli instigating initiation of a lesion, could be invoked to explain the development and the natural prevention of an FD lesion at different sites.32

Therefore, in the present study, we investigated the occurrence of GNAS mutations in clonal adherent mutant hBMSCs and hTBCs, respectively derived from histologically abnormal and normal bone of the same patient with FD. In addition, we used PCR-RFLP to detect the occurrence of mutations at the Arg201 codon in hBMSCs and hTBCs. PCR-RFLP analyses of positive control DNA from the mutant hBMSCs demonstrated the presence of an R201H mutation in GNAS gene, consistent with the frequency of the mutant allele in this DNA sample, as determined previously.24 On the other hand, hTBCs used as a control showed no mutation in the Gsα gene at the Arg201 codon. Our results further confirmed that fibrous tissue in the abnormal marrow spaces of FD lesions is composed of mutant cells with BMSCs. In addition, stromal cell cultures from the abnormal FD bone marrow carry the causative Gsα gene mutation.18,32,33

In 1988, Shoyab et al. first discovered the glycoprotein amphiregulin in the concentrated conditioned medium of MCF-7 breast cancer cells treated with phorbol 12-myristate-13-acetate (PMA).13 Amphiregulin belongs to the EGF family which includes EGF, TGFα, HB-EGF, betacellulin, and various heregulins. These factors mediate biological functions of epithelial and mesenchymal cells through the EGFRs.10 Amphiregulin is bifunctional because it inhibits the growth of many human tumor cells but stimulates the proliferation of other cells, such as normal fibroblasts, osteoblast lineage, keratinocytes, and cells associated with microvascular walls in FD tissues with mosaic-activated mutation of GNAS.11,12,34 Similarly to EGF, amphiregulin is produced as a precursor transmembrane protein that undergoes proteolytic cleavage to yield the mature protein.17 So far, there have been no reports of amphiregulin production or function in mutant hBMSCs of FD. However, EGF has been shown to have several effects on FD or on mutant cells of FD: It stimulates osteoblast proliferation, decreases alkaline phosphatase and collagen production, changes bone nodule formation, and, yet, has catabolic effects on bone, i.e., similar to amphiregulin, bifunctional effects.

Therefore, in this study, we first detected the gene expression of amphiregulin in hBMSCs and hTBCs by means of RT-PCR and Western blotting. The results showed that hBMSCs constitutively expressed amphiregulin and EGFR protein compared with hTBCs. The growth of hBMSCs was stimulated by the exogenous addition of amphiregulin and inhibited by AG-1478 (an EGF receptor tyrosine kinase inhibitor). Our results clearly demonstrated that amphiregulin is a growth stimulator for hBMSCs. Taken together with the observation that an EGFR tyrosine kinase inhibitor inhibits the mitogenic stimulation by amphiregulin, these findings suggest that amphiregulin can function through an extracellular autocrine loop that involves EGFR in hBMSCs. Recently, Shigeishi et al. demonstrated that amphiregulin stimulates mesenchymal cell proliferation of human osseous dysplasia.16 Moreover, amphiregulin-null mice significantly displayed less tibial trabecular bone than wild-type mice.17 To further confirm the possible mediation of EGFR-mediated signaling pathways, amphiregulin mRNA expression and protein secretion were analyzed by RT-PCR and Western blotting. The results showed that AG-1478 almost completely blocked amphiregulin-induced amphiregulin mRNA and protein expression.

However, the mechanism by which amphiregulin stimulates hBMSCs differentiation is not well understood. The amphiregulin promoter contains a cAMP-responsive element, and several reports have demonstrated that expression of amphiregulin is regulated by
cAMP. Qin and Partridge showed that the amphiregulin expression is regulated through the CREB phosphorylation pathway. Activation of the PKA/CREB pathway, in which Gα stimulates production of cAMP and activation of PKA, leads to activation of multiple transcription factors, including CREB, and Cbfa1. Specifically, PKA is able to phosphorylate CREB at Ser-133, this phosphorylated CREB is required for the up-regulation of the AP family member, c-Fos, and the c-Fos gene product Fos is overexpressed in FD lesions. Fos overexpression in transgenic mice results in bone lesions reminiscent of FD.

In conclusion, by using PCR-RFLP, we successfully identified the Gα mutations in clonal adherent mutant hBMSCs and hTBCs, respectively derived from histologically abnormal and normal bone of the same patient with FD. Furthermore, we evaluated the gene expression and protein secretion of amphiregulin from hBMSCs and hTBCs. The growth of hBMSCs was stimulated by the exogenous addition of amphiregulin and inhibited by AG-1478 (an EGFR tyrosine kinase inhibitor). These results indicate that biologic effects of amphiregulin are at least partly mediated through EGFR-mediated signaling pathways.

REFERENCES


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