A Novel Alternatively Spliced Fibroblast Growth Factor Receptor 3 Isoform Lacking the Acid Box Domain Is Expressed during Chondrogenic Differentiation of ATDC5 Cells*

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To determine the role of fibroblast growth factor (FGF)-FGF receptor (FGFR) signaling in chondrogenesis, we analyzed the gene expression of alternatively spliced FGFRs during chondrogenic differentiation of ATDC5 cells in vitro. Two isoforms of FGFR3 were expressed in these cells. One was the complete form of FGFR3 (FGFR3) already reported, and the other was a novel one that lacks the acid box domain (FGFR3ΔAB). The gene of FGFR3ΔAB was expressed in undifferentiated ATDC5 cells. In contrast, the transcripts of FGFR3 were not detectable in undifferentiated cells but increased during cellular condensation, which is an obligatory step for chondrogenic differentiation. FGFR1 and FGFR2 expression was higher than that of FGFR3 in undifferentiated cells. The gene expression of cell cycle inhibitor p21 was induced during cell condensation and correlated best with the expression of FGFR3 among the FGFR isoforms expressed. The differential expression of FGFR3 isoforms during chondrogenesis suggests that these isoforms may play different roles in the regulation of growth and differentiation in chondrocytes. To define the mitogenic response of FGFR3ΔAB and FGFR3 to FGFs, their cDNAs were stably transfected into mouse Ba3 pro-B cells. FGFR3 preferentially mediates the mitogenic response to FGF1 and poor response to FGF2. In contrast, FGFR3ΔAB mediated a higher mitogenic response to FGF2 as well as to FGF1. In addition, FGFR3ΔAB responds to FGF1 at lower concentrations of heparin than FGFR3 does. These results suggest that the acid box plays an important role in the regulation of FGFR3 to mediate biological activities in response to FGFs.

The fibroblast growth factor (FGF) family consists of four closely related members, the amino acid sequences of which are highly conserved both between different members of the family and throughout evolution (1), They regulate a multitude of cellular processes, including cell growth, differentiation, migration, and survival (2, 3).

The structure of FGFRs has three different parts: an extracellular portion with three Ig-like domains, a single transmembrane portion, and a split tyrosine kinase domain inside the cell. The overall complexity of the receptor is increased by the existence of additional isoforms generated by alternative mRNA splicing (4–8). These receptors possess an alternative sequence for the C-terminal half of the third Ig domain (IgIII), encoded by a separate 5′-exon IIIa and 3′-exon, either IIib or IIIC, which determines ligand specificity (1, 7, 8). In the segment between the first Ig domain (IgI) and the second Ig domain (IgII), there is a cluster of the acidic residues, which is referred to as the “acid box,” similar to that found in adhesion molecules such as E-cadherin/uvomorulin (9, 10). Another major alternative splicing event results in FGFR isoforms differing in the IgI and acid box domains (4–6). FGFRs lacking IgI have a higher ligand affinity for some FGF ligands, although it is not known if ligand binding specificity is affected by removal of the IgI domain (6). Distinct expression patterns of these isoforms have also been reported in cell differentiation (11), embryonal development (12), and tumor progression (13), suggesting that there are functional differences between these isoforms.

Activation of three members of the FGFR family, FGFR1, FGFR2, and FGFR3, by FGFs induces mitogenic responses in various cell types (14). In addition, a gain of functional mutations in FGFR3 causes early cessation of cell growth in chondrocytes (15, 16). These mutations cause autosomal dominant disorders of skeletal development such as hypochondroplasia (17), achondroplasia (18, 19), and thanatophoric dysplasia types I and II (20, 21). These mutations activate receptor signaling by either inducing ligand-independent receptor dimerization (22, 23) or relieving the constraints for autophosphorylation of receptor-tyrosine kinase kinase (21, 24). Analysis of FGFR3-deficient mice generated by targeted disruption of the FGFR3 gene revealed that these mice have longer than average bones, expansion of their growth plate, and increased chondrocyte proliferation (25, 26). Moreover, the introduction of the achondroplasia mutation (G380R) into the murine FGFR3 gene resulted in a dominant dwarf phenotype that exhibited many of the features of human achondroplasia (27). These results strongly suggested that FGFR3 is a negative regulator of bone

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The abbreviations used are: FGF, fibroblast growth factor; FGFR, FGF receptor; IL-3, interleukin 3; FBS, fetal bovine serum; bp, base pair(s); kb, kilobase; PCR, polymerase chain reaction; RT-PCR, reverse transcription-PCR.
growth, in contrast to the common role of FGFRs in stimulation of cell proliferation. The role of FGFR3 in growth inhibition is cell-type-specific, in which the receptors utilize signaling through STAT1 activation. Ligand stimulation of FGFR3 in a rat chondrosarcoma cell line (28) and the thaparotrophic dysplasia type II mutant (K650E) of FGFR3 expressed in 293 cells (29) increased the phosphorylation of STAT1 and its translocation to the nucleus. One of the consequences of STAT1 activation is expression of p21 (30), a general inhibitor of cyclin-dependent kinases, which causes growth arrest in chondrocytes.

To elucidate the role of the FGF-FGFR signaling system in normal bone growth, we chose ATDC5 cells as an in vitro chondrogenesis model system. ATDC5 cells have been shown to be very useful for molecular analysis of early and late phase differentiation of chondrogenesis, because the cells display the sequential transitions of their phenotype in a synchronous manner in vitro (31–33). Taking advantage of the sequential differentiation of ATDC5 in culture, we studied the gene expression of FGFRs during early chondrocyte differentiation.

Our study showed temporal regulation of FGFR1, FGFR2, and FGFR3 gene expression during chondrogenic differentiation. We also analyzed the expression of alternatively spliced isoforms of these receptors differing in IgI and acid box domains. During the study, we isolated and cloned the cDNA of a novel splice variant of FGFR3, designated “FGFR3ΔAB,” which lacks the acid box domain in the extracellular part of FGFR3. To determine whether FGFR3ΔAB has the potential to stimulate cell proliferation, and if so, whether it has a different ligand specificity than that of FGFR3, we compared the mitotic cell proliferation, and if so, whether it has a different ligand specificity than that of FGFR3.

Hybridization was performed for 16 h at 42 °C with the appropriate probe (10 μg/dm ρm hybridization solution containing 50% formamide, 5× SSPE (20× SSPE contains 3.6 M NaCl, 0.2 M NaH₂PO₄, 20 mM EDTA, pH 7.7), 5× Denhardt’s solution, 0.5% (w/v) SDS, and 40 μg/ml sonicated denatured salmon sperm DNA (Stratagene, La Jolla, CA). RNA was isolated and prepared to a specific activity of 5 × 10⁶ cpm/μg. A random primer DNA labeling kit (Ready to go labeling kit; Amersham Pharmacia Biotech) using [α-32P]dCTP (3000 Ci/mmol, PerkinElmer Life Sciences). Templates were as follows: a 364-bp HinClI fragment of pT7Blue 2-mouse FGFR1 for FGFR1 mRNA, a 1.1-kb BamHI fragment of pT7Blue2-mouse FGFR2 for FGFR2 mRNA, a 0.97-kb BsuI fragment of pT7Blue2-mouse FGFR3 for FGFR3 mRNA, and a 0.7-kb purified PCR fragment for glyceraldehyde-3-phosphate dehydrogenase mRNA. The nylon membranes were washed once for 15 min at 42 °C in 2× SSPE and 0.1% SDS, once for 30 min at 42 °C in 1× SSPE and 0.1% SDS, and twice for 15 min at room temperature in 0.1× SSPE and 0.1% SDS. The membranes were exposed to BioMax MS film (Eastman Kodak Co.) at −80 °C with a TranScreen L-E intensifying screen (Kodak).

Reverse Transcription–PCR—First strand cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase (First strand cDNA synthesis kit; Amersham Pharmacia Biotech). The RNA (5 μg in 8 μl) was heated at 65 °C for 10 min and then cooled on ice. Then, 5 μl of the first strand cDNA synthesis reaction mixture (153 μM Tris-HCl, pH 8.3, 204 mM KCl, 27 mM MgCl₂, 5.4 mM each dNTP, and 0.24 mg/ml bovine serum albumin), together with 1 μl of 0.2 M diithiotreitol and 0.2 μg/ml random d(N)₆ primer were added to the RNA solution. After incubation at 37 °C for 1 h the mixture was heated to 95 °C for 5 min and then chilled on ice. PCR was performed in a reaction mixture consisting of 25 μl of cDNA, 50 mM KCl, 10 mM Tris-HCl, pH 9.0, 0.1% Triton X-100, 200 μM each dNTP, 0.625 units of Taq DNA polymerase (Promega), and 25 pmol each of the forward and reverse sequences of primers. The reaction was carried out for 25–30 cycles of 1 min at 94 °C, 2 min at 60 °C, and 3 min at 72 °C, with an extra 7-min extension at 72 °C for the last cycle (RoboCycler Gradient40, Stratagene). The optimal Mg²⁺ concentrations, cycles, and the forward and reverse primers used for analysis of FGFR and other gene markers are summarized in Table I. Aliquots of the PCR products (10 μl) were electrophoresed on 2.5% agarose gels (NuSieve GTG/SeaKem in Tris acetate EDTA buffer, pH 8.0, and stained with 0.5 μg/ml ethidium bromide. A linear amplification dependent on the amount of RNA was obtained under the above conditions (from 2.5 to 40 ng of RNA). The amounts of mRNA were adjusted in each RT-PCR reaction by checking amplification of the glyceraldehyde-3-phosphate dehydrogenase transcript. The identity was confirmed by hybridization with the AmpliTaq cycle sequence kit (Applied Biosystems, Inc., Foster City, CA) using the dye terminator method and a 310 genetic analyzer (Applied Biosystems, Inc.).

Cloning and Sequencing of the Mouse Acid Box-deleted Form of FGFR3 cDNA—3 μg of total RNA that was extracted from undifferentiated ATDC5 cells was used for the reverse transcription, which was performed using avian myeloblastosis virus reverse transcriptase (SuperScript II RNA LAPCR kit) and the oligo(dT) primer at 50 °C for 1 h. The cDNA was amplified using the forward primer (5′-CCGAGGGTCTTGGAGGCGCATGAGTATG) and the reverse primer (5′-TCAATGCGCTCAGGTTAAAACCGGCTTC) before being extracted with phenol/chloroform and precipitated with cold ethanol. The DNA was then ligated into the pT7Blue2 vector (Novagen), and competent Escherichia coli cells (Nova Blue) were transformed with the ligated vector. The plasmids of positive clones were extracted, and the nucleotide sequences of their inserts were determined in both orientations with an AmpliTaq cycle sequence kit (Applied Biosystems, Inc.), as described above.

FGFR Expression Plasmids and Stable Expression—Full-length cDNAs encoding the FGFR3ΔAB and FGFR3 were cloned into the pBKSv expression vector (Stratagene). First strand cDNA was synthesized using the total RNA derived from ATDC5 cells. The full-length cDNAs encoding FGFR3 were amplified by LA PCR, using a pair of primers (forward, 5′-CGACAATCTGGAGGCACATGGTACTCC-3′; reverse, 5′-CTAGCAGCGCCATGAAATTGCTG-5′). The resulting DNA was then cloned into the pT7Blue2-T vector and sequenced. The full-length pT7Blue2-T-FGFR3ΔAB plasmids obtained were linearized and a following fragments were isolated and ligated to yield the two pBKSv plasmids: an SpeI-BamHI restriction fragment of FGFR3 from the pT7Blue2-T plasmids or Nhel-BamHI fragment of FGFR3ΔAB with an Nhel-BamHI fragment of the pBKSvLac expression vector, in which the lac promoter was removed. To express these FGFRs in BaF3 cells, 4 × 10⁶ cells were incubated with 20–30 μg of pBKSv-FGFR3 or pBKSv-FGFR3ΔAB plasmids for 10 min at 4°C. The BaF3 cells were

Acid Box-deleted FGFR3
200 mM sucrose, 2 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. The cells were plated at 1 × 10^5 cells per well in a total volume of 300 µl of medium in 24-multiwell plates. Various concentrations of IL-3. The cells were washed twice with RPMI 1640 containing 10% FBS but lacking D-glucose. After washing the medium, the BaF3 cells were selected with 10 ng/ml FGF1 and 10 µg/ml heparin in RPMI 1640 containing 10% FBS without the conditioned medium from WEHI-3 cells.

**Immunoblotting**—The cells expressing each FGFR3 isoform were homogenized in isotonic buffer containing 50 mM HEPES buffer, pH 8.0, 200 mM sucrose, 2 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged at 100,000 g for 1 h at 4 °C. The cell membranes were then electrophoresed through SDS-7.0% polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore Co.). The membranes were incubated with anti-C-terminal FGFR3 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and then with horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology) before finally being developed with enhanced chemiluminescence substrate solution (Super Signal West Pico; Pierce) and exposed to BioMax MS film (Kodak) to visualize immunoreactive bands.

**Cell Growth Assays**—Cell proliferation was assayed by counting the number of BaF3 cells stably expressing FGFR3AB or FGFR3. Cells were washed twice with RPMI 1640 containing 10% FBS but lacking IL-3. The cells were plated at 1 × 10^5 cells per well in a total volume of 500 µl of medium in 24-multiwell plates. Various concentrations of FGFs were added in the presence or absence of the indicated concentrations of heparin. After 3 days, the viable cells were harvested and counted by a Coulter Counter ZM type, Beckman Coulter Co.). Experiments were performed at least in triplicate, and the results are expressed as means ± standard deviations.

**RESULTS**

**Formation of Cartilage Nodules by ATDC5 in Vitro**—ATDC5 cells were cultured in the medium containing 10 µg/ml insulin (the differentiation medium) as described under “Experimental Procedures.” As reported previously, the presence of insulin resulted in an increased number of chondrocytes in the culture, which promoted more efficient chondrogenic differentiation in ATDC5 cells compared with that in the absence of insulin (32). At the beginning of culture, ATDC5 cells were undifferentiated and did not express chondrogenic markers. The cells stopped growing at confluence reached 4–5 days after the culture was started, but remained undifferentiated with a fibroblastic morphology. A transient condensation of cells with an elongated spindle-like morphology preceded the formation of the nodules. ATDC5 cells reentered the growth state through the cellular condensation followed by the formation of cartilage nodules from day 7 to day 10, with a cell doubling time of 48 h. The cartilage nodular structures seen from day 14 were composed of proliferating chondrocytes with a round morphology. This postconfluent growth of cells continued until day 21, when typical cartilage nodules were formed all over the culture. This cell state is called early phase differentiation (32). To visualize the chondrogenic differentiation, Alcian blue staining of ATDC5 cells was performed as shown in Fig. 1. The Alcian blue positive cells produce the proteoglycan aggregan, which is a major component of cartilage. Alcian blue positive cells were not seen until day 10 (Fig. 1, a and b). During the postconfluent growth phase between days 10 and 21, Alcian blue-positive cells appeared to expand by the accumulation of cartilage matrix resulting from the ongoing chondrogenesis (Fig. 1, c and d).

**Expression of Cartilage-characteristic Extracellular Matrix Genes and p21 Cyclin-dependent Kinase Inhibitor Gene during Chondrogenic Differentiation of ATDC5 Cells**—Chondrogenic differentiation of ATDC5 cells was further characterized by the expression of cartilage-characteristic extracellular matrix genes such as aggregan and type II collagen. As shown in Fig. 2, transcripts of these genes were undetectable by RT-PCR in the cells cultured with the differentiation medium from day 3 until day 7. These transcripts became detectable after day 10 and gradually increased until day 21. At the same time, the cartilage nodules first appeared at day 10, and their number also gradually increased until day 21. The mRNA of p21 cyclin-dependent kinase inhibitor was first detected on day 7 when the cells were in the condensation stage, and its expression gradually increased until day 21. This correlates well with the appearance of cartilage nodules and the increase in number of mature chondrocytes in culture.

**Expression of FGFR1, FGFR2, and FGFR3 Genes during Chondrogenic Differentiation of ATDC5 Cells**—Fig. 3 shows the time course of changes in FGFR mRNA by Northern blot analysis. FGFR1 (4.0 kb) and FGFR2 (4.5 kb) mRNAs were detected on all days tested. In contrast, FGFR3 mRNA was not detected in undifferentiated ATDC5 cells (day 3). By means of Northern blot analysis, the 4.5 kb-FGFR3 transcript was weakly detected on day 7, when the cells reached postconfluence, and the levels of FGFR3 expression gradually increased until day 21. The expression levels of FGFR1 and FGFR2 mRNAs appeared to greatly increase until day 21 during formation of cartilage nodules (Fig. 3) and continued even until day 42 during late phase differentiation, when the number of hypertrophic chondrocytes increased in the culture (data not shown). FGFR4 transcripts were not detected at any phase in ATDC5 cells (data not shown).

**Identification of the Acid Box-deleted Isoform of FGFR3—**
was then isolated. 5 different differentiation medium) for 1, 3, 5, 7, 10, 14, and 21 days. Total RNA
10 gene.

ATDC5 cells were grown in the presence of 10 gene.

matrix genes and the p21 cyclin-dependent kinase inhibitor
phoresed on a 1.2% agarose gel. After blotting to Hybond-N

described under “Experimental Procedures.” The primer pairs used were

specific for collagen type II, aggrecan, p21 cyclin-dependent kinase
m

in 15-

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l reaction mixtures, and an aliquot was used for PCR as de-
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exogenously added heparin, FGF cannot stimulate a mitogenic
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mine the ligand specificity of each FGFR. In the absence of
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DAB is expressed not only in ATDC5 cells but
m

DAB.” Interestingly, only the 392-bp PCR band was
m

an alternatively spliced variant of FGFR3, and we designated it
m

A

Acid Box-deleted FGFR3
m

s were detected, one of which contains IgI (FGFR1
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9

-IgII in each FGFR, respectively (Table I).

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FIG. 2. Expression of cartilage-characteristic extracellular matrix genes and the p21 cyclin-dependent kinase inhibitor gene. ATDC5 cells were grown in the presence of 10 µg/ml insulin (the differentiation medium) for 1, 3, 5, 7, 10, 14, and 21 days. Total RNA was then isolated. 5 µg of total RNA was used for reverse transcription in 15-µl reaction mixtures, and an aliquot was used for PCR as described under “Experimental Procedures.” The primer pairs used were specific for collagen type II, aggrecan, p21 cyclin-dependent kinase inhibitor, and glyceraldehyde-3-phosphate dehydrogenase.

m

Because FGFR3 has been shown to be a glycosyl protein (43, 44), the molecular mass (125 kDa) of both FGFR3 isoforms (FGFR3, 88 kDa; FGFR3
m

D

of FGFR3
m

D

AB was not detectably different from that of FGFR3
m

D

AB.

FIG. 3. Northern hybridization analysis of FGFR1, FGFR2, and FGFR3 mRNA levels. 10 µg of total RNA was denatured and electrophoresed on a 1.2% agarose gel. After blotting to Hybond-N membranes, each membrane was hybridized with 32P-labeled FGFR1, FGFR2, FGFR3, or glyceraldehyde-3-phosphate dehydrogenase cDNA.

Next, the expression of the alternatively spliced isoforms of FGFRs that differ in the presence or absence of IgI and the acid box was examined by RT-PCR. We used the forward and reverse primers, which annealed to the signal sequence region and to the 3'-half of IgII in each FGFR, respectively (Table I). In FGFR1 and FGFR2, several isoforms were detected, as reported previously (4, 5). The two types of FGFR1 variants were detected, one of which contains IgI (FGFR1a; 605 bp) and the other of which lacks IgI (FGFR1b; 338 bp) (Fig. 4). In contrast, three types of FGFR2 variants were detected. These are the complete form (633 bp), an isoform that lacks IgI (366 bp), and an isoform that lacks IgI and the acid box (288 bp) (Fig. 4). We extracted all the DNA fragments and confirmed their sequences. The expression levels of these FGFR1 and FGFR2 isoforms were increased during chondrogenic differentiation of ATDC5 cells, but the ratio of them remained unchanged throughout. It was found that all isoforms expressed in ATDC5 cells had only the IIc exon (data not shown). This result corresponds to the previous observation that the IIc isoform of FGFRs is expressed mainly in mesenchymal lineages, whereas the IIb isoform expression is restricted to epithelial lineages (39). In the case of FGFR3, two PCR bands (446 bp and 392 bp) were detected (Fig. 5A). It revealed that the 446-bp fragment was derived from the previously reported complete form of FGFR3 that contains IgI (36) and that the 392-bp fragment lacked the acid box between IgI and IgII in the extracellular domain. The 392-bp fragments were thought to be derived from an alternatively spliced variant of FGFR3, and we designated it “FGFR3ΔAB.” Interestingly, only the 392-bp PCR band was detected by RT-PCR using cDNA from the undifferentiated ATDC5 cells (day 2). This PCR band was also detected using cDNA from the rat rib cartilage-derived chondrocytes, suggesting that FGFR3ΔAB is expressed not only in ATDC5 cells but also in chondrocytes in vivo (Fig. 5B).

Sequence of the Mouse Acid Box-deleted Form of FGFR3 cDNA—Because FGFR3ΔAB appears to be novel, the full-length cDNA of FGFR3ΔAB from RNA of undifferentiated ATDC5 cells was cloned further. The forward and reverse primers for amplification of the cDNA by RT-PCR were based on the mouse FGFR3 sequence (36) (Fig. 6A). FGFR3ΔAB encoded a polypeptide consisting of 782 amino acids, with an amino acid sequence identical to that of mouse FGFR3 (800 amino acids) except for the absence of the acid box region in the extracellular domain (Fig. 6B). Sequence comparison studies between FGFR3ΔAB and genomic FGFR3 suggested that there is alternative splicing of the acid box coding exon and the 5'-half of the IgII coding exon (40) (data not shown). It has been previously reported that FGFR3 has two alternatively spliced isoforms in the 3'-half of IgIII in the extracellular domain. These are IIc and IIb isoforms, which differ in the ligand specificity (41, 42). The novel FGFR3ΔAB and FGFR3 expressed in ATDC5 cells have the IgIIc domain (Fig. 6C).

Mitogenic Activity of BaF3 Cell Lines Expressing FGFR3 Splice Variants—To test whether FGFR3ΔAB has the potential to stimulate the cell proliferation, and if so, whether it has different ligand specificity from that of FGFR3, both isoforms were stably expressed in BaF3 cells. BaF3 cells are a pro-B cell line that is IL-3-dependent. They do not express endogenous FGFRs, but when transfected with an FGFR cDNA, they exhibit a dose-dependent proliferative response to FGFs (8, 36). Following selection, clonal cell lines expressing FGFR3 or FGFR3ΔAB were analyzed for their receptor expression and mitogenic response to various FGF ligands. Receptor expression levels were assessed by Western blotting (Fig. 7). The size of FGFR3ΔAB was not detectably different from that of FGFR3 (Fig. 7). The predicted molecular mass of the mature receptors (FGFR3, 88 kDa; FGFR3ΔAB, 86 kDa) differ only by ~2 kDa. Because FGFR3 has been shown to be a glycosyl protein (43, 44), the molecular mass (125 kDa) of both FGFR3 isoforms appeared to be larger than the estimated one. From this experiment, we concluded that both cell lines express comparable numbers of receptors. Both BaF3 cell lines could proliferate in response to FGFs in culture conditions, despite the absence of IL-3. The mitogenic response of both BaF3 cell lines was compared at various concentrations of FGFs in the presence of 10 µg/ml heparin (Fig. 8). Heparin was added to the cultures to substitute for the heparan sulfate proteoglycans that determine the ligand specificity of each FGFR. In the absence of exogenously added heparin, FGF cannot stimulate a mitogenic
response in BaF3 cells expressing FGFRs (8, 36, 42), because BaF3 cells do not express heparan sulfate proteoglycans, which support the FGF-1/FGFR complex formation (45) and are expressed in a tissue- or cell-specific manner (46). The parental BaF3 cells did not proliferate in the presence of FGFs and heparin (data not shown). Among the FGFs tested, FGFR3 preferentially responded to FGF1, whereas FGFR3ΔAB exhibited a higher response to FGF9, FGF4, and FGF2 than to FGF1. To assess the relative mitogenic activity of FGFs on each FGFR3 isoform and to make comparisons between two isoforms, we normalized the data in Fig. 8, A and B to that of FGF1. Because FGF1 is known as a universal FGFR ligand, the mitogenic response to FGF1 was used as a 100% control in each cell line (42). The relative mitogenic response to FGF1 was averaged at two different concentrations (0.312 and 1.25 mM) to reduce the influence of experimental error (Table II). FGFR3 shows a 22.7-fold, 2.9-fold, and 3.6-fold lower response to FGF2, FGF4, and FGF8, respectively, than to FGF1. The differential response of FGFR3 to FGF1 and FGF2 is in agreement with the previous paper, which showed that mouse FGFR3 preferentially binds to FGF1 (36). In our study, the response of FGFR3ΔAB to FGF2 and FGF4 was equal to that of FGF1 (105.2 and 112.4%, respectively). The response to FGF9 and FGF8 was also increased compared with FGFR3 (FGF9, from 27.6 to 70.0%; FGF8, from 63.2 to 119.2%). Next, we determined the heparin requirement of FGFR3 and FGFR3ΔAB. Both FGFR3- and FGFR3ΔAB-expressing BaF3 cells demonstrate an absolute requirement for heparin in their response to FGF1 (Fig. 9). After 3 days of incubation in the absence of heparin, the number of FGFR3ΔAB- and FGFR3-expressing BaF3 cells decreased from 10,000 cells/well to 627 ± 142 cells/well and 1,187 ± 83 cells/well, respectively. The half-maximal response to FGF1 and FGF2 was observed at 0.6 and 0.2 μg/ml, respectively. These results are consistent with the fact that FGF1 has a lower affinity for heparin than does FGF2 (47). In contrast, FGFR3 cells showed the half-maximal response to FGF1 at a concentration of 2.8 μg/ml, and their response to FGF2 was poor at these concentrations of heparin.

| Gene     | Sequence (5'→3') | Product size [Mg^2+] | Annealing temp. [°C] | No. of cycles |
|----------|------------------|----------------------|----------------------|---------------|
| FGFR1    | TTCTGGGCTGTGCTGGTCAC | 605, 338            | 1.75                 | 60            | 30            |
| FGFR2    | TATGATGCTCCAGGTGGCAT | 633, 288            | 1.75                 | 60            | 30            |
| FGFR3    | ACTAGTGTTCTGCGTGGCGGT | 446, 392            | 3.5                  | 60            | 30            |
| GAPDH    | ACCACAGTCCATGCCATCAC | 452                 | 1.0                  | 60            | 30            |
| Aggrecan | TGGTGGAATGCAGAGACCG | 481                 | 1.75                 | 60            | 30            |
| Collagen type II | CACACTGTTAGTGACCAGACG | 172              | 1.75                 | 60            | 30            |
| p21      | TCCTGGTATGTCGCCAGTG  | 437                 | 1.5                  | 60            | 30            |

Table I

PCR primers for amplification of FGFR and other cDNAs

Fig. 4. RT-PCR analysis of FGFR1 and FGFR2 isoforms alternatively spliced in their IgI and IgII domains. ATDC5 cells were grown in 100-mm plates with differentiation medium. Total RNA was isolated on days 3, 5, 7, 10, 14, and 21. 5 μg of total RNA was used for reverse transcription in 15 μl of reaction mixture, and aliquots were used for PCR as described under “Experimental Procedures.” The primer pairs used are summarized in Table I.

Fig. 5. RT-PCR analysis of the alternatively spliced isoform of FGFR3 expressed during chondrogenic differentiation of ATDC5 cells and rat rib cartilage-derived chondrocytes. A, ATDC5 cells were grown in the differentiation medium for 21 days. Total RNA was isolated on days 2, 7, 14, and 21 before the RT-PCR was performed as described above. B, the rat chondrocytes were harvested from rib cartilage, and total RNA was isolated as described under “Experimental Procedures.” Lane M, molecular weight marker, dX174/HinfI; lane 1, rat chondrocytes; lane 2, undifferentiated ATDC5 cells (day 2).
DISCUSSION

Gene Expression and Biological Roles of FGFRs in Chondrogenesis—

Our results indicated that in cultures of ATDC5 cells a single population of progenitor cells undergoes chondrogenesis without the influence of the environmental factors involved in vivo. This system allowed us to investigate the molecular mechanism of chondrogenic differentiation in vitro. Interestingly, gene expression of the cyclin-dependent kinase inhibitor p21 was first seen during cell condensation (around days 7–10), which was followed by increased levels of collagen type II and aggrecan gene expression. These results demonstrated that ATDC5 cells gradually stop proliferating as they differentiate into proliferating chondrocytes and finally into mature chondrocytes in a manner typical of growth plate chondrocytes. The levels of FGFR1 and FGFR2 gene expression were high in undifferentiated ATDC5 cells, and the expression levels of these FGFRs increased markedly during chondrogenic differentiation (Fig. 3). In contrast, the level of FGFR3 expression was very low in undifferentiated ATDC5 cells, compared with levels of FGFR1 and FGFR2 expression, but gradually increased during cell condensation. FGFR1 and FGFR2 were thought to stimulate cell proliferation prior to cell condensation. After 2 weeks in culture, the level of FGFR3 transcripts was increased, indicating that FGFR3 is expressed in proliferating chondrocytes.
ating and mature chondrocytes. This was consistent with the high levels of FGFR3 expression observed in developing mice: in the prebone cartilage rudiments as well as in cartilage during endochondral ossification (48). The increased level of FGFR3 transcripts correlated well with the increase in cyclin-dependent kinase inhibitor p21 gene expression, as the proliferating chondrocytes were further differentiating and maturing to form cartilage nodules. Our findings supported a model in which the expression of FGFR3 may lead to growth inhibition in chondrocytes, despite the higher levels of FGFR1 and FGFR2 expression. Because the levels of FGFR1 and FGFR2 expression increased markedly after cell condensation, FGFR1 and FGFR2 also must have received FGF signals during the differentiation process. Thus, these receptors may share the ligands available during chondrogenesis. This raises several questions, including what the consequences of signaling from FGFR1 and FGFR2 are, whether this signaling stimulates growth of chondrocytes, and whether this signaling accelerates the differentiation of chondrocytes and growth arrest. It will also be interesting to further investigate the cross-talk between the signals from FGFR1, FGFR2, and FGFR3.

Expression of Alternatively Spliced Isoforms of FGFRs Differing in IgI, Acid Box, and IgIII Domains during Chondrogenesis—Various alternatively spliced isoforms of FGFR1 and FGFR2 differing in IgI and acid box domains were expressed during chondrogenesis, but the expression ratios of these isoforms were unchanged (Fig. 4). In contrast, that of FGFR3 isoforms was differentially regulated. Whether these isoforms have different roles in chondrogenesis has not yet been clarified. The temporal expression of FGFR3 is consistent with its role in mediating growth arrest of mature chondrocytes. FGFR3 isoforms have different roles in mesodermal cell migration and growth during limb development (13). FGFR2 isoforms differing in the IgIII domain mediate a reciprocal regulation loop between FGFs expressed in mesenchyme and ectoderm during the early development of vertebrate limbs (3, 49, 50). This regulation was shown to be dependent on the cell type-specific differential expression of the FGFR2 isoforms IIIb (FGFR2b) and IIIc (FGFR2c) (39, 51, 52). FGFR1, FGFR2, and FGFR3 isoforms expressed in this system had the IIIc exon, which is specific for mesenchymal cells. During this study, we found that a novel isoform of FGFR3, FGFR3ΔAB, was expressed in ATDC5 cells. FGFR3ΔAB seemed to be generated by alternative splicing that deletes exon 4 encoding the acid box (40). FGFR3ΔAB was shown to be expressed in rat chondrocytes taken from cartilage tissue, suggesting that this form is present in vivo (Fig. 5B). It is interesting to determine whether FGFR3 and FGFR3ΔAB have distinct biological activities and signal transduction pathways in chondrocytes. The factors that control the alternative splicing of FGFRs in a cell- and time-specific manner should be identified in the near future.

Biological Activities and Heparin Requirements of FGFR3ΔAB and FGFR3—Binding of FGF and heparin to the receptor induces FGF-FGFR dimerization and receptor-tyrosine kinase autophosphorylation that initiates signal transduction events inside the cell (24). The biological activity of FGFR3 is cell type-specific. Activation of FGFR3 leads to cell proliferation (e.g. BaF3 cells and NIH3T3 cells), cell transformation (53), and inhibition of chondrocyte proliferation (28). In this study, we chose BaF3 cells in which to compare the biological activities of FGFR3ΔAB and FGFR3. Both receptors mitogenically respond well to FGF1 and FGF9 in the presence of 10 μg/ml heparin (Fig. 8). Heparin is required for high affinity binding of FGF to the FGFR in cells that are unable to synthesize cell surface heparan sulfate (45). Heparin interacts with FGFs and independently of FGF ligand with a specific sequence of FGFR in the N-terminal region of the IgII domain, which is composed of a cluster of basic and hydrophobic residues (K18K in FGFR1) (54). The basic heparin binding site is well conserved among the four FGFRs and corresponds to the sequence from Arg134 to Arg151 in FGFR3 (Fig. 6B). The acid box is located next to the heparin binding site. A dimeric model
Acid Box-deleted FGFR3

requires lower concentrations of heparin compared with FGFR3. The acid box may bind to the basic heparin binding region on the IgII domain, thereby competing with heparin for FGFR binding. It may also affect FGF-FGFR interactions. These results suggest that the binding activity of FGFR3 to heparin may be modulated by the presence of the acid box and that as a consequence the mitogenic activity of the receptor is regulated.

Using FGF1 as an internal standard, we found that FGFR3ΔAB showed higher responses to FGF2, FGF4, and FGF8 than did FGFR3 (Table II). The low mitogenic response of FGFR3 to FGF2 is consistent with the results of a previous study using BaF3 cells expressing the mouse full-length FGF3 (11). It has also been reported that a soluble FGFR3c did not bind to FGF2 but bound to FGF1 (36). However, the mitogenic responses of a chimeric receptor, which was engineered to contain the entire extracellular domain of FGFR3 and the tyrosine kinase domain of FGFR1, were equivalent to FGF1 and FGF2 (42). The mitogenic responses of FGFR3ΔAB to FGF2 and other FGFs in our study were similar to those of the chimeric receptor. Many studies have suggested that the ligand binding domains reside in IgII and IgIII but do not include the acid box (8, 56, 57). The increased responsiveness of FGFR3ΔAB to FGF2 may be due to a conformational change caused by the complete deletion of the acid box, because the protein folding of the ligand binding region in FGFR3 may be strongly affected by the presence of the acid box. Mapping ligand binding studies in chimeric FGFR1 and FGFR3 suggested a two-binding region model, i.e. proximal and distal binding sites (58). In this model, the distal binding site, the C-terminal half of FGFR1 Ig domain IIIc, is important for FGF2 binding, and the proximal binding site in FGFR1, the IgII loop and the IgI-IgII linker region, is cooperatively linked to the distal binding site. In contrast, the distal binding site in FGFR3 was poor for binding to FGF2. This may account for the decreased binding of FGFR3 to FGF2. In the crystal model mentioned above (55), FGF2 was shown to interact with seven amino acids (Asp154, Lys155, and Leu157-Pro161) within the basic heparin binding site that were thought to be the responsible residues in the proximal binding site. In addition, FGF8 and FGF9 were also shown to bind to a broad region spanning the IgII loop and IgI-IgII linker sequence in FGFR3. Thus, deletion of the acid box may enhance not only heparin binding but also the ligands binding to the proximal binding site in FGFR3. It is necessary to determine whether the increased mitogenic responses of FGFR3ΔAB to FGF2 and other FGFs are due to the increased ligand binding ability of this isoform, and if so, we should analyze the mechanism by which the acid box regulates FGF binding.

Biological Significance of FGF and FGFR Expression during Chondrogenesis—FGF2 is abundant in cartilage. It was originally extracted from bovine scapular cartilage and purified as a cartilage-derived growth factor (59). FGFR3ΔAB showed higher sensitivity to FGF2 than did FGFR3. FGFR3ΔAB may stimulate the signal cascades in mesenchymal cells during early development of the limb bud, utilizing FGF2 that is also expressed in the mesenchyme (60). During chondrogenesis, FGFR3ΔAB may modulate FGFR3 signaling by forming a heterodimer between these two isoforms. Ligand specificity of FGFR isoforms may also be regulated in a cell type-dependent manner by the expression of specific cell surface heparan sulfate proteoglycans (46, 61). Our results suggest that FGFR3ΔAB is likely to be the preferred receptor for FGF under restricted heparan sulfate proteoglycan conditions and that the acid box plays an important role in the regulation of FGFR3 to mediate biological activities in response to FGFs. Therefore, it

has been deduced from the crystal structure of the bacterially expressed extracellular domains IgII and IgIII of FGFR1 in complex with FGF2 (55). In this model, a positively charged canyon on the surface of the dimeric receptors is formed by the heparin binding site on IgII. Binding of heparin to the canyon stabilizes the dimeric structure of the FGF-FGFR complex. This hypothesis is consistent with the results of previous studies demonstrating that FGFR1 lacking IgI and the acid box exhibited higher binding affinity for FGF and heparin (56). This also agreed with our results indicating that FGFR3ΔAB

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Acid Box-deleted FGFR3

TABLE II

| FGFR ligand | FGF1a | FGF2 | FGF4 | FGF6 | FGF8 | FGF9 |
|-------------|-------|------|------|------|------|------|
| FGFR3       | 100.0 ± 5.7 | 4.4 ± 0.8 | 34.0 ± 1.9 | 1.5 ± 0.2 | 27.6 ± 1.0 | 63.2 ± 1.8 |
| FGFR3ΔAB    | 100.0 ± 1.2 | 105.2 ± 2.5 | 112.4 ± 3.2 | 7.8 ± 0.4 | 70.0 ± 4.3 | 119.2 ± 4.2 |

a All values are given ± S.D.

and FGFR3 play different roles in regulation of the growth and differentiation of chondrocytes.

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REFERENCES

1. Johnson, D. E., and Williams, L. T. (1993) Adv. Cancer Res. 60, 1–41
2. Baird, A., and Klagsbrun, M. (1991) Cancer Cells 3, 239–243
3. Martin, G. R. (1996) Genes Dev. 12, 1571–1586
4. Eisemann, A. A., Graziani, G., Gronick, S. R., and Ron, D. (1991) Oncogene 6, 1195–1200
5. Champion-Arnaud, P., Ronsoni, C., Gilbert, E., Genzani, M. C., Houssaint, E., and Breathnach, R. (1991) Oncogene 6, 579–587
6. Johnson, D. E., Lu, J., Chen, H., Werner, S., and Williams, L. T. (1990) Mol. Cell. Biol. 10, 4728–4736
7. Werner, S., Duan, D. H., De Vries, C., Peter, K. G., Johnson, D. E., and Williams, L. T. (1992) Mol. Cell. Biol. 12, 82–88
8. Chelliah, A. T., McEwen, D. G., Werner, S., Xu, J., and Orntz, D. M. (1994) J. Biol. Chem. 269, 11620–11627
9. Kemler, R., Ozawa, M., and Rigwild, M. (1989) Curr. Opin. Cell Biol. 1, 892–897
10. Chaudhuri, M. M., Moscatelli, D., and Basilico, C. (1995) J. Cell. Physiol. 157, 209–214
11. Bansal, R., Kumar, M., Murray, R., and Pfeiffer, S. E. (1996) Mol. Cell. Neurosci. 7, 263–275
12. Xu, X., Li, C., Takahashi, K., Slavkin, H. C., Shum, L., and Deng, D.-X. (1999) Proc. Natl. Acad. Sci. U. S. A. 95, 1195–1200
13. Ramsden, D. A., and Breathnach, R. (1991) Oncogene 6, 915–922
14. Maniyama, T., Miwa, Y., and Kikuta, Y. (1996) Cell Differ. 30, 109–116
15. Shukunami, C., Shigeno, C., Atsumi, T., Ishizeki, K., Sato, K., and Hirakawa, Y. (1996) J. Cell Biol. 133, 457–468
16. Shukunami, C., Ohno, O., Sakuda, M., and Hirakawa, Y. (1998) Exp. Cell Res. 241, 1–11
17. Kurukawa, T., Sasada, R., Iwane, M., and Igarashi, K. (1987) FEBS Lett. 213, 586–184
18. Miyaomoto, M., Narno, K., Seko, C., Matsumoto, S., Kondo, T., and Kurakawa, T. (1993) Mol. Cell. Biol. 13, 4251–4259
19. Orntz, D. M., and Leder, P. (1992) J. Biol. Chem. 267, 16055–16011

will be important to compare the functions of these isoforms when they are expressed in prechondrocytes and chondrocytes.

Further studies are required to examine the native ligands of the FGFR3 isoforms as well as the signaling cascade activated upon FGF-FGFR binding in chondrogenesis. Similar studies on FGFR1 and FGFR2 isoforms and the cross-talk between FGFR1, FGFR2, and FGFR3 signaling are also important. These studies will provide insight into whether FGFR3ΔAB

FIG. 9. Heparin dependence for FGF1 and FGF2 mitogenic activity. BaF3 cells expressing FGFR3 (A) or FGFR3ΔAB (B) were plated in 24-well plates at a density of 1 × 10^4 cells/well and then increasing concentrations of heparin and 10 ng/ml heparin (0.645 nM) FGF1 (filled squares) or 10 ng/ml heparin (0.541 nM) FGF2 (filled circles) were added. After 3 days, the cells were harvested, and the cell number was counted by a Coulter Counter. The results are presented as a percentage of the cell number in the presence of FGF1 and 10 μg/ml heparin. The number in the absence of heparin is subtracted from all the data. The numbers of BaF3 expressing FGFR3 obtained after the growth assay were as follows: FGF1 without heparin, 1,187 ± 142 cells/well; FGF1 and 10 μg/ml heparin, 44,173 ± 1,714 cells/well; FGF2 and 10 μg/ml heparin, 42,180 ± 1,367 cells/well.
37. Shimomura, Y., Yoneda, T., and Suzuki, F. (1975) *Calcif. Tissue Int.* **19**, 179–187.
38. Chomczynski, P., and Sacchi, N. (1987) *Anal. Biochem.* **162**, 156–159.
39. Orr-Urtreger, A., Bedford, M. T., Burakova, T., Arman, E., Zimmer, Y., Yayon, A., Givol, D., and Lonai, P. (1993) *Dev. Biol.* **158**, 475–486.
40. Peter-Castro, A. V., Wilson, J., and Altherr, M. R. (1997) *Genomics* **41**, 10–16.
41. Ornitz, D. M., Xu, J., Colvin, J. S., McEwen, D. G., MacArthur, C. A., Coulier, F., Gao, G., and Goldfarb, M. (1996) *J. Biol. Chem.* **271**, 15292–15297.
42. Keegan, K., Johnson, D. E., Williams, L. T., and Hayman, M. J. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 1095–1099.
43. Keegan, K., Meyer, S., and Hayman, M. J. (1991) *Oncogene* **6**, 2229–2236.
44. Yayon, A., Klagesbrun, M., Esco, J. D., Leder, P., and Ornitz, D. M. (1991) *Cell* **64**, 841–848.
45. Kan, M., Wu, X., Wang, F., and McKeehan, W. L. (1999) *J. Biol. Chem.* **274**, 15947–15952.
46. Klagesbrun, M., and Shing, Y. (1985) *Proc. Natl. Acad. Sci. U. S. A.* **82**, 805–809.
47. Peters, K., Ornitz, D., Werner, S., and Williams, L. (1993) *Dev. Biol.* **155**, 423–430.
48. Niswander, L., Tickele, C., Vogel, A., Booth, I., and Martin, G. R. (1993) *Cell* **73**, 579–587.
49. Crossly, P. H., and Martin, G. R. (1995) *Development* **121**, 439–451.
50. Orr-Urtreger, A., Givol, D., Yayon, A., Yarden, Y., and Lonai, P. (1991) *Development* **113**, 1419–1434.
51. Xu, X., Weinstein, M., Li, C., and Deng, C.-X. (1999) *Cell Tissue Res.* **296**, 33–43.
52. Webster, M. K., and Donoghue, D. J. (1997) *Mol. Cell. Biol.* **17**, 5739–5747.
53. Keegan, K., Kan, M., Xu, J., Crabb, J. W., Hou, J., and McKeehan, W. L. (1991) *Science* **250**, 1918–1921.
54. Plotnikov, A. N., Schlessinger, J., Hubbard, S. R., and Mohammadi, M. (1999) *Cell* **98**, 641–650.
55. Wang, F., Kan, M., Xu, J., Yan, G., and McKeehan, W. L. (1995) *J. Biol. Chem.* **270**, 10231–10235.
56. Zimmer, Y., Givol, D., and Yayon, A. (1993) *J. Biol. Chem.* **268**, 7899–7903.
57. Chellaiah, A., Yuan, W., Chellaiah, M., and Ornitz, D. (1999) *J. Biol. Chem.* **274**, 54765–54774.
58. Klagesbrun, M., Langer, R., Levenson, R., Smith, S., and Lillehei, C. (1977) *Exp. Cell Res.* **105**, 99–108.
59. Savage, M. P., and Fallon, J. F. (1995) *Dev. Dyn.* **202**, 343–353.
60. Ornitz, D. M. (2000) *Bioessays* **22**, 108–112.
A Novel Alternatively Spliced Fibroblast Growth Factor Receptor 3 Isoform Lacking the Acid Box Domain Is Expressed during Chondrogenic Differentiation of ATDC5 Cells

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