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Short Contribution

Developmental expression of splicing variants of fibroblast growth factor receptor 3 (FGFR3) in mouse

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ABSTRACT A characteristic feature of the fibroblast growth factor receptor (FGFR) family is the structural diversity generated by alternative splicing. The FGFR3 gene encodes two splice variants because of the mutually exclusive use of the exons IIIb and IIIc. In the present study we examined the expression of the two different splice forms IIIb and IIIc of FGFR3 in developing mouse embryos (12 days p.c., 14 days p.c., 20 days p.c.). The overall level of the IIIc exon splice product surpassed that of the IIIb exon form. The IIIc mRNA was detected in the developing brain and in the spinal cord. Outside the nervous system very strong expression was observed in the vertebra and in all other bony structures. In contrast, the IIIb splice form was restricted to epithelial structures with no expression detected in the central nervous system and bone.

KEY WORDS: *FGFR3 splice forms, mouse development, tissue expression, in situ hybridization*

The fibroblast growth factor receptors are members of a tyrosine kinase receptor family. Four unlinked genes that encode FGF receptors (FGFR1-FGFR4) are currently known. The putative protein structure of the four FGFRs is similar, comprising an extracellular region with two or three immunoglobulin (Ig)-like domains, a transmembrane domain and an intracellular tyrosine kinase domain (Johnson and Williams, 1993).

A characteristic feature of the family is the structural diversity generated by alternative splicing. In the case of FGFR3 two splice variants are known, generated by differential use of two exons encoding an approximately 50 amino acid long variable region in the C-terminal half of the third Ig-like domain. These two forms are referred to as FGFR3 IIIb and FGFR3 IIIc.

In the last years, mutations in the FGF receptor genes have been identified as the underlying causes of several human disorders of bone growth and patterning (Wilkie *et al.*, 1995). While mutations in the FGFR1 and FGFR2 genes have been associated with Pfeiffer syndrome, Crouzon syndrome, Apert syndrome and Jackson-Weiss syndrome (Wilkie *et al.*, 1995), specific mutations in FGFR3 gene have been found in patients with the related chondrodysplasias Achondroplasia (ACH, Shiang *et al.*, 1994), Hypochondroplasia (HCH, Bellus *et al.*, 1995), Thanatophoric dysplasia (TD, Tavormina *et al.*, 1995), and recently in a specific Crouzon phenotype (Meyers *et al.*, 1995).

We used exon-specific oligonucleotides as probes for RNA *in situ* hybridization studies to investigate the expression pattern of FGFR3 at various stages during mouse development, focusing on the selective expression pattern of the two splice variants.

The two splice forms showed distinct expression patterns. The expression pattern of the FGFR3 IIIc form was clearly different from that of the IIIb variant.

At embryonic day (E) 12 the most intense expression of the FGFR3 IIIc splice form was in the hindbrain, in the vertebra and in the bones of the extremities. Faint labeling was also observed in the liver (Fig. 1A). No expression of the FGFR3 IIIb form was observed E12. At E14 the expression of the IIIc-variant was characterized by abundant labeling of the germinal epithelium of the developing central nervous system, vertebra, ribs and spinal cord (Fig. 1B), whereas the FGFR3 IIIb labeling was weak and confined to gastrointestinal and urinary tract epithelia (Fig. 1C). At E20 decreased FGFR3 IIIc expression in the central nervous system was observed, but labeling was still detected in the ventral spinal cord (Fig. 1D). In addition, strong labeling was observed in the vertebrae (Figs. 1D and 2D) and in the ribs and bones of the extremities (Fig. 1D). Distinct expression at this stage of development was also observed in the cartilage of the trachea and the submandibulary gland whereas only very weak labeling was

Abbreviations used in this paper: ACH, achondroplasia; FGFR, fibroblast growth factor receptor; HCH, hypochondroplasia; Ig, immunoglobulin; TH, thanatophoric dysplasia.

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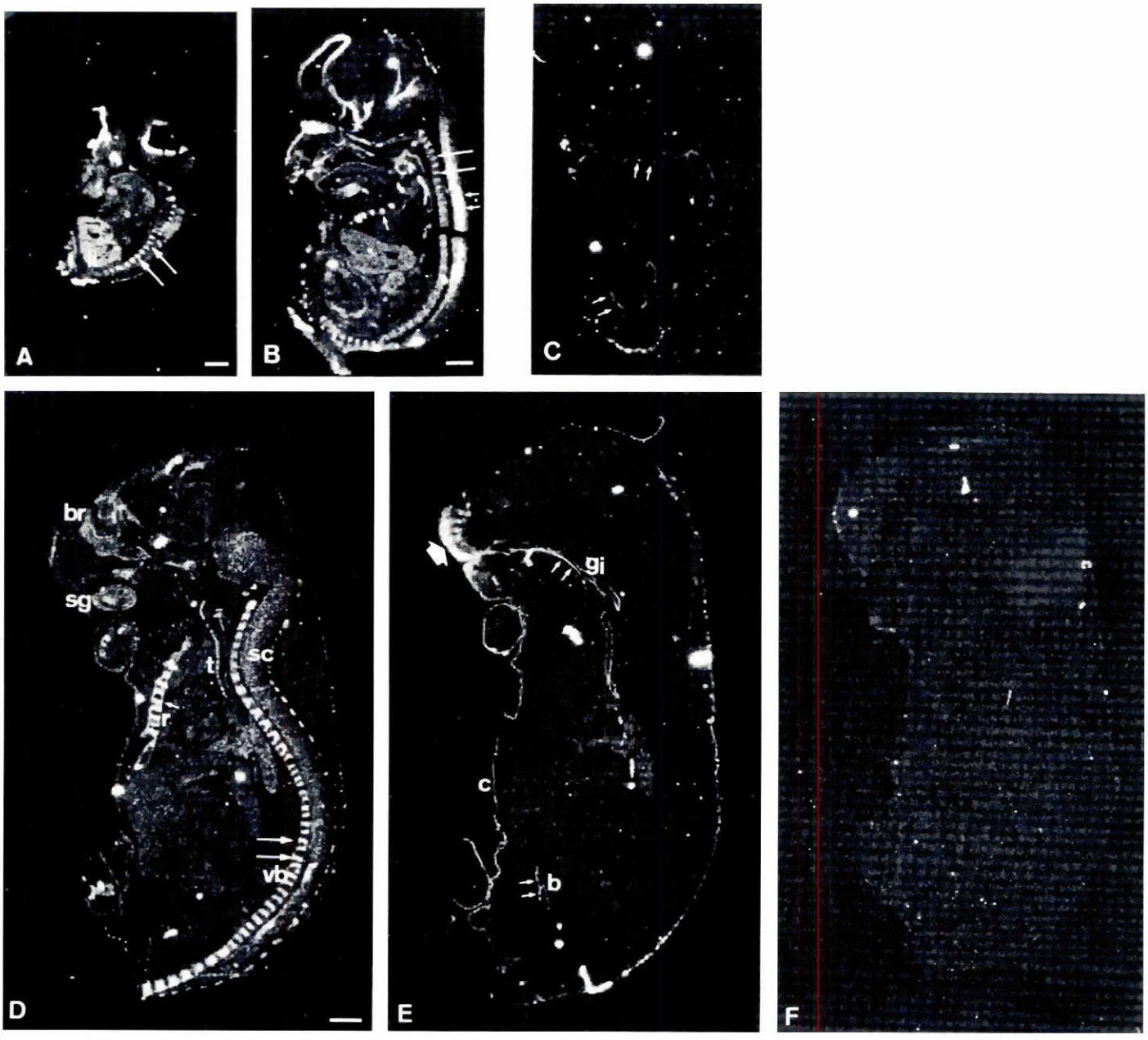

Fig. 1. Darkfield X-ray autoradiographs of the developing mouse embryo hybridized with FGFR3 IIIc specific (A,B,D), FGFR3 IIIb specific (C and E) and a sense probe (F). *Sagittal sections were taken from the midline of the day E12* **(A)**, *E14* **(B and C)** *and E20* **(D-F)** *embryos.* **(A)** *Very intense labeling of FGFR3 IIIc mRNA is observed in developing vertebra (arrows) as well as the developing central nervous system. Distinct labeling is also observed in the liver.* **(B)** *At E14 very intense labeling is seen over the developing vertebra (long arrows), ribs and bones of the extremities, as well as in the spinal cord (short arrows) and the epithelial layer of the developing central nervous system.* **(C)** *FGFR3 IIIb mRNA labeling is confined to epithelial structures of the gastrointestinal tract (short arrows) and urinary bladder (short arrows). No labeling is seen in the central nervous system or bony structures.* **(D)** *At E20 FGFR3 IIIb labeling is less intense but distinct in the developing brain stem and spinal cord, whereas developing vertebra (long arrows), ribs (short arrow) and bones of the extremities display very intense labeling. Distinct labeling is also present in cartilage of the trachea and faint labeling can be seen in the submandibulary gland.* **(E)** *Labeling with the FGFR3 IIIb specific probe is confined to the gastrointestinal (short arrows), bronchial and urinary (short arrows) epithelia as well as the epithelial cell layer of the epidermis. Particularly strong labeling is observed over epithelial cells surrounding the developing whiskers (big arrow). Note complete absence of labeling in the central nervous system and bony structures. In* **(F)** *a sense probe is hybridized to an adjacent section with no apparent labeling of any structure. gi, gastrointestinal tract; sc, spinal cord; sg, submandibulary gland; li, liver; b, bladder; vb, vertebral body; br, brain; r, ribs; t, trachea; c, cutis.*

observed over epithelial cells of the epidermis. No labeling was observed over urinary tract epithelia (Fig. 2B). At E14 the expression of the IIIb variant was confined to epithelial structures, including gastrointestinal, bronchial and urinary tract epithelia, as well as the epithelial cell layer of the epidermis (Figs. 1E and 2A). Very specific and intense expression in the epithelial cells surrounding

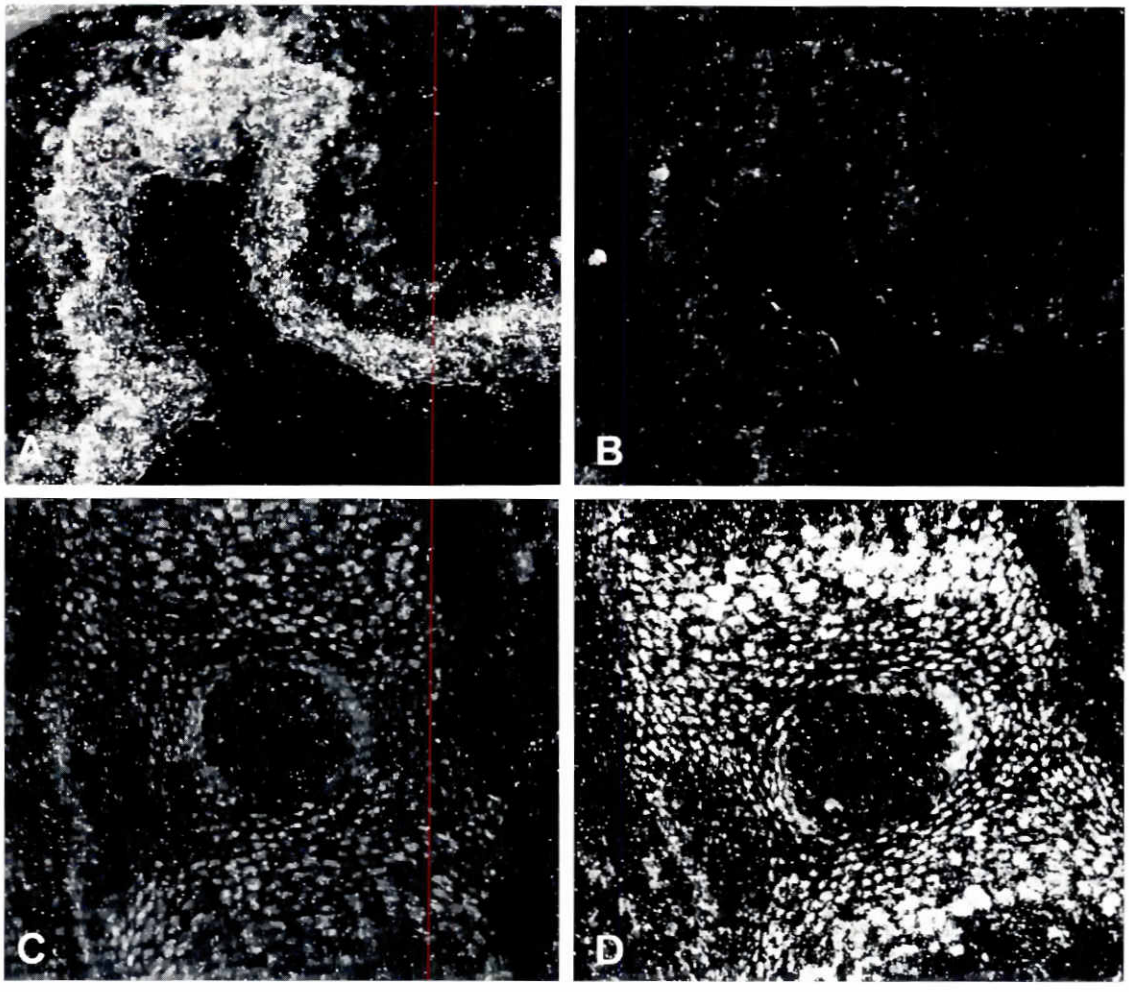

Fig. 2. FGFR3 expression in developing bladder (A,B) and vertebra (C,D). *Sections from E20 were hybridized with antisense oligonucleotides for FGFR3 IIIb* **(A,C)** *or FGFR3 IIIc* **(B,D). (A)** *Intense labeling of FGFR3 IIIb mRNA is seen in the epithelium of the urinary bladder whereas in* **(B)** *no labeling is observed using the probe selective for FGFR3 IIIc mRNA.* **(C)** *No labeling using the FGFR3 IIIb splice variant is seen in any bony or cartilage structures of the developing vertebra, whereas in* **(D)** *intense labeling of chondrocytes is seen using the FGFR3 IIIc probe. Similar labeling is observed in all bony structures examined. Magnification, 400x.*

the developing whiskers was detected with the FGFR3 IIIb probe (Fig. 1E). No labeling was found over bone (Figs. 1E and 2C). No specific signals were detected using the sense control probe (Fig. 1F).

These results are in agreement with previous observations (Peters *et al.*, 1993), using a probe which did not distinguish the two splice variants of FGFR3. They showed that outside the nervous system the highest level of FGFR3 expression was found in the cartilage rudiments of developing bones, with FGFR3 transcription confined to the resting cartilage at the growth plate during endochondral ossification (Peters *et al.*, 1993). They also found an intense expression in differentiating hair cells of the developing cochlear duct. They were however unable to detect FGFR3 mRNA in other types of sensory epithelia. The reason for these discrepancies might be due to usage of different hybridization probes. Other studies using Northern hybridizations and RT-PCRs, have shown that the IIIb splice variant is expressed at high levels in epithelial structures, while the IIIc variant is expressed predominantly in nonepithelial cells and tissues (Chellaiah *et al.*, 1994; Murgue *et al.*, 1994). The tissue-specific expression pattern of FGFR3 IIIb is similar to that of KGFR (FGFR2 IIIb) expression in epithelial tissue (Orr-Urtreger *et al.*, 1993).

Cross hybridization between the specific FGFR3 IIIb and IIIc oligonucleotides and the appropriate segments of the FGFR2 gene can be excluded, because the homology is only 51% and 57%, respectively. It is therefore possible that these receptors share a common ligand and have redundant function in epithelial tissue.

While specific mutations in the FGFR3 gene can be correlated with the clinical phenotypes of ACH, HCH and TD, it has to be investigated whether these defects also involve the expression pattern or formation and processing of the different splice forms. Further expression studies need to be done, to study the variant FGFR3 splice forms in patients with these chondrodysplasias. It is furthermore possible that other clinical phenotypes could be caused by mutations affecting selectively one or the other splice form. For example, an almost exclusively epithelial phenotype could correlate with mutations in FGFR3 IIIb.

Experimental Procedures

Preparations of probes

Oligonucleotide probes with sequence complementary to mouse mRNA encoding FGFR3 IIIa/IIIb exon (5'- CAC ATT CTC ACT GAT CCA GGA CTT GAG TAC AGT GAC GTA GGG C3') (nucleotides 1178-1158 + 214-193), FGFR3 IIIc/juxtamembrane exon (5'- GTT TCC ATC AGC TCC TCC TCA GCT GGC AGC ACC ACC AGC CAC GC3') (nucleotides 2612-2591 + 2137-2116) (Chellaiah *et al.*, 1994) and a FGFR3 sense probe (5'-GTT CCA CTG CAA GGT GTA CAG TGA CGC ACA GCC CCA CAT CCA GTG GCT CAA GCA CGT GGA-3') (nucleotides 854-913) (Keegan *et al.*, 1991)

were synthesized and purified through NAP-25 columns at Pharmacia Biotech, Uppsala (Sweden). The oligonucleotides were labeled at the 3' end with α-^{35}S-dATP (DuPont, NEN, USA) using terminal deoxynucleotidyl transferase (Amersham, Buckinghamshire, UK) to a specific activity of 2-4x10^9 cpm/μg.

In situ hybridization

In situ hybridization of 12 days p.c., 14 days p.c., 20 days p.c. mouse embryos (Balbc) was performed using frozen sectioned material as previously described (Schalling *et al.*, 1990). The tissue was covered with a hybridization buffer containing 50% formamide, 4xSSC, 1xDenhardt's solution, 1% sarcosyl, 0.02 M phosphate buffer (pH 7.0), 10% dextran sulphate (Pharmacia, Biotech), 500 μg/ml salmon-sperm DNA, 200 mM dithiothreitol and 1x10^7 cpm/ml of one of the labeled probes. After hybridization the sections were sequentially rinsed in four changes of 1xSSC at 55°C for 1 h, dried, and exposed to Amersham Hyperfilm-ß-max. Some sections were exposed in NTB2 nuclear track emulsion (Kodak), and analyzed in a Zeiss Axiophot microscope (Carl Zeiss, Oberkochen, Germany) using TMX 100 black- and white film (Kodak).

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