Vol. 194, No. 1, 1993 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1993 Pages 512-518

EXON SKIPPING CAUSES ALTERATION OF THE COOH-TERMINUS AND DELETION OF THE PHOSPHOLIPASE Cγ1 INTERACTION SITE IN THE FGF RECEPTOR 2 KINASE IN NORMAL PROSTATE EPITHELIAL CELLS*

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Received June 7, 1993

SUMMARY. Polymerase chain reaction analysis revealed an mRNA in rat prostate that results from alternate splicing of exon 16 in the heparin-binding fibroblast growth factor receptor kinase type 2 gene (FGFR2). The absence of exon 16 and the shift in reading frame at the exon 15-17 junction predicts an expression product (FGFR2-2) with a unique COOH-terminus that does not exhibit the major autophosphorylation site (tyrosine 789) required for interaction of phospholipase Cγ1 with the full-length FGFR2-1 isoform. Nuclease protection analysis revealed that the FGFR-2 splice variant is expressed in quantities equal to or greater than the FGFR2-1 isoform in normal prostate tissue. When combined with the same FGFR2 extracellular domain, co-expression of the two COOH-terminal variants may mediate effect of the same FGF ligand on different signal transduction pathways.

The tyrosine kinase glycoprotein component of the heparan sulfate fibroblast growth factor receptor complex (FGFR) is composed of four genes that exhibit splice variations resulting in structural isoforms that vary in the ligand-binding extracellular, the intracellular juxtamembrane and the intracellular tyrosine kinase domains (1,2). Variants in the extracellular domain affect

*This work was supported by US Public Health Service grants CA37589 and DK40739.

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Abbreviations used are: FGFR, fibroblast growth factor (FGF) receptor (exons are numbered according to ref. 5); PCR, polymerase chain reaction; nt, nucleotides.

0006-291X/93 $4.00

interaction with heparan sulfate, affinity for the same ligand and specificity for different ligands (3). The intracellular kinase domain exhibits multiple candidate tyrosine autophosphorylation sites of which two have been identified as autophosphorylated and important in the interaction with and phosphorylation of signal transducers (3,4). Phosphotyr-766 in the COOH-terminus of the FGFR1 kinase is obligatory and sufficient for binding of phospholipase Cγ1 (PLCγ1) while phosphotyr-653 within the catalytic domain of the kinase is required for phosphorylation and presumably activation of PLCγ1 (3). Although 14 single site splice variations in the FGFR1 gene have been reported from diverse tissues and cells, only one occurs in the intracellular kinase domain (1,3). An alternate donor site splice at the exon 12-13 junction (5) in the FGFR1 gene in a human hepatoma cell results in a kinase-defective isoform devoid of tyr residues 653 and 766. Regulated combinatorial splicing of the low-affinity extracellular domain with the kinase- and phosphorylation site-defective intracellular domain and heterodimerization with the kinase monomers appears to underlie the suppression of phosphorylation of PLCγ1 which correlates with the suppression of growth of the hepatoma cell line at high concentrations of FGF (3).

Although activated in tumor epithelial cells, the FGFR1 gene is normally expressed in non-parenchymal mesenchymal cell types in adult tissues with a well-defined epithelium and stroma (6). In contrast, the FGFR2 gene is specifically expressed in epithelial cells (6,7). Here we report a splice variant of the FGFR2 gene (FGFR2-2) in adult rat prostate whose predicted product exhibits a largely intact kinase domain containing the FGFR2 counterpart (tyr-753) of FGFR1 tyr-653, but does not exhibit the FGFR2 counterpart (tyr-789) of FGFR1 tyr-766 in the COOH-terminus. The variant results from alternate splicing of FGFR2 exon 16 (5) and is expressed at levels equal to or greater than the full-length FGFR2-1 kinase that exhibits both tyrosine autophosphorylation sites in adult prostate tissue.

MATERIALS AND METHODS

Characterization of COOH-terminal variants of FGFR2 from rat prostate. Methods for purification of poly(A+)RNA, reverse

transcription and the polymerase chain reaction (PCR) have been described (6,7). The PCR was carried out at 94 °C for 1 min, 55 °C for 2 min and 72 °C for 3 min using template and sense and anti-sense primers described in the text. PCR products were end-filled with Klenow enzyme and cloned into the EcoRV site of pSK Bluescript vector (Stratagene). cDNAs were sequenced in both directions by the dideoxy chain-termination method.

Nuclease protection analysis of FGFR2-2. The FGFR2-2 cDNA was excised from the pSK vector with SacI at a site within the exon 17 coding sequence 65 bp downstream of the exon 15-17 junction. The cDNA was cloned into the SacI site of the pSK vector which was then linearized by treatment with BglII which cut at a site which is 111 bp upstream of the 3'-end of exon 15 which is in both isoforms of FGFR2. A [^{32}P]-labeled antisense probe was synthesized with T3 polymerase and RNAase protection was performed as described (6,7). Reaction mixtures were digested with RNAase A and T1 at 30 °C for 30 min and analyzed on 5% acrylamide DNA sequencing gels containing 8 M urea.

RESULTS

Characterization of COOH-terminal variants of FGFR2 in rat prostate. Partial cDNAs coding for the intracellular COOH-terminus of the FGFR2 gene were generated in the PCR using a sense primer (5'-TGACAGACTTTGGACTCGCCAGAG-3') which is complementary to coding sequence within kinase subdomain VII and antisense primer (5'-GCAGAATTCTTGGGGACAGGCAGGCAGA-CACAGTCA-3') beginning 18 bp downstream of the stop codon for the full-length rat FGFR2-1 isoform with single-stranded cDNA from normal rat ventral prostate tissue as template. In addition to the expected 560-bp band coding for the FGFR2-1 isoform, the analysis revealed a 460-bp fragment which was verified to be an FGFR2 cDNA by Southern blot. Cloning and sequence analysis confirmed that the 560-bp band coded for the intact rat FGFR2-1 isoform including the 106-bp sequence of exon 16 (Fig. 1A). The sequence analysis revealed that the shorter 460-bp band did not contain the exon 16 sequence and defined the novel FGFR2-2 mRNA (Fig. 1B).

Relative expression of the FGFR2 variants in rat prostate and a mouse keratinocyte cell line. Expression of the FGFR2-2 isoform relative to the FGFR2-1 isoform was determined by nuclease protection using a rat antisense probe which consisted of 111 nucleotides (nt) of the 3' sequence of exon 15 followed by 65 nt of the 5' sequence of exon 17. The analysis revealed that intensity of the 176-nt

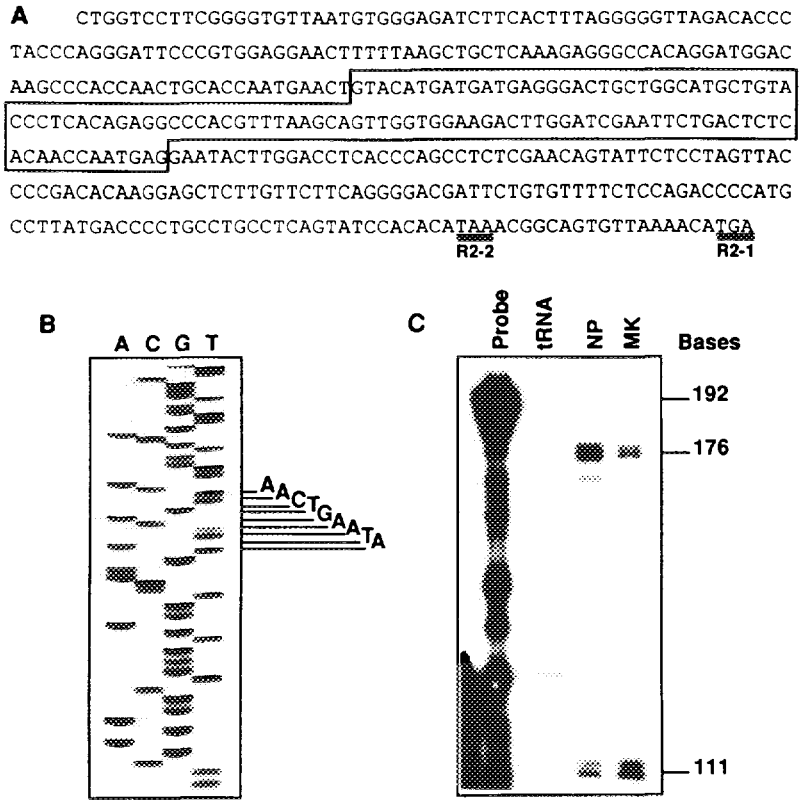

Fig. 1. Nucleotide sequence and expression of the rat FGFR2 splice variants. A. Coding sequence for the FGFR2-1 intracellular domain beginning with the 5'-end of exon 15. Exon 16 which is deleted in FGFR2-2 is boxed. Stop codons for FGFR2-2 and FGFR2-1 are indicated. The complete nucleotide sequences coding for the intracellular domains of rat FGFR2-1 and FGFR2-2 have been assigned Genbank nos. L19109 and L19110, respectively. Nucleotide sequences for extracellular domain variants of FGFR2 that have been cloned from rat prostate have been assigned Genbank nos. L19104, L19105, L19107, L19108 and L9111. B. Sequence analysis across the exon 15-17 junction in the FGFR2-2 cDNA. The antisense primer described in the text was used in the sequencing reaction. C. Nuclease protection analysis of FGFR2-2 expression. NP, total RNA from normal rat ventral prostate tissue; MK, total RNA from a non-malignant mouse keratinocyte cell line. Each analysis contained 20 μg of RNA.

band characteristic of the FGFR2-2 isoform was equal to or greater than that of the 111-nt band indicative of the FGFR2-1 isoform in normal rat ventral prostate tissue (NP) (Fig. 1C). Application of the same analysis to RNA preparations from a mouse keratinocyte line (MK) indicated that both isoforms are co-expressed, however, expression of the FGFR2-1 isoform appears to exceed the FGFR2-2 isoform in the cell line (Fig. 1C).

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r R2-2  ////////////////////////////////NTWTSPSLSNSILLVTPTQGALVLQGTILCFLQTPCLMTPACLSIHT
r R2-1  YMMMRDCWHAVPSQRPTFKQLVEDLDRILTLTTNEEYLDLTQPLEQYSPSYPDTRSSCSSGDDSVFSPDPMPYDPCLPQYPHINGSVKT
m R2-1  ▲753.....▲789.....E.....
h R2-1  .....S.....E.....
h tk25  .....S....P...C...P. ∫
h k-sam .....PPNPSLMSIFRK
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Fig. 2. Comparison of the deduced amino acid sequences of variant cDNAs coding for the COOH-terminal intracellular domain of the FGFR2-2 gene. Deduced sequences beginning at the 3'-end of exon 15 are indicated. Slashes indicate residues encoded in exon 16 which are deleted in FGFR2-2. Dots indicate residues in the mouse (m) and human (h) sequences that are identical to rat (r). Tyr-753 and tyr-789 in FGFR2-1 are indicated. The sequence for mouse R2-1 is from ref. 10, human R2-1 and *tk25* from ref. 8 and human *k-sam* from ref. 9.

Deduced sequence of the FGFR2-2 isoform. Fig. 2 compares the deduced amino acid sequence of the intracellular COOH-terminal domain of rat FGFR2-2 from the 3'-end of exon 15 to the rat, human and mouse FGFR2-1 isoforms and two other reported COOH-terminal variants of the human FGFR2 gene (8,9). The ligation of exon 15 to exon 17 in FGFR2-2 results in a shift in reading frame that codes for 47 unique residues. The deletion of exon 16 results in deletion of two candidate tyrosine phosphorylation sites, tyr-753 and tyr-789 (Fig. 2), resulting from ligation of exons 15-16-17 in the FGFR2-1 mRNA.

DISCUSSION

Here we report a new splice variant of the FGFR2 gene (FGFR2-2) from normal rat prostate whose predicted translation product results in a unique intracellular COOH-terminal sequence devoid of two candidate tyrosine autophosphorylation sites (tyr-753 and tyr-789) that are in the previously reported FGFR2-1 isoform. Phosphotyr-789 is the counterpart of phosphotyr-766 in the FGFR1 gene that is required for interaction with PLCγ1 (3, our unpublished results). The FGFR2-2 mRNA arises by alternate splicing of exon 16 and appears to be the most abundant isoform expressed in resting adult rat ventral prostate. Two cDNAs coding for COOH-terminal variants of the FGF-R2 gene from specific human tumor tissue have been reported previously (8,9), but neither have been detected in other cells and tissues (8). One variant, *tk25*, appears to result from an alternate acceptor site splice at the exon 16-17 junction

(8). The predicted product is an isoform with an intact exon 16 coding sequence with a truncated COOH-terminus downstream of tyr-789 (8, Fig. 2). The expression and mode of generation of the second human variant, *k-sam* (9), which predicts a truncated COOH-terminus due to a shift in reading frame upstream of tyr-789, has not been verified (9). We have been unable to detect the rat counterpart of either the *tk25* or *k-sam* variants in rat prostate cell and tissues by the approach described in this report.

The FGFR2 gene is specifically expressed in the epithelial cells of both normal rat prostate and non-malignant, androgen-responsive, well-differentiated model rat prostate tumors which are composed of both epithelial and stromal cell compartments (6,7). Exclusive splicing of exon 6 versus exon 7 which codes for the COOH-terminus of the juxtamembrane Ig-like disulfide loop in the extracellular domain of the FGFR2 gene in epithelial cells results in a specific isoform, FGFR2(IIIb) (5,8,9), which recognizes stromal cell-derived FGF-7 ligand and appears to be the reception end of a directionally-specific communication system from stromal to epithelial cells (6,7). FGF-7 potentially causes both the growth and terminal differentiation of prostate and other epithelial cells (6,7). Since the FGFR2(IIIb) extracellular domain isoform is exclusively expressed in normal prostate and non-malignant prostate tumor epithelial cells that are capable of differentiation (6,7), the ratio of expression of the FGFR2-1 and FGFR2-2 intracellular COOH-terminal isoforms which are expected to interact with different signal transduction substrates may explain the dual role of FGF-7 on cell growth and differentiation.

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