Advances in Brief

Mutations in *Fibroblast Growth Factor Receptor 2* and *Fibroblast Growth Factor Receptor 3* Genes Associated with Human Gastric and Colorectal Cancers[1]

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Abstract

Autosomal dominant disorders of skeletal and cranial development have been linked to *fibroblast growth factor receptor* (*FGFR*) *2* and *FGFR3*. Here we report two identical mutations in *FGFR2* that cause craniosynostosis syndromes, Crouzon, Apert, and Pfeiffer in gastric carcinoma. A missense mutation (Ser267Pro) in exon IIIa and a splice site mutation (940−2A→G) in exon IIIc were detected in gastric cancer patients. Interestingly, these heterozygous somatic mutations are identical to the germinal activating mutations in *FGFR2* reported previously in craniosynostosis syndromes. In addition, the two novel mutations of *FGFR3* in colorectal carcinomas were identified. All identified mutations occurred at highly conserved sequences, not only in the FGFR family of molecules, but also throughout evolution and clustered in the immunoglobulin-like loop-III domain, highlighting the functional importance of this domain. Our results indicate that *FGFR2* and *FGFR3*, in addition to their potential role in skeletal dysplasias, play an important role in tumorigenesis.

Introduction

FGFR[3] encodes a transmembrane tyrosine kinase receptor involved in signaling via interaction with the family of fibroblast growth factors. Currently, four members of the FGFR family (FGFR1–4) have been identified which are composed of an extracellular Ig-like domain, a transmembrane domain, and an intracellular kinase domain (1). They regulate a multitude of cellular processes, including cell growth, differentiation, migration, and survival, and have been implicated in pathological processes including angiogenesis, wound healing, and tumorigenesis (2–4). A number of autosomal dominant skeletal dysplasias are associated with mutations in FGFR1, -2, and -3 (reviewed in Ref. 5). These include achondroplasia (*FGFR3*; the most common genetic form of dwarfism; Ref. 6), Crouzon syndrome (*FGFR2*; Ref. 7), Apert syndrome (*FGFR2*; Ref. 8), Pfeiffer syndrome (*FGFR1* and *FGFR2*; Ref. 9), and thanatophoric dysplasia types I and II (*FGFR3*; Ref. 10). Most, although not all, of the mutations causing skeletal abnormalities involve conserved residues that are predicted to play an important structural role in the correct folding of FGFRs, and substitutions with other residues result in constitutive activation of FGFRs with ligand-independent dimerization (11).

Materials and Methods

Materials. Surgically resected tumors and corresponding normal tissues were obtained from patients who underwent surgery at the Department of Surgery, Seoul National University Hospital (Seoul, Korea). All tissue samples were immediately snap-frozen after surgery, and these were kept in liquid nitrogen. Genomic DNA was prepared by proteinase K digestion and phenol/chloroform extraction and then ethanol precipitation, as described (2).

Mutation Analysis of *FGFR2* Gene. Genomic DNA was prepared from solid tumors by conventional techniques. PCR amplifications of FGFR2 exons IIIa and IIIc were performed using primers reported previously (12, 13). The amplifications were carried out in a GeneAmp PCR system 9600 (Perkin-Elmer Corp., Norwalk, CT). The reaction profile consisted of one cycle at 94°C for 1 min and then 35 cycles at 94°C for 1 min, 58°C for 30 s, and 72°C for 1 min, with a final extension of 5 min at 72°C. To sequence the PCR products containing the DNA insertions, the fragments were cloned using a TOPO TA Cloning Kit according to the manufacturer's protocol (Invitrogen) and we sequenced the cloned DNA using internal gene-specific primers in both directions.

Mutation Analysis of *FGFR3* Gene. DNA samples were amplified for single-strand conformational polymorphism analysis of the *FGFR3* gene using PCR under the same conditions as reported previously (14). Putative mutations were analyzed further by cloning into plasmids and sequencing the cloned DNA.

DNA Sequencing. Automated sequencing was performed using dideoxy terminator cycle sequencing (Applied Biosystems) and an Applied Biosystems model 377 DNA sequencer (Perkin-Elmer, Foster City, CA).

GenBank Accession Nos. Human FGFR3 (*Homo sapiens*), P22607; mice FGFR3 (*Mus musculus*), A48991; Rat FGFR3 (*Rattus norvegicus*), B54846; *Drosophila* FGFR homologue 1 (*Drosophila melanogaster*), Q07407; nematode egl-15 protein (*Caenorhabditis elegans*), g3877838; yeast Cdc 15p (*Saccharomyces cerevisiae*), g349757; human FGFR1 (flg), g183879; human FGFR2 (bek), g339711; human FGFR4, g182571.

Results and Discussion

Somatic Mutations of *FGFR2* in Gastric Carcinomas Are Identical to Germinal Activating Mutations in Craniosynostosis Syndromes. *FGFR2* was first identified as an amplified gene from the human gastric cancer cell line KATOIII (15). It is preferentially amplified and overexpressed in the undifferentiated or diffuse type of gastric cancers. Its overexpression has been correlated with poor prognosis in gastric cancer (16). Thus far, no mutations in *FGFR2* have been reported to be associated with human carcinoma. We screened 30 human primary gastric carcinomas and matched normal gastric tissues for FGFR2 mutations in the third Ig-like domain by direct sequencing exons IIIa and IIIc (exons 8, 10), which are frequently observed to be mutated in craniosynostosis. Of the 30 gastric cancer tissues, two heterozygous somatic mutations were found: one missense mutation (136C) detected at exon IIIa resulting in the replacement of Ser by Pro at codon 267; and an A to G transition (105C) in the 3′ splicing acceptor site of the intron adjacent to exon IIIc of the *FGFR2,* disrupting the consensus sequence required for the normal splicing (Fig. 1). All identified FGFR2 mutations were identical to the germinal activating mutations that are responsible for

Crouzon syndrome (7), Apert syndrome (8), and Pfeiffer syndrome (Refs. 17, 18; Fig. 2*A*). Noncysteine craniosysnostosis mutations like S267P have been proposed to disrupt the formation of the native third Ig-like loop disulfide bond, which in turn allows the unbonded cysteine residues to participate in aberrant intermolecular dimerization resulting in constitutive receptor activation (11). Ser_{267} is a highly conserved residue throughout evolution except for Thr (another polar, hydroxy-containing amino acid) substitution (Fig. 3*A*).

Mutations of FGFR3 Occur in Highly Conserved Residues. Recently, a number of identical mutations that cause thanatophoric dysplasia, the most common neonatal lethal skeletal dysplasia, were reported in association with human bladder and cervix cancers (3). We performed PCR-single-strand conformational polymorphism analysis of the entire coding region of *FGFR3* and the intronic sequences flanking each of its exons for mutation in genomic DNA from 40 primary CRCs to screen for DNA variants before direct sequence analysis. Sequences of abnormally migrating bands revealed three sporadic mutations in 2 of 40 colorectal tumor samples. The two somatic mutations occurred in a third Ig-like loop region (one in exon 7 and one in exon 9). The tumor DNA from CRC 386C had a G to A transition at the first nucleotide of codon 322 (Fig. 1), which results in the substitution of Lys for Glu. The crystal structure of FGFR1 reveals that Glu_{322} is a primary residue involved in direct FGF2-FGFR1 interaction (Fig. 2*B*; Ref. 19). The multiple sequence alignment of the FGFR family and FGFR3 homologues from various species was used to infer its contribution to the phenotype. Fig. 3*B* shows the alignment of amino acid sequences from the six species in regions relevant to
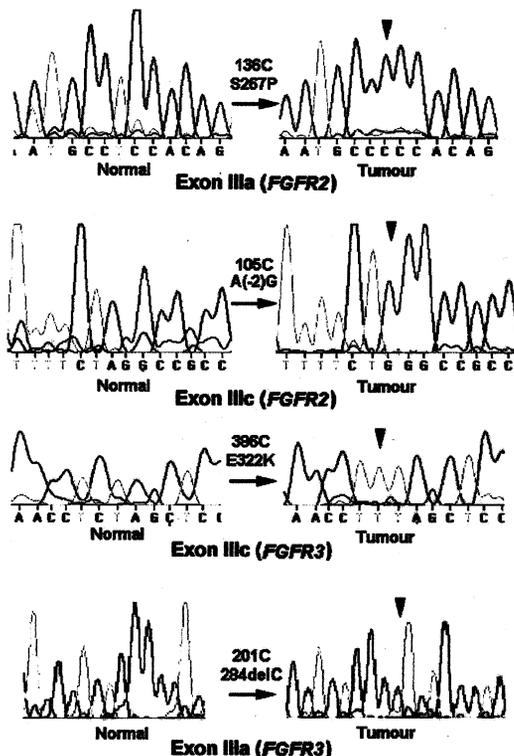

Fig. 1. Somatic mutations of *FGFR2* and *FGFR3* in human carcinomas. Sequence electropherograms of mutations (*arrowheads*) found in human primary gastric carcinomas and CRCs with the corresponding normal sequences. All mutations were not found in corresponding normal DNAs, demonstrating the somatic nature of these mutation. An one-base substitution of T to C at the first position of codon 267 of *FGFR2* in gastric carcinoma (136C); an one-base substitution of A to G at position −2 of the acceptor splice site of the FGFR2 exon IIIc in gastric carcinoma (105C); an one-base substitution of G to A at the first position of codon 322 in tumor DNA from human primary CRC (386C); an one-base deletion of C at the first position of codon 284 in tumor DNA from human primary CRC (201C). All sequences shown are in the forward direction except for 386C and 201C.
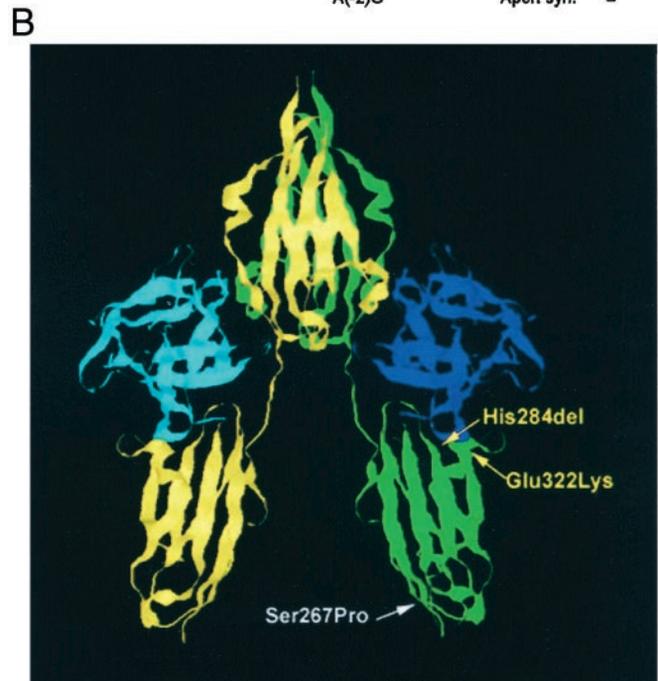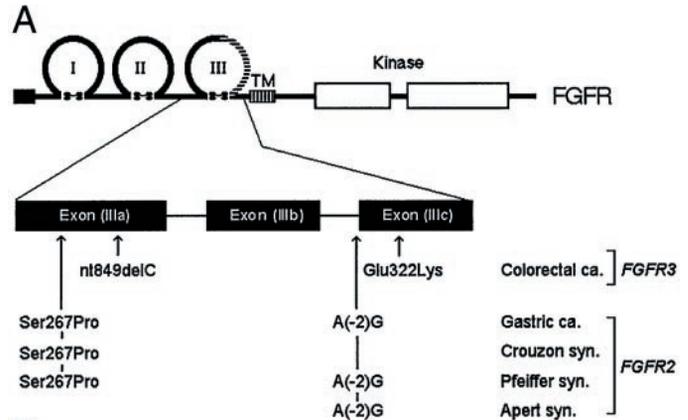

Fig. 2. *A,* the location of mutations are shown with the schematic structure of FGFR (*I-III*, Ig-like domains; *TM*, transmembrane domain; *Kinase*, two tyrosine kinase domains; ▤, the second half of the Ig-like loop III domain. Mutations were found in the Ig-like loop III of FGFR. *B,* position of mutations in the tertiary structure of the dimeric FGF-FGFR complex. *Arrow,* positions of *FGFR2* (*white*) and *FGFR3* (*yellow*) mutations. The two FGF2 molecules are shown in *cyan* and *blue* and dimeric FGFR1 molecules are shown in *yellow* and *green*, respectively. The image was prepared from the X-ray crystallographic coordinates taken from the Molecular Modeling Database.[4]

Glu_{322}. Glu_{322} is indeed a highly conserved residue from yeast to human and its FGFR family. Therefore, substitution of Glu_{322} with the positively charged lysine side chain will disrupt its interaction with FGF, resulting in an impairment of its capability to control cell growth, and will lead to tumors. However, the possibility cannot been ruled out that this FGFR3 mutation inactivates FGFR3 function. These experiments are in progress in our laboratory. In one case, in CRC 201C, we detected a nonsense mutation (849delC) in exon 7 causing a frameshift and premature termination (Fig. 1 and Fig. 3*C*).

For tumor suppressor proteins, highly conserved residues in human and mice have been instrumental in highlighting domains of particular biological relevance in which mutations tend to cluster (20). In this study, we show that the residues that predispose to tumors are a set of amino acids that are conserved not only in the FGFR family of molecules but also throughout evolution. Our findings stress that the phenotypic expression of *FGFR2* and *FGFR3* mutations is related to

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[4] Internet address: http://www.ncbi.nlm.nih.gov/structure/MMDB/mmdb.html.

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A

	259
H. sapiens FGFR2	Q A G L P AN **A S** T V V **G G** D V E
H. sapiens FGFR1	Q A G L P AN K T V A L G S N V E
H. sapiens FGFR3	Q A G L P AN Q T A V L G S D V E
H. sapiens FGFR4	Q A G L P AN T T A V V G S D V E
M. musculus FGFR2	Q A G L P AN **A S** T V V **G G** D V E
R. norvegicus FGFR2	Q A G L P AN **A S** T V V **G G** D V E
D. melanogaster FGFR homolog 2	V V - - P Q N Q **T** V K V N G S L V
C. elegans egl-15	V P N I L AN Q S V N I N D T A T
Crouzon syndrome	Q A G L P AN **A P** T V V **G G** D V E
Pfeiffer syndrome	Q A G L P AN **A P** T V V **G G** D V E
Gastric ca. 136C	Q A G L P AN **A P** T V V **G G** D V E

B

	314
H. sapiens FGFR3	A N T T D - - - - - - **K E** L **E** V L S L H
H. sapiens FGFR1	V N T T D - - - - - - K E M E V L H L R
H. sapiens FGFR2	V N T T D - - - - - - K E I E V L Y I R
H. sapiens FGFR4	I N S S D - - - - - - E V E E V L Y I R
M. musculus FGFR3	A N T T D - - - - - - **K E** L **E** V L S L H
R. norvegicus FGFR3	I N S S N - - - - - - - - - A E V L A L F
D. melanogaster FGFR homolog 1	- - - - - - - - - - - - - D K D Q L D F V
C. elegans egl-15	M D T F D K A H V H H V G D E S T L T I
Colorectal ca. 386C	A N T T D - - - - - - **K E** L **K** V L S L H

C

	279
H. sapiens FGFR3	V Y S D **A** Q P **H** I Q **W** L K
H. sapiens FGFR1	V Y S D P Q P H I Q W L K
H. sapiens FGFR2	V Y S D **A** Q P **H** I Q **W** I K
H. sapiens FGFR4	V Y S D **A** Q P **H** I Q **W** L K
M. musculus FGFR3	V Y S D **A** Q P **H** I Q **W** L K
R. norvegicus FGFR3	V Y S D **A** Q P **H** I Q **W** I K
C. elegans egl-15	V V S D L L P **H** I I **W** V R
Colorectal ca. 201C	V Y S D **A** Q P T S S G S S T **W** R *

Fig. 3. The effects of the mutations on the primary structure of FGFRs and the level of evolutionary conservation of the domains in which the mutations occur. *A,* in 136C (gastric carcinoma), the identical missense mutation that causes Crouzon and Pfeiffer syndromes results in a substitution of a highly conserved Ser to a Pro. Ser_{267} is highly conserved throughout evolution except for Thr (another polar, hydroxy-containing amino acid) substitution. *B,* in 386C (CRC), substitution of a highly conserved Glu (from *C. elegans* to man) by a Lys as a result of a missense mutation. *C,* in 201C (CRC), one-base deletion leads to 9 out-of-frame amino acids starting at the highly conserved His_{284} and a stop codon (∗). Alignments were constructed using the CLUSTALW program. The primary amino acid sequences shows a 92% (mouse), 68% (rat), 42% (*Drosophila*), 41% (*C. elegans*), and 29% (yeast) identity to the human FGFR3 protein, respectively. The mutated amino acids and the conserved residues corresponding to the mutated residues are highlighted in **bold**.

the degree of interspecies conservation of the residues. The analysis of expressed mutant mRNAs in cell culture or transgenic animals and the analysis of the corresponding signaling pathways will elucidate further the role of FGFRs in tumorigenesis.

FGFR represents an important example of a single gene that causes different human developmental and tumoral diseases. The finding of identical mutations in skeletal dysplasias and gastric carcinoma is the first evidence for the involvement of the somatic mutations of *FGFR2* in gastric cancer, and suggests that a similar mechanism of ligand-independent constitutive activation of the *FGFR2* product is responsible for both diseases.

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