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Constitutive receptor activation by Crouzon syndrome mutations in fibrobast growth factor receptor (FGFR) 2 and FGFR2/Neu chimeras

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ABSTRACT **Crouzon syndrome is an autosomal dominant condition primarily characterized by craniosynostosis. This syndrome has been associated with a variety of amino acid point mutations in the extracellular domain of fibroblast growth factor receptor 2 (FGFR2). FGFR2/Neu chimeras were generated by substituting the extracellular domain of Neu with that of FGFR2 containing the following Crouzon mutations: Tyr-340→His; Cys-342→Tyr; Cys-342→Arg; Cys-342→Ser; Ser-354→Cys; and Δ17 (deletion of amino acids 345–361). Each of the mutant chimeric FGFR2/Neu constructs stimulated focus formation in NIH 3T3 cells, indicating that Crouzon mutations can stimulate signal transduction through a heterologous receptor tyrosine kinase. *In vitro* kinase assay results indicate that FGFR2 receptors containing Crouzon mutations have increased tyrosine kinase activity and, when analyzed under nonreducing conditions, exhibited disulfide-bonded dimers. Thus the human developmental abnormality Crouzon syndrome arises from constitutive activation of FGFR2 due to aberrant intermolecular disulfide-bonding. These results together with our earlier observation that achondroplasia results from constitutive activation of the related receptor FGFR3, leads to the prediction that other malformation syndromes attributed to FGFRs, such as Pfeiffer syndrome and Thanatophoric dysplasia, also arise from constitutive receptor activation.**

The human fibroblast growth factor receptor (FGFR) family is composed of four widely expressed receptor tyrosine kinases that play important roles in growth and development (1). These receptors possess three extracellular immunoglobulin-like domains, a single transmembrane domain, and an intracellular tyrosine kinase domain (see Fig. 1*B*). Various mutations in three of these receptors, FGFR1, FGFR2, and FGFR3, have recently been associated with several human developmental syndromes (2–21). Mutations located in the extracellular domain of FGFR2 have been linked to one such disorder called Crouzon syndrome (2, 6, 13, 14, 19, 20, 22), an autosomal dominant condition that is characterized by craniosynostosis, maxillary hypoplasia, shallow orbits, and ocular proptosis.

The Crouzon mutations described by Reardon *et al.* (2) result in single amino acid substitutions within or near the third immunoglobulin-like region, except for one mutation that generates a new donor splice site, leading to the deletion of 17 amino acids in the juxtamembrane region (20, 23). Four of the point mutations either destroy or create a Cys residue that may potentiate intermolecular disulfide bond formation, leading to receptor homodimerization. Studies of other unrelated receptors, such as the epidermal growth factor receptor (24), the erythropoietin receptor (25, 26), and RET (27), demonstrate that similar mutations that create unpaired extracellular Cys residues result in receptor homodimerization and constitutive activation.

In this work, we have examined six distinct Crouzon mutations and demonstrate that each one leads to constitutive receptor activation. When the FGFR2 extracellular domain, containing any of the Crouzon mutations, is fused to the Neu receptor tyrosine kinase, the chimeric FGFR2/Neu construct is able to drive proliferation of NIH 3T3 cells in a focus formation assay. Moreover, full-length FGFR2 receptors containing a Crouzon mutation exhibit an increase in tyrosine kinase activity and form ligand-independent dimers. These data indicate that the human developmental abnormality known as Crouzon syndrome arises from constitutive activation of FGFR2 due to aberrant intermolecular disulfide-bonding.

MATERIALS AND METHODS

Construction of FGFR2 Clones Containing Crouzon Mutations. All constructs contain the extracellular domain of FGFR2, transmembrane and intracellular domains derived either from FGFR2 or from Neu. Clones designated as FNN contain the transmembrane and intracellular domain of Neu; clones designated as FFN contain the transmembrane derived from FGFR2 and intracellular domain of Neu; clones designated as FFF contain transmembrane and intracellular domain from FGFR2.

In the map of the FNN constructs shown in Fig. 1*A*, the region from *Hin*dIII to *Nci*I was derived from a pair of complementary synthetic oligonucleotides (D984/985), which include FGFR2 sequences starting at nucleotide (nt) 166 and ending at the *Nci*I site at nt 241–245 in the standard FGFR2 sequence, EMBL Data Library accession #X52832 (28). The region from *Hpa*I to *Nhe*I was derived from a second pair of oligonucleotides (D1021/1022), which incorporated the following restriction sites into the FGFR2 extracellular domain without changing the resulting amino acid sequence: *Bst*BI (TTCGAA for TTTGAG, nt 1179–1184); *Eco*RI (GAATTC for TAATTC, nt 1214–1219); and *Nhe*I (GCTAGC for GCTTCC, nt 1290–1295).

Pairs of complementary oligonucleotides encoding wild-type (wt) (D1060/1061), Tyr-340→His (D1064/1065), Cys-342→Tyr (D1062/1063), Cys-342→Arg (D1164/1165), and Cys-342→Ser (D1166/1167) were ligated between the *Bst*BI and *Eco*RI sites. Oligonucleotides encoding Ser-354→Cys (D1078/1079) were ligated between the *Eco*RI and *Nhe*I sites. Oligonucleotides (D1146/1147) encoding the Δ17 splicing variant (20, 23), a deletion of 17 amino acids (residues 345–361), were ligated between the *Bst*BI and *Nhe*I sites. Each of

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Abbreviation: FGFR, fibroblast growth factor receptor.

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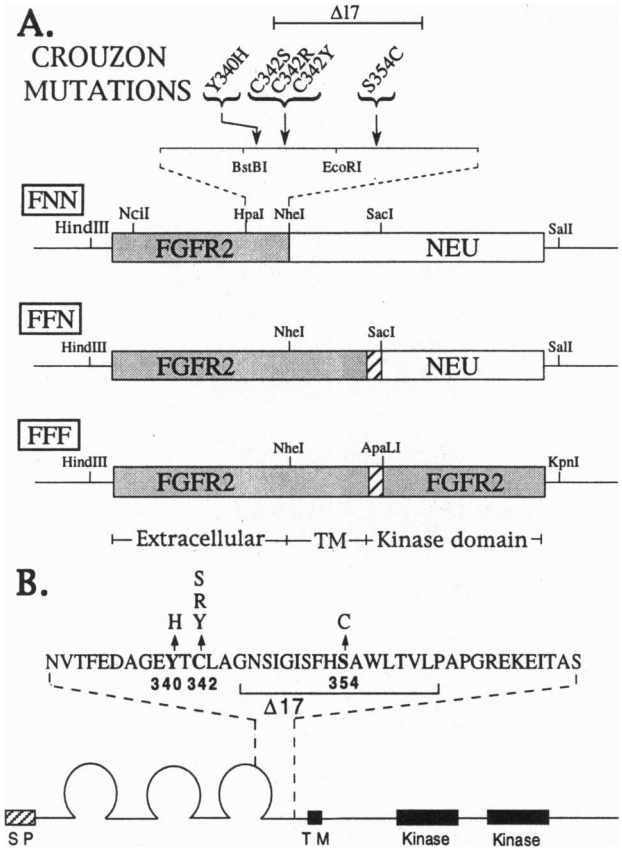

FIG. 1. Structure of FGFR2 constructs. (*A*) Chimeric receptors between FGFR2 and Neu were constructed as shown. Constructs designated FNN consist of the extracellular domain from FGFR2, and the transmembrane domain and kinase domain from Neu. Constructs designated FFN consist of the extracellular and transmembrane domains from FGFR2, and the kinase domain from Neu. Constructs designated FFF consist of extracellular, transmembrane and kinase domains from FGFR2. The stippled region shown in the FFN and FFF constructs represents an epitope tag, recognized by the mAb P5D4. (*B*) The structure of FGFR2 is shown schematically, including the signal peptide (SP), three Ig-like regions, the transmembrane domain, and the kinase domains within the intracellular domain. The various Crouzon mutations studied here are shown near the third Ig-like region in the extracellular domain of FGFR2.

these partial FGFR2 constructs was ligated into the vector pSV2Neu(*Nhe*I/*Sac*I) (29) between the *Hin*dIII and *Nhe*I sites, generating final FNN constructs.

The FFN constructs were derived by substitution of a pair of complementary oligonucleotides (D1134/1135), encoding the FGFR2 transmembrane domain, between the *Nhe*I and *Sac*I sites of each FNN construct. These oligonucleotides also encode an epitope tag for the monoclonal antibody (mAb) P5D4 (30) in the intracellular juxtamembrane region; this sequence (KLKHTKKRQIYTDIEMNRLGK) extends between residue Cys-398 of FGFR2 (28), and Glu-700 of Neu (31) in the FFN clones.

In FFF constructs, the *Hin*dIII-*Nhe*I fragment (containing the extracellular FGFR2 domain) was derived from the appropriate FNN construct; the *Nhe*I-*Apa*LI fragment was derived from a pair of complementary oligonucleotides (D1314/1315), encoding the FGFR2 transmembrane domain and epitope tag; the *Apa*LI-*Kpn*I fragment was isolated from the FGFR2 cDNA clone TK14 (32). The vector pcDNA3 (Invitrogen) was utilized for expression of the FFN and FFF constructs. For all constructs, sequences derived from oligonucleotides were confirmed by dideoxy nt sequencing.

Transformation Assays. NIH 3T3 cells were used in transformation assays as previously described (29).

Immunoprecipitations, Kinase Assays, and Immunoblotting. NIH 3T3 cells were transfected with each construct and prepared as previously described (29). Immunoprecipitation was performed with antiserum specific for either the C terminus of FGFR2 (polyclonal FGFR2/bek C-17, Santa Cruz Biotechnology), or with mAb P5D4 (30). Immune complexes were collected on Protein A-Sepharose beads, and extensively washed.

Kinase assays on immunoprecipitates from NIH 3T3 cells transfected with each expression construct were performed as previously described (29). Products were washed extensively with Nonidet P-40 lysis buffer and RIPA (10 mM NaH_2PO_4, 150 mM NaCl, 1% DOC, 1% Nonidet P-40, 0.1% SDS, 10 μg/ml Aprotinin), and analyzed either by 6.0% SDS/PAGE or by 4–8% SDS/PAGE and autoradiography (33).

Immunoprecipitates obtained with FGFR2 antisera (polyclonal FGFR2/bek C-17, Santa Cruz Biotechnology) from unlabeled NIH 3T3 cells transfected with each expression construct were electrophoresed through a 6.0% SDS/PAGE gel, and transferred to nitrocellulose. Membranes were incubated with FGFR2 antisera (polyclonal FGFR2/bek C-17, Santa Cruz Biotechnology) followed by horseradish peroxidase-conjugated goat anti-rabbit IgG and developed by the Enhanced Chemiluminescence Kit (ECL, Amersham) according to the manufacturer's instructions.

Indirect Immunofluorescence. Two days after transfection, NIH 3T3 cells were fixed with 3% paraformaldehyde in PBS, permeabilized with 0.5% Triton X-100 in PBS, and subjected to immunofluorescence. FNN constructs were detected with antiserum C-18 directed against the C terminus of Neu (Santa Cruz Biotechnology), and a fluorescein-conjugated goat anti-rabbit secondary antibody (Boehringer Mannheim). FFN constructs were detected with P5D4 (30), and a fluorescein-conjugated goat anti-mouse secondary antibody. FFF constructs were detected with antiserum FGFR2/bek C-17, and fluorescein-conjugated goat anti-rabbit secondary antibody.

RESULTS

Transforming Activity of FGFR2/Neu Chimeric Receptors. FGFR2/Neu chimeras were generated by substituting the extracellular domain of Neu with that of FGFR2 containing either wt or the following mutant sequences: Tyr-340→His; Cys-342→Tyr; Cys-342→Arg; Cys-342→Ser; Ser-354→Cys; and Δ17 (a deletion of residues 345–361) (Fig. 1). The first set of chimeras retained the wt Neu transmembrane domain, and are referred to as FNN constructs. In these constructs, activation of the Neu kinase domain can be readily analyzed in a focus formation assay, whereas assays for activation of FGFR2 kinase domain are less well characterized. To assay whether these FGFR2 mutations activate this heterologous reporter system, focus assays were performed using NIH 3T3 cells. Expression of all FNN chimeras containing Crouzon mutations resulted in significant numbers of foci of transformed cells (Table 1).

To exclude any effects due to the Neu transmembrane domain present in the FNN constructs, another set of constructs was designed to incorporate the transmembrane domain of FGFR2, and are referred to as FFN constructs. These constructs also incorporated a convenient epitope tag derived from the vesicular stomatitis virus glycoprotein (VSV-G), against which the mAb P5D4 (30) is available. FFN constructs were generated for wt FGFR2 sequences and the three Crouzon mutations: Tyr-340→His, Cys-342→Tyr and Ser-354→Cys. The FFN constructs containing mutations in the extracellular domain demonstrated significant transforming activity (Table 1). NIH 3T3 cells transformed by the FFN constructs are shown in Fig. 2*C–F*). The positive results obtained from focus formation assays using both types of constructs, FNN and FFN, clearly indicate that the mutations

Table 1. Transformation activity of Crouzon syndrome mutations in FGFR2/Neu Chimeras

Clone	FNN construct	FFN construct
Wild-type	–	–
Δ17	+	ND
Tyr340→His	++	++
Cys342→Ser	++	ND
Cys342→Arg	++	ND
Cys342→Tyr	++	++
Ser354→Cys	+++	++
Neu (Val664→Glu)	+++	+++

NIH 3T3 cells were transfected with either FNN or FFN constructs and scored for foci after 14 days. Oncogenic Neu, activated by the Val664→Glu mutation, served as the positive control (100%). All assays were repeated a minimum of three times and average values are presented. The transforming activity of FNN and FFN constructs are presented as percentages with respect to the positive control: –, 0–9%, +, 10–19%; ++, 20–39%; +++, 40–100%. ND, not determined.

involved in Crouzon syndrome cause increased signal transduction through a heterologous receptor tyrosine kinase. This suggests that Crouzon syndrome arises from constitutive activation of the FGFR2 receptor.

Cell Surface Expression of FGFR2 Proteins. Cell surface expression of the FNN and FFN chimeric receptors containing wt FGFR2 sequences or the following Crouzon mutants: Tyr340→His, Cys-342→Tyr and Ser-354→Cys receptors was assayed in transiently transfected NIH 3T3 cells (Fig. 3, left and middle columns, respectively). In addition to staining of the proteins in the ER/Golgi, Fig. 3 demonstrates staining of the edges and surface of the cells. Cell surface localization of these chimeric FGFR2/Neu receptors demonstrates that the Crouzon mutations in the extracellular region of FGFR2 do not grossly interfere with the ability of a chimeric receptor to localize appropriately to the plasma membrane. Some of the mutant receptors also exhibited increased localization on intracellular membranes, suggesting the possibility that the rate of transport through the secretory pathway may be altered for some of the Crouzon mutant receptors.

Crouzon Mutations in Full-Length FGFR2. Full-length FGFR2 constructs (see Fig. 1*A*), designated FFF, were constructed in which the extracellular domain consisted of the wt FGFR2 sequence or, the Crouzon mutations Tyr-340→His, Cys-342→Tyr or Ser-354→Cys. These constructs were all inactive in focus forming assays (data not shown). This indicates that Crouzon mutations assayed in the tagged FGFR2 background are unable to promote unregulated growth in NIH

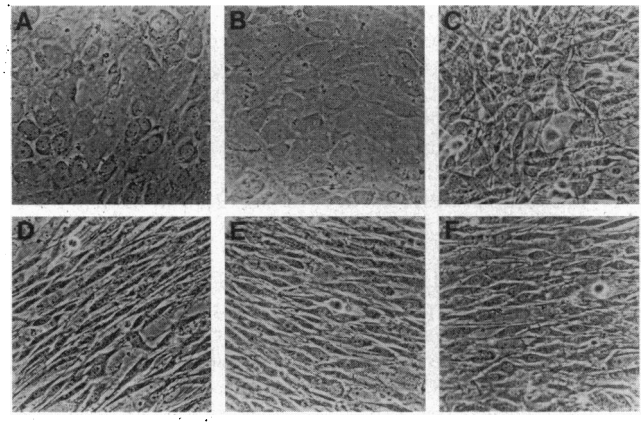

FIG. 2. Chimeric FGFR2/Neu constructs (FFN) containing Crouzon mutations are transforming in NIH 3T3 cells. (*A*) Mock-transfected cells (nontransformed); (*B*) wt FFN (nontransformed); (*C*) Crouzon mutation Tyr-340→His; (*D*) Crouzon mutation Cys-342→Tyr; (*E*) Crouzon mutation Ser-354→Cys; (*F*) Neu containing activating mutation Val-664→Glu, as positive control.

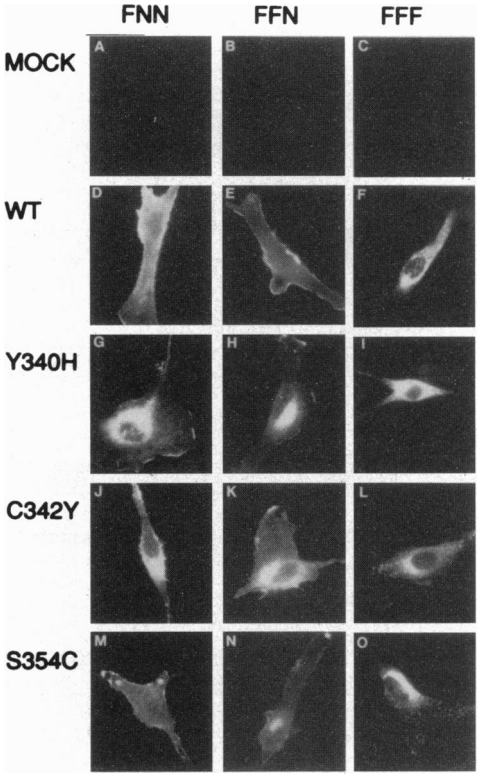

FIG. 3. Indirect immunofluorescence of cells expressing FGFR2 proteins. (*A–C*) Mock transfected cells; (*D–F*) wt FGFR2 sequence in extracellular domain; (*G–I*) Crouzon mutation Tyr-340→His; (*J–L*) Crouzon mutation Cys-342→Tyr; (*M–O*) Crouzon mutation Ser-354→Cys. (*Left*) FNN constructs were detected using polyclonal antiserum C-18 directed against the C terminus of Neu. (*Center*) FFN constructs were detected using monoclonal P5D4, which recognizes the epitope tag. (*Right*) FFF constructs were detected using polyclonal antiserum C-17 directed against the C terminus of FGFR2/bek. Intracellular as well as cell surface staining is evident, since the various antisera used all recognize intracellular epitopes.

3T3 cells, although we cannot totally exclude the possibility that the epitope tag itself may have affected this assay. This is unlikely, however, since the same tag had no adverse effect on transformation assays of the FFN constructs above. The FFF receptors were also examined by indirect immunofluorescence in transiently expressing cells, using an antibody against the C terminus of FGFR2, and exhibited cell surface localization (Fig. 3, right column).

Kinase Assay and Aberrant Dimerization of Crouzon Receptors. Cells were transfected with either the wt FFF construct or with the Ser-354→Cys mutant, lysed, and subjected to immunoprecipitation with the polyclonal antibody FGFR2/bek C-17, or with mAb P5D4 (30). Immune complexes were tested for kinase activity in the presence of [γ-^{32}P]ATP and examined by SDS/PAGE. As shown in Fig. 4, a significant increase in autophosphorylation for the Ser-354→Cys receptor was observed when compared with the wt receptors, as evidenced by increased labeling of an approximately 135 kDa protein corresponding in size to FGFR2 (34). To examine whether the increased kinase activity correlated with increased covalent dimer formation we analyzed immunoprecipitated protein by SDS/PAGE under reducing and nonreducing conditions. The Ser-354→Cys receptors exhibited the expected 135 kDa reduced band and a novel nonreduced band of approximately 270 kDa, consistent with covalent dimerization of the mutant receptors (Fig. 5). Similar results were also obtained with the constructs containing the Crouzon mutations Tyr-340→His and Cys-342→Tyr (data not shown).

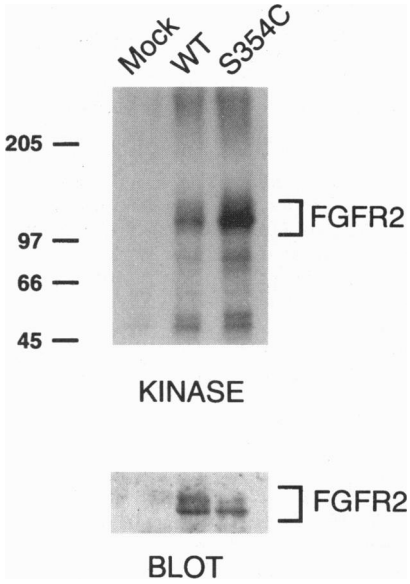

FIG. 4. NIH 3T3 cells transiently expressing either a control (Mock), wt FFF FGFR2 (wt), or the Ser-354→Cys Crouzon FFF FGFR2 mutation (S354C) were lysed and immunoprecipitated with FGFR2 antiserum C-17 against FGFR2/bek. *(Upper)* Kinase assay. Immunoprecipitates were subjected to an *in vitro* kinase assays using [γ-^{32}P]ATP, and analyzed by a 6% SDS/PAGE gel and autoradiography. Cells expressing the Ser-354→Cys Crouzon mutant receptor construct exhibited significantly increased autophosphorylation relative to the wild type. *(Lower)* Immunoblot. Immunoprecipitates were electrophoresed through a 6% SDS/PAGE gel, transferred to a nitrocellulose membrane, and incubated with FGFR2 antiserum followed by horseradish peroxidase-conjugated secondary antiserum and ECL development. Molecular mass markers in kDa are indicated. Levels of receptor expression are similar.

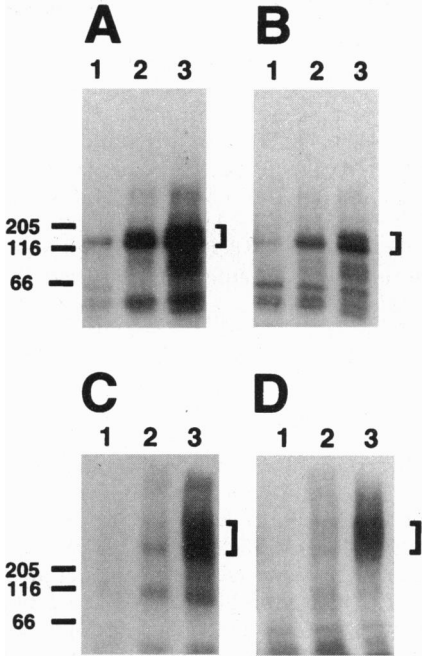

FIG. 5. Kinase assay of immunoprecipitated FGFR2 receptors. Receptors were isolated from transiently transfected NIH 3T3 cells and subjected to *in vitro* kinase assay using [γ-^{32}P]ATP. Samples were then analyzed by SDS/PAGE on a 4–8% gradient under reducing conditions, with 20% 2-mercaptoethanol in the sample buffer, or under nonreducing conditions. Molecular mass standards in kDa are indicated. Brackets indicate either monomer receptors of ≈135 kDa, or dimeric receptors of ≈270 kDa. (*A*) FFF clones immunoprecipitated with antiserum C-17 against FGFR2/bek, analyzed under reducing conditions. (*B*) FFF clones immunoprecipitated with mAb P5D4 that recognizes the epitope tag, analyzed under reducing conditions. (*C*) FFF clones immunoprecipitated with antiserum C-17 against FGFR2/bek, analyzed under non-reducing conditions. (*D*) FFF clones immunoprecipitated with mAb P5D4 that recognizes the epitope tag, analyzed under nonreducing conditions. (*A–D*) Lane 1, mock transfected cells; lane 2, FFF wt; lane 3, FFF with Ser-354→Cys Crouzon mutation.

DISCUSSION

Neu as a Reporter for Receptor Activation. In this study, we have exploited the ability of the intracellular kinase domain of Neu (31, 35, 36) to serve as a reporter for receptor activation mediated by mutations in the extracellular domain of the heterologous receptor FGFR2. Similar results were obtained in transformation assays for FNN and FFN constructs, which differed only in the origin of the transmembrane domain (Neu vs. FGFR2). This indicates that chimeric constructs containing the transmembrane domain derived from wt (unactivated) Neu can serve as a reliable reporter to assay for activating mutations in the extracellular domain of heterologous receptors. This strategy is similar to the use of Neu as a reporter for transmembrane dimerization mediated by the achondroplasia mutant of FGFR3; in these constructs, the mutant FGFR3 transmembrane domain containing a single Gly-380→Arg mutation was substituted in place of the Neu transmembrane domain, resulting in activation of the resulting chimeric receptor (29).

Constitutive Activation of FGFRs. Results reported here demonstrate that Crouzon syndrome mutations in the extracellular domain of FGFR2 result in constitutive receptor activation. The mutations examined include the following: Tyr-340→His, Cys-342→Tyr, Cys-342→Arg, Cys-342→Ser, Ser-354→Cys, and the Δ17 splicing mutant (20, 23), which represent those initially described by Reardon *et al.* (2). When incorporated into FGFR2/Neu chimeras, Crouzon mutations were sufficiently activating to result in transformation of NIH 3T3 cells. When examined by immunoprecipitation and *in vitro* kinase assay, mutant receptors exhibited aberrant disulfide bonding in comparison with wt receptors when analyzed by SDS/PAGE under nonreducing conditions. Both wt receptors and receptors with Crouzon mutations were expressed normally at the cell surface when examined by indirect immunofluorescence, suggesting that Crouzon mutations do not lead to gross changes in the subcellular localization of receptors.

Mutations within FGFR2 have also been found to be associated with three other syndromes: Pfeiffer syndrome (9, 18), Jackson-Weiss syndrome (6, 19, 22), and Apert syndrome (11, 21). Although each syndrome exhibits craniosynostosis as one of their defining features, they each have distinct characteristics; for example, Apert syndrome patients exhibit symmetric syndactly of the hands and feet. One interesting aspect of the mutations that cause these related syndromes is their similarity. Surprisingly, the identical FGFR2 mutation Cys342→Arg has been associated with the clinically distinct Crouzon syndrome, Pfeiffer syndrome, and Jackson-Weiss syndrome (2, 9, 19). The fact that the same mutation in FGFR2 gives rise to distinct phenotypes demonstrates the complexity of the role that FGFR2 plays in developmental processes. It also highlights the important role that other factors must play in controlling these developmental processes.

The following observations provide compelling support for the hypothesis that the clinically distinct skeletal abnormalities involving FGFRs all result from constitutive receptor activation leading to downstream signal transduction. First, recent work from this laboratory demonstrated that the achondroplasia mutation in FGFR3 (Gly-380→Arg) is activating (29) and the Crouzon mutations in FGFR2, as shown here, are also activating. Second, a correlation exists between mutations

within a given FGFR and a common set of phenotypes, e.g., craniosynostosis resulting from FGFR2 mutations, and abnormal long bone development resulting from FGFR3 mutations. Third, some of the various FGFR mutations which give phenotypes consistent with constitutive FGFR2 and FGFR3 activation are very similar, e.g., the mutation of the highly conserved Ser-Pro dipeptide in the linker region between Ig-like domain II and III in FGFR1, FGFR2, and FGFR3 causing Pfeiffer syndrome, Apert syndrome, and Thanatophoric Dysplasia type I, respectively.

Activation of Other Receptors by Aberrant Dimerization. The phenomenon of receptor activation as a result of aberrant intermolecular disulfide bond formation has also been shown to result from mutations in three other proteins: RET (27), the erythropoietin receptor (25, 26), and the epidermal growth factor receptor (24). RET, a receptor-like tyrosine kinase that is involved in multiple endocrine neoplasia type 2A, becomes constitutively activated when Cys-634 is changed to Tyr, Arg, or Trp (27). The erythropoietin receptor is activated by the replacement of an extracellular Arg-129 by Cys (25, 26). Finally, epidermal growth factor receptor can be activated by placing a novel Cys residue in the extracellular juxtamembrane region (24). The activation of each of these proteins is associated with the presence of detectable disulfide-linked dimers, which allows for constitutive activation and increased signaling through their respective pathways.

Developmental Role of FGFR Expression. The developmental regulation of the FGF family, which presently includes four distinct receptors (FGFRs) and nine distinct growth factor molecules (FGFs), remains largely uncharacterized. The FGFRs have distinct patterns of expression during embryogenesis and development, suggesting that each receptor mediates different developmental responses to FGFs. Alternative splicing of FGFR2 results in two variants, designated keratinocyte growth factor receptor and bek, which exhibit distinct developmental patterns of expression. Keratinocyte growth factor receptor appears to have a role in skin development, while bek is preferentially expressed in osteogenesis (37). Expression of bek is specifically observed in the developing ossicles of middle ear, maxilla, mandibula, and in the frontal bones of the skull (37). Unregulated signaling through mutant FGFR2 should alter the normal development of these areas, and could account for craniosynostosis and other milder phenotypes such as conductive hearing defects observed in approximately 50% of Crouzon patients (38).

Other Crouzon Syndrome Mutations. Recently, several new Crouzon syndrome mutations have been discovered (6, 13, 14, 19, 20, 22). Although many of these mutations create or destroy Cys residues, indicating that their mechanism of action involves the creation of ligand-independent dimers through aberrant disulfide-bond formation, as shown here, there are some mutations that do not directly involve Cys residues. These mutations may indirectly affect neighboring Cys residues, leading to aberrant disulfide bond formation or, alternatively, there may exist a secondary mechanism of FGFR2 activation. While the original Crouzon mutations were solely found in exon IIIc, which encodes part of the third Ig-like domain and occurs only in the bek isoform of FGFR2 (39–42), several of the more recently discovered mutations, e.g., Ser-267→Pro, Cys-278→Phe, Gln-289→Pro or Tyr-328→Cys, lie in the upstream exon U (or exon 7), which is present in both the keratinocyte growth factor receptor and bek isoforms (6, 14, 22). This is quite intriguing because these mutations, potentially expressed both as keratinocyte growth factor receptor and as bek transcripts, result in a phenotype clinically indistinguishable from the originally described Crouzon mutations, which seem to affect only the bek transcripts. Further definition of the role that FGFRs have in development and the signal transduction pathways downstream of the different FGFRs will clearly be necessary to fully understand the developmental consequences of activating receptor mutations.

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