A Homeo-interaction Sequence in the Ectodomain of the Fibroblast Growth Factor Receptor*

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Fen Wang, Mikio Kan, Kerstin McKeehan, Jun-Hyeog Jang, Shuju Feng, and Wallace L. McKeenan‡

From the Center for Cancer Biology and Nutrition, Albert B. Alkek Institute of Biosciences and Technology and Department of Biochemistry and Biophysics, Texas A&M University, Houston, Texas 77030-3303

Interaction of fibroblast growth factor receptors (FGFR) sufficient for a trans-phosphorylation event in which one intracellular domain is substrate for the other is essential for signal transduction. By analysis of the direct interaction of recombinant constructions co-expressed in baculoviral-infected insect cells, we identified a 17-amino acid sequence that is required for the stable interaction between ectodomains of FGFR. The sequence ERSPHRPILQAGLPANK (Glu160–Lys176) connects immunoglobulin modules II and III. In insect cells, the interaction between Glu160–Lys176 domains occurs independently of intact heparin or FGF binding domains. The sequence is not required for the binding of heparin or FGF-1, but is essential for mitogenic activity of the FGFR kinase in mammalian cells. The results support a model in which the homeo-interaction between Glu160–Lys176 in the ectodomain contributes to the interaction between intracellular domains in mammalian cell membranes (Kan, M., Wang, F., Kan, M., To, B., Gabriel, J. L., and McKeenan, W. L. (1996) J. Biol. Chem. 271, 26143–26148). We propose that the Glu160–Lys176 domain plays a pivotal role in restriction of the interaction between kinases by pericellular matrix heparan sulfate proteoglycan and divalent cations. Restrictions are overcome by FGF or constitutively by diverse gain of function mutations which cause skeletal and craniofacial abnormalities.

trans-Phosphorylation of tyrosines between fibroblast growth factor receptor (FGFR) kinase domains is thought to be necessary for activation of FGFR. It derepresses the access of substrates to the catalytic site of the tyrosine kinase, and creates binding sites containing phosphotyrosine for recruitment of substrates that relay signals to the intracellular compartment (1–3). Recombinant FGFR kinases without the ectodomain exhibit autophosphorylation activity of which a part is assumed to occur by an intermolecular mechanism. However, the intracellular kinase domain with or without the COOH-terminal tail sequence does not exhibit sufficient mutual affinity to demonstrate oligomers in solution (2, 4–6). The FGFR kinase with an intact ectodomain exhibits an intrinsic ability to self-associate (1). In cell membranes where concentration and mobility are restricted, primary control of the proximity and the activity of the intracellular FGFR kinase domains occurs by the interaction of the extracellular domains with heparan sulfate and FGF.

Recently, we proposed a model in which interaction between kinase domains is restricted by conformation due to interaction of Ig loop II with divalent cations and heparan sulfate (5). This model predicts an interface in the FGFR ectodomain whose interaction is modified by occupation of each FGFR monomer by an FGF and contributes to the relationship between intracellular kinase domains in a physiological context. In this report, using deletion and site-directed mutagenesis and analysis of the self-association of recombinant FGFR ectodomains co-expressed in insect cells, we localize the interaction domain of FGFR to the sequence ERSPHRPILQAGLPANK (Glu160–Lys176). This domain lies between Ig loop II and loop III and by homology to other Ig motifs extends into the NH₂ terminus of loop III. In addition to constituting a homeo-interaction domain between FGFR monomers, the sequence likely plays a role in maintenance of the structural relationship between Ig loops II and III and may contribute to the high affinity binding of some members of the FGFR family.

Heterozygous mutations of the counterpart residues to Arg161 or Ser162 to cysteine in FGFR3 result in disulfide links between receptors and cause the neonatal lethality and profound dwarfism associated with thanatophoric dysplasia (6, 7). Mutations at the counterpart of Ser162 to tryptophan in the FGFR2 gene and Pro163 to arginine in the FGFR1, FGFR2, and FGFR3 genes cause less severe abnormalities (8). Both types of mutations are thought to induce FGF-independent FGFR activity presumably by subversion of restrictions on dimerization and trans-activation of FGFR kinases. We propose that the Glu160–Lys176 sequence is the primary sequence within FGFR ectodomains whose interaction between FGFR ectodomains is modified by the presence of FGF in the context of normal cell membranes.

EXPERIMENTAL PROCEDURES

Oligonucleotide Primers—Oligonucleotides that have not been described elsewhere are listed below, were synthesized on an Applied Biosystems PCRMate and recovered by the vendor’s instructions or purchased from Integrated DNA Technologies, Inc. (Coralville, IA). Numbering is based on +1 for the first nucleotide of the initiation codon in the FGFR1 β cDNA. Restriction sites are underlined and nucleotides

‡ To whom correspondence should be addressed: Center for Cancer Biology and Nutrition, Albert B. Alkek Institute of Biosciences and Technology and Department of Biochemistry and Biophysics, Texas A&M University, 2121 W. Holcombe Blvd., Houston, TX 77030-3303.

1 The abbreviations used are: FGFR, FGF receptor; FGF, fibroblast growth factor; GST, glutathione S-transferase; Sf9, Spodoptera frugiperda; FBS, fetal bovine serum; PCR, polymerase chain reaction; PBS, phosphate-buffered saline; bp, base pair(s).

2 F. Wang, M. Kan, and W. L. McKeenan, unpublished results.
not in the coding sequence for FGFR or glutathione S-transferase (GST) constructs are shown in Fig. 1A. cDNAs coding for the full-length FGFR1α1, FGFR1β1, and FGFR1β2 tyrosine kinases have been described (1, 9, 10). Murine FGFR3 was a gift from Dr. David Ornitz (11). The 5'-noncoding sequence was removed from the transcript by digestion with Klenow enzyme followed by BstI. The cDNA fragment was ligated at the BclI site with a polylinker chain reaction (PCR) fragment coding for the COOH-terminal of FGFR3 generated with sense primer R31 and antisense primer R32 using the FGFR3 cDNA as the template. The resultant full-length cDNA sequence of FGFR3 containing short non-coding sequences was cloned into pBlueScript SK vector at Smal and EcoRI sites. The human FGFR4 cDNA beginning 42 bp upstream of the translation initiation site and ending 123 bp downstream of the end of the coding sequence of the transmembrane domain was generated by reverse transcriptase-PCR from human hepatoma HepG2 cells using sense primer R41 and antisense primer R42. Coding sequence for the intracellular domain of FGFR4 was generated from the same cDNA pool by the sense primer R43 beginning 15 bp upstream of the coding sequence of the transmembrane domain and an antisense primer R44 ending 18 bp downstream of the translation stop codon. The PCR fragment coding for the ectodomain was treated with EcoRI and cloned into pBlueScript SK at an EcoRI site. The PCR fragment coding for the intracellular domain was digested with EcoRI and XhoI. The two cDNAs were cloned and ligated at the AclI site to yield the full-length coding sequence.

For construction of FGFR1β variants fused to GST, the cDNA sequence for first was generated using sense G1 and antisense G2 primers, respectively, and 5′-G5ST-PVL 1392 (12) as the template in a PCR. After treatment with KpnI and BamHI, the GST coding sequence was ligated in frame with partial cDNAs coding for wild type or mutant FGFR1β cDNAs at the KpnI site and the constructs were cloned into pBlueScript SK vector at Xbal and BamHI sites. The cDNAs coding for mutants of cDNA and GST were expressed in a baculovirus system. The mammalian cell line was transfected with the pBlueScript SK vector containing the GST fusion protein sequence coding for full length FGFR1 or antisense primer R44 ending 18 bp downstream of the translation stop codon. The PCR fragment coding for the transmembrane domain was generated by reverse transcriptase-PCR from human hepatoma HepG2 cells using sense primer R41 and antisense primer R42. Coding sequence for the intracellular domain of FGFR4 was generated from the same cDNA pool by the sense primer R43 beginning 15 bp upstream of the coding sequence of the transmembrane domain and an antisense primer R44 ending 18 bp downstream of the translation stop codon. The PCR fragment coding for the ectodomain was treated with EcoRI and cloned into pBlueScript SK at an EcoRI site. The PCR fragment coding for the intracellular domain was digested with EcoRI and XhoI. The two cDNAs were cloned and ligated at the AclI site to yield the full-length coding sequence.

For construction of recombinant FGFR—FGFR1, the yeast two-hybrid system was used. The yeast two-hybrid vectors pGBT9 and pGAD424 were transformed into yeast strain EGY48 (13, 14) or the FGFR1 extending 26 amino acid residues past the transmembrane domain was generated by reverse transcriptase-PCR from human hepatoma HepG2 cells using sense primer R41 and antisense primer R42. Coding sequence for the intracellular domain of FGFR4 was generated from the same cDNA pool by the sense primer R43 beginning 15 bp upstream of the coding sequence of the transmembrane domain and an antisense primer R44 ending 18 bp downstream of the translation stop codon. The PCR fragment coding for the transmembrane domain was generated by reverse transcriptase-PCR from human hepatoma HepG2 cells using sense primer R41 and antisense primer R42. Coding sequence for the intracellular domain of FGFR4 was generated from the same cDNA pool by the sense primer R43 beginning 15 bp upstream of the coding sequence of the transmembrane domain and an antisense primer R44 ending 18 bp downstream of the translation stop codon. The PCR fragment coding for the ectodomain was treated with EcoRI and cloned into pBlueScript SK at an EcoRI site. The PCR fragment coding for the intracellular domain was digested with EcoRI and XhoI. The two cDNAs were cloned and ligated at the AclI site to yield the full-length coding sequence.

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FIG. 1. Expression and activity of recombinant FGFR constructions. A, schematic of recombinant constructs. Ig modules I, II, and III are indicated showing putative disulfides. The secretory signal sequence (S), the transmembrane domain (TM), and the two parts of the kinase domain (K1 and K2) are indicated. The acidic box sequence is hatched and the heparin-binding domain is indicated by double lines. The Glu1156-Lys1776 domain is indicated in bold. The GST sequence fused at Thr314 of FGFR1 is indicated by the cross-hatched oval. AD, Gal-4 activation domain; BD, Gal-4 DNA binding domain. FGFR is numbered throughout this study beginning with the initiator methionine of the FGFR1 isoform as residue 1. B, conservation of the inter-loop II and III sequence among FGFRs. The junction of loop II, the inter-loop sequence, and loop III are indicated by molecular models is indicated (5, 21). The single residue mutations associated with craniofacial and skeletal syndromes are indicated (22, 23).

RESULTS

A Heparin/Heparan Sulfate- and FGF-independent Interaction Domain Resides in the FGFR8 Ectodomain—Previously we demonstrated that FGFR1β interacts with FGFR1α in insect cells co-infected with the two constructs with sufficient affinity to survive detergent-extraction and co-precipitation with an FGFR1α-specific antibody (1). FGFR does not interact under the same conditions with a variety of components at high concentrations in the analytical system including serum proteins, immunoglobulins, recombinant fusion partners, host cell proteins and co-expressed members of other transmembrane receptor families (1, 12). This suggested the presence of an interaction domain in FGFR1β and FGFR1α that differs by presence or absence of a naturally occurring Ig domain.

FGFR1α and FGFR1β are natural variants that differ by presence or absence, respectively, of an alternately spliced NH2-terminal Ig module.
FGFR Dimerization

Fig. 2. Kinase domain- and ligand-independent interaction of FGFR1α and FGFR1β. A, interaction of FGFR independent of the kinase domain. Lysates from 2 × 10^6 Sf9 cells infected with the recombinant baculovirus bearing the constructs indicated at the top were divided into two portions. The first portion was used to determine how much FGFR1α (125 kDa) and FGFR1β (95 kDa) reacted with FGFR1GST (68 kDa) by purification on GSH-Sepharose beads. Lower bands of doublets in the FGFR1β products are due to proteolytic cleavage of the NH₂ terminus at the COOH terminus of the acidic box sequence (13). The immobilized products were analyzed by immunoblot with anti-FGFR1 antibody M5G10 (top panel). The second portion was used to determine the total amount of each product that was present in the extract by purification on protein A-Sepharose beads bearing anti-FGFR1 monoclonal antibodies M2F12 and M17A3. The immobilized products were analyzed by immunoblot with anti-FGFR1 monoclonal antibody M5G10 (bottom panel). Immobilized products are indicated on the right. Ab denotes the immobilizing mouse antibody detected by the goat anti-mouse Ig conjugated to alkaline phosphatase. The presence (+) or absence (−) of 200 ng/ml FGF-1 and 25 μg/ml heparin during the analysis is indicated at the bottom. Densitometric scanning indicated that 10 and 33% of total FGFR1α and total FGFR1β, respectively, were complexed to FGFR1GST (top panel). The ratios of total FGFR1α:FGFR1GST and FGFR1β:FGFR1GST = 2:0 (lower panel). B, ligand-independent interaction of the FGFR1β ectodomain. Sf9 cells that were co-infected with FGFR1βGST (68 kDa) and FGFR1β (95 kDa) constructs were incubated with the indicated components for 30 min prior to cell lysis. The FGFR complexes were purified on GSH-Sepharose beads and analyzed by immunoblot as described in A. Total antigen indicates the amount of both expression products in the cell lysate which was purified on protein A-beads bearing the A50 anti-FGFR1 serum. FGFR was detected with anti-FGFR1 monoclonal antibody M17A3. Scanning densitometry indicated that 35% of total FGFR1β was complexed to FGFR1GST, the ratio of total FGFR1β:FGFR1βGST = 2:0 and the mean ratio of FGFR1β:FGFR1βGST in the purified complexes = 0.4 (range 0.35–0.54).

FGFR1 interacts directly with FGFR2, FGFR3, and FGFR4. The FGFR isoforms indicated at the top (95, 110, 115–120, and 115 kDa, respectively) were co-expressed with FGFR1βGST and the complexes purified on GSH-Sepharose beads. After release from the beads by incubation with GSH, the bound FGFR antigens were detected by anti-FGFR1 serum (A50), rabbit anti-FGFR2 serum, rabbit anti-FGFR3 serum, or anti-FGFR4 serum. The right lane indicates an immunoblot of an Sf9 extract after infection with all four FGFR and analysis with a mixture of the four antisera.

were expressed at low levels at a ratio of about 1 to 1 (1). However, a significant portion of the two isoforms of FGFR interacted independent of heparin and FGF at the lowest level of expression that could still be accurately measured (1). When either FGFR1α or FGFR1β were co-expressed at a high level and at a ratio of approximately 2 to 1 FGFR1βGST (Fig. 2A), no effect of added heparin and FGF on the amount of either isoform that co-precipitated with FGFR1βGST could be demonstrated (Fig. 2B). Scanning densitometry indicated that less than 10% of FGFR1α and 35% of FGFR1β complexed with FGFR1βGST under the conditions. FGFR2α, FGFR3α, and FGFR4α also complexed with the FGFR1βGST product under the same conditions (Fig. 3).

These results show that in the absence of restrictions imposed by concentration or other factors in mammalian cells, a domain resides in the FGFR1βGST product that interacts with another FGFR independent of heparin and FGF. The domain required for the interaction is potentially conserved among the four FGFR genes (Fig. 1B).

The Acidic Box Sequence and Ig Loop II Are Not Involved in the Interaction of FGFR Ectodomains—The ectodomain of FGFR exhibits a characteristic sequence domain called the acidic box. It is located at the NH₂ terminus of FGFRβ and constitutes the inter-loop sequence between loops I and II in FGFRα. It is not essential for the binding of heparin/heparan sulfate or FGF (10, 15). Although the acidic box is alternately spliced in FGFRβ (15, 25), it is always present in the FGFRα isoform (15). Loop II of FGFR contains an essential heparan sulfate-binding domain in its NH₂ terminus. The domain together with the connecting sequence between Ig loops II and III and a segment of the NH₂ terminus of loop III constitutes a common high affinity binding site for FGF-1, FGF-2, FGF-7, and possibly all members of the FGF family of polypeptides (13, 14). To determine whether the acidic box or loop II were involved in the interaction between FGFR ectodomains, we deleted the acidic box and loop II from the FGFR1α ectodomain. This resulted in a construct composed of loop I, the inter-loop sequence that normally connects loops II and III (FGFR1αΔII) plus the transmembrane domain and 17 residues of the intracellular juxtamembrane (Fig. 1A). A similar construct [FGFR1αΔIIΔIII] was also built in which the acidic box and the heparin-binding domain were present at the NH₂ terminus of loop I (Fig. 1A). Both constructs missing the entire Ig loop II module were unable to bind FGF (Fig. 1B), but retained the ability to interact with intact FGFR1α (Fig. 4) and FGFR1β (data not shown). Unless a separate interaction domain lies within Ig loop I of the FGFRα isoform, these results suggested that an FGFR interaction domain resides between the COOH terminus of loop II and the COOH terminus of the intracellular juxtamembrane sequence fused to GST (FGFR1βGST) showed that an interactive domain was upstream of most of the FGFR intracellular domain (Figs. 1 and 2A).

Heparin and FGF increased the interaction of FGFR1β with FGFR1α2 in the membranes of insect cells when both
To further confirm the absence of an interaction domain within the acidic box and loop II, we prepared a construct of FGFR1GST from which loop III and the inter-loop II-III sequence between Val(159) and Thr(268) was deleted (FGFR1βIIIGST). The association of FGFR1β with the construct after purification on GSH-beads was markedly reduced relative to FGFR1βGST (Fig. 5, top panel). However, the FGFR1βIIIGST product composed of the acidic box, loop II, the extracellular juxtamembrane and transmembrane domains of FGFR retained high affinity for heparin (Fig. 6A) and FGF-1 (Fig. 6B). The binding of FGF-2 to the construction was reduced, but still detectable under the same conditions. No binding of FGF-7 could be detected under the same conditions. However, specific binding of FGF-7 to the FGFR1βIIIGST product was observed when radiolabeled FGF-7 was increased 10-fold in binding assays and exposure of autoradiographs was prolonged (Fig. 6B). These results suggest that the loop II-heparin complex exhibits a binding domain for all three FGF polypeptides, but that the highest affinity binding of FGF-2 and FGF-7 requires the inter-loop II/III sequence and a segment of the NH₂ terminus of loop III as reported previously (14). These results show that the acidic box and loop II do not support the direct interaction between FGFR. The heparin-and FGF-binding domains associated with loop II are neither required nor are they sufficient for the interaction. These data further suggest that Ig module II independently composes a heparin and FGF-1 binding site and contributes to the binding site for FGF-2 and FGF-7. This suggests that a global disruption of the Ig loop II structure when divorced from downstream domains unlikely underlies its failure to associate with other FGFR. More likely an FGFR interaction domain lies downstream of the COOH terminus of loop II.

**The FGFR Interaction Domain Resides within the Inter-loop II/III Sequence**

To determine whether loop III contributed to the FGFR interaction domain, we constructed a variant of FGFR1GST in which the sequence between Glu(29) and Leu(168) was deleted (FGFR1βIIIGST). This results in a construction comprised of loop III, the extracellular juxtamembrane sequence, the transmembrane sequence and six residues of the intracellular juxtamembrane domain. The mutant FGFR1βIIIGST exhibited no detectable heparin or FGF-1 binding (Fig. 6A). However, the construction complexed with FGFR1β1, but to less extent than FGFR1βGST (Fig. 5, middle and lower panels). To determine whether the interaction domain overlapped or resided downstream of the main FGF binding domain (14), Sf9 cells were infected with viruses bearing the secreted FGFR1 fragments SFRIK1(176) or SFRIK(159) together with FGFR1βGST. As described previously (14), a complex of heparin and SFRIK(159) exhibits an equal affinity for FGF-1, FGF-2 and FGF-7, while the heparin-SFRIK(176) complex exhibits a reduction in affinity for all three FGFs. Both secreted FGFR1 fragments interacted with FGFR1βGST (Fig. 7). Taken together with the fact that structural domains upstream of the COOH terminus of loop II do not participate in the interaction between FGFR, these results suggest that the interaction domain lies in the sequence **ERSPHRPILQAGLP**. This sequence connects Ig loops II and III, while homology to characterized Ig structures, extends into the NH₂ terminus of loop III. The results also suggest that sequence domains downstream of Lys(176) are not required for the stable homo-interaction defined by these analyses.

To confirm that the inter-loop sequence composed a sequence-specific interactive interface when in the full-length FGFR ectodomain, we constructed mutants FGFR1βS1 bearing the scrambled sequence **160IPHPRESR167** instead of the wild-type sequence **160ERSPHRP167** and FGFR1βS2 bearing the scrambled sequence **160NQALLGA175** instead of the wild-type sequence **160LQAGLPAN175** (Fig. 1). The FGFR1βS1 construct lost ability to bind both FGF-1 and FGF-2. However,
FGFR1β1S2 bound FGF-1 and FGF-2 (Fig. 6B). Both constructions exhibited a marked decrease in the homo-interaction between mutant monomers (Fig. 6A). Scanning densitometry indicated that less than 2% of FGFR1β1S1 was complexed with FGFR1β1S1GST and less than 5% of FGFR1β1S2 associated with FGFR1β1S2GST when the pairs were expressed at a ratio of 1 to 1.5 (Fig. 6A). The heterotypic interaction of both mutants with the wild type ectodomain FGFR1β was considerably increased over the homotypic interaction between mutants (Fig. 6B). Moreover, the heterotypic interaction between mutant FGFR1β1S1 and FGFR1β1S2 was also greater than the homotypic interaction between mutants. These results suggest that (i) both segments, ERSPHRPI and LQAGLPAN of the Glu160–Lys176 domain are involved in a common domain, and (ii) heterotypic interactions in trans between the Glu160–Lys176 domain and two different FGF may occur.

A Sequence Containing the Inter-loop II-III Domain Glu160–Lys176 Interacts in the Yeast Two-hybrid Expression System—To determine whether the Glu160–Lys176 structural domain would interact when present in an unrelated protein, the amino acid sequence between Glu160 and Lys176 from FGFR1 was fused to the Gal4 promoter activation domain and DNA binding domain. The two constructs, Gal4AD-IL and Gal4BD-IL (Fig. 1), were tested for ability to support growth and activate the Gal4 promoter indicated by expression of β-galactosidase in HF7C yeast cells. Only cells co-infected with the two constructs supported growth of HF7C cells in Trp- and Leu-deficient medium and yielded the characteristic blue colonies indicative of the presence of β-galactosidase (Fig. 9). Individual constructs expressed alone or with unrelated products fused to the activating or DNA binding partner yielded negative results.

Requirement for the Intact Glu160–Lys176 Sequence for Mitogenic Activity of FGFR1—To determine impact of the Glu160–Lys176 sequence on mitogenic activity of FGFR, recombinant FGFR1β1, FGFR1β1S2, and FGFR1β1ΔIII with full-length kinase domains were expressed in BaF3 cells which exhibit little or no endogenous FGF and do not respond to FGF (24) (Fig. 10). Clonal cultures derived from the transfected BaF3 population that displayed about 10000 specific FGF-1 binding sites per cell were selected in binding assays using radiolabeled FGF-1 (Fig. 10, A, inset, and B). Only cells expressing the wild type FGFR1β1 exhibited a response to FGF-1 (Fig. 10A). A second set of clonal cultures from an independently transfected BaF3 population expressing about 20000 sites per cell were examined at twice the cell density as the experiment in Fig. 10, A and B (Fig. 10C). Although the base level of FGF-independent DNA synthesis increases under these conditions, the cells expressing intact FGFR1β1 still exhibited the largest response to FGF-1. These results confirm that the intact Glu160–Lys176 sequence is not required for binding of FGF-1, but is required for mitogenic activity. The results further suggest that the interaction of ectodomains mediated by the Glu160–Lys176 sequence is essential for mitogenic activity.

**DISCUSSION**

Auto-phosphorylation of tyrosines in FGFR tyrosine kinases is an obligatory event for FGF signal transduction (1, 3, 26). A recent structural analysis of the FGFR kinase (4) suggests that, similar to the insulin receptor kinase (27), activation of
the FGFR kinase requires trans-phosphorylation of tyrosine in a cis-acting autoinhibitory domain within the kinase domain. Therefore, the level of activity of FGFR simply depends on concentration or proximity of FGFR molecules sufficient to sustain an enzyme-substrate relationship between intracellular domains. When free of restraints imposed by concentration, position and co-factors in mammalian cell membranes, the FGFR tyrosine kinase self-activates through auto-phosphorylation independent of FGF and pericellular heparan sulfate (1, 26). This activity is also independent of other structural domains of the transmembrane receptor including the ectodomain (2, 3) which contains the heparan sulfate and FGF binding sites (13, 14). The activation and presence of FGF is normally required for activity of the FGFR signal transduction complex in mammalian cell membranes. Therefore, it is important to understand the role of the ectodomain and its ligands which include FGF and heparan sulfate in control of the enzyme-substrate relationship between the intracellular FGFR kinases. Here we show that the FGFR ectodomain exhibits a homo-interaction domain that is within the 17-amino acid sequence ERSPHRPI of the Glu160–Lys176 domain to activation and DNA binding domains. When the binding site is not occupied by FGF, the relationship (inactive state I) between ectodomains of adjacent molecules is unstable. However, the occupancy of a binding site by FGF results in a conformational change which “closes” the FGFR dimer (5). When the binding site is occupied by FGF, the enzyme-substrate relationship between adjacent kinases is such that a homeo-interaction domain that is within the 17-amino acid sequence ERSPHRPI of the Glu160–Lys176 domain to activation and DNA binding domains. When the binding site is not occupied by FGF, the relationship (inactive state I) between ectodomains of adjacent molecules is unstable.
domain in the ectodomain may play a pivotal role, possibly as a contact interface, in control of the enzyme-substrate relationship between FGFR kinases by heparan sulfate and FGF in the context of cell membranes.

A variety of autosomal dominant point mutations occurs in FGFR1, FGFR2, and FGFR3 that results in craniofacial and skeletal abnormalities of graded severity. These include achondroplastic, hypochondroplastic, Apert, Crouzon, Jackson-Weiss, and Pfeiffer syndromes in adults and lethal thanatophoric dysplasia (22). The diverse mutations appear to be gain-of-function mutations (7, 28–31). None to date occur within loop II and most occur between the COOH terminus of loop II and the COOH terminus of the transmembrane domain (22). Some mutations disrupt and some have no effect on binding of FGF (10, 28, 30, 31). Several mutations in the ectodomain result in new cysteines capable of forming disulfides between adjacent FGFR (7, 22, 28). The most severe syndrome is thanatophoric dysplasia which is characterized by neonatal death and profound dwarfism. The phenotype is caused by (i) a mutation of lysine to glutamate in the kinase repressor loop within the kinase domain of FGFR3 (31); (ii) cysteine substitutions in the FGFR3 counterparts of Arg161 and Ser162 in the G160–Lys176 domain of FGFR1 (7, 23); (iii) cysteine substitutions just NH2-terminal to the transmembrane domain (23); or (iii) non-cysteine mutations which cause an extension of the distal COOH-terminal sequence (23). The severity of the cysteine substitutions in the G160–Lys176 sequence suggests the sequence may comprise a contact domain in which disulfides form more efficiently between FGFR dimers than in other non-contact domains. This suggests a model for how autosomal dominant mutations at different sites within FGFR can cause diverse phenotypes of graded severity from achondroplastic dwarfism to thanatophoric dysplasia. In cells in which 50% of FGFR bear mutations between the COOH terminus of loop II and the intracellular domain, a maximum of 25% of total heparan sulfate-FGFR complexes that are present as mutant-mutant complexes can be FGF-independent and constitutively active. Since occupancy of both FGFR is required for transphosphorylation in the model, the 50% mutant-wild type and 25% wild type-wild type complexes remain FGF-dependent. The constitutively active fraction of FGFR and thus severity of the phenotype will depend on the degree that an individual mutation can subvert the forces which prevent a sustained enzyme-substrate relationship between intracellular kinases within the mutant-mutant complexes. In the case of non-cysteine mutations, the impact will depend on the extent that the mutation disrupts the relationship between loops II and III that prevents sustained interaction of FGFR contact domains, particularly G160–Lys176. The impact of cysteine substitutions will depend on location of the cysteine and efficiency of disulfide bond formation between mutant FGFR. This is consistent with the differences in severity of phenotype caused by cysteine substitutions at residues Arg161 and Ser162 within the G160–Lys176 contact domain relative to the mutation of Ser162 to tryptophan and adjacent Pro163 to arginine. A large percentage of the mutant-mutant complexes may be locked in an enzyme-substrate relationship through stable disulfide bonds that form efficiently during the normally transient contact between G160–Lys176 domains. This is in contrast to cysteine residues in non-contact domains or indirect structural changes caused by the Ser162 to tryptophan and Pro163 to arginine in the G160–Lys176 contact domain. The fact that thanatophoric dysplasia also results from cysteine substitutions at the NH2 terminus of the transmembrane domain of FGFR3 also suggests contact between FGFR at that domain. If the domain is a contact point in FGFR dimers, its intrinsic and independent
affinity for self-association is too low for detection by the methods used in this report. In contrast to the cysteine mutations in the two subdomains in the ectodomain above, an activating K650E mutation in the repressor loop of the kinase domain of FGFR3 results in a still more profound level of constitutive FGFR activity and associated phenotype (31). If the sole role of trans-phosphorylation is to derepress the active site of the FGFR kinase, then this model predicts that in contrast to the ectodomain mutations which are capped at 25%, a larger percentage (potentially the 25% mutant-mutant plus the 50% mutant-wild type) of FGFR complexes could be FGF-independent.

Last, this model explains the tissue specificity of diverse heterozygous FGFR mutations and lack of effect on other tissues where the same FGFR isoform is believed to play a regulatory role. For example, the FGFR2 gene exhibiting mutations in the Glu160–Lys176 domain associated with Apert syndrome is widely expressed in the epithelium of adult parenchymal or- 294 tissues where the same FGFR isoform is believed to play a regulatory role. For example, the FGFR2 gene exhibiting mutations in the Glu160–Lys176 domain associated with Apert syndrome is widely expressed in the epithelium of adult parenchymal or- 294 tissues where the same FGFR isoform is believed to play a regulatory role. For example, the FGFR2 gene exhibiting mutations in the Glu160–Lys176 domain associated with Apert syndrome is widely expressed in the epithelium of adult parenchymal or-

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Fen Wang, Mikio Kan, Kerstin McKeehan, Jun-Hyeog Jang, Shuju Feng and Wallace L. McKeehan

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