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Profound Ligand-Independent Kinase Activation of Fibroblast Growth Factor Receptor 3 by the Activation Loop Mutation Responsible for a Lethal Skeletal Dysplasia, Thanatophoric Dysplasia Type II

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Thanatophoric dysplasia type II (TDII) is a neonatal lethal skeletal dysplasia caused by a recurrent Lys-650→Glu mutation within the highly conserved activation loop of the kinase domain of fibroblast growth factor receptor 3 (FGFR3). We demonstrate here that this mutation results in profound constitutive activation of the FGFR3 tyrosine kinase, approximately 100-fold above that of wild-type FGFR3. The mechanism of FGFR3 activation in TDII was probed by constructing various point mutations in the activation loop. Substitutions at position 650 indicated that not only Glu but also Asp and, to a lesser extent, Gln and Leu result in pronounced constitutive activation of FGFR3. Additional mutagenesis within the β10-β11 loop region (amino acids Tyr-647 to Leu-656) demonstrated that amino acid 650 is the only residue which can activate the receptor when changed to a Glu, indicating a specificity of position as well as charge for mutations which can give rise to kinase activation. Furthermore, when predicted sites of autophosphorylation at Tyr-647 and Tyr-648 were mutated to Phe, either singly or in combination, constitutive kinase activity was still observed in response to the Lys-650→Glu mutation, although the effect of these mutations on downstream signalling was not investigated. Our data suggest that the molecular effect of the TDII activation loop mutation is to mimic the conformational changes that activate the tyrosine kinase domain, which are normally initiated by ligand binding and autophosphorylation. These results have broad implications for understanding the molecular basis of other human developmental syndromes that involve mutations in members of the FGFR family. Moreover, these findings are relevant to the study of kinase regulation and the design of activating mutations in related tyrosine kinases.

Fibroblast growth factor receptors (FGFRs) play an important role in regulating biological processes, including proliferation, differentiation, angiogenesis, and embryonic development. The four members of the FGFR family are highly related at the amino acid level, with each protein having an extracellular ligand-binding domain composed of three immunoglobulin-like domains, a transmembrane spanning region, and a cytoplasmic tyrosine kinase domain that is split by the kinase insert region. In response to ligand binding by members of the fibroblast growth factor family, FGFRs dimerize, resulting in autophosphorylation of the kinase domain and interaction with and phosphorylation of effector signalling proteins (for reviews, see references 11 and 12).

Recently, several human congenital skeletal disorders have been shown to result from point mutations in members of the FGFR family. For instance, a variety of mutations mapping to the region between the second immunoglobulin loop and the transmembrane domain of FGFR2 cause the highly related craniosynostosis disorders known as Crouzon, Jackson-Weiss, Apert, and Pfeiffer syndromes. Pfeiffer syndrome also results from a mutation at a similar position in FGFR1 (for a review, see reference 19). Point mutations in different domains of FGFR3 are implicated in several clinically related forms of dwarfism, including achondroplasia, hypochondroplasia, and the neonatal lethal syndrome thanatophoric dysplasia (TD). Achondroplasia, the most common form of genetic dwarfism, is associated with a Gly-380→Arg mutation in the transmembrane domain of FGFR3 (25, 29). Some cases of hypochondroplasia, a milder form of achondroplasia, map to an Asn-540→Lys substitution in the proximal half of the split tyrosine kinase domain (4).

The most severe of the FGFR3 disorders, TD, has an estimated frequency of 1 in 20,000 to 1 in 50,000 births (1, 22). Affected individuals exhibit extreme shortening of the limbs and ribs, reduction in height of the vertebrae, and facial and skull anomalies. Infants born with TD usually die shortly after birth from respiratory failure due to severe thoracic and abdominal malformations (28). In the few individuals who have survived for several years with medical intervention, there are also readily apparent deficiencies in central nervous system development (16). Histologically, in TD there is a generalized disorganization of endochondral ossification at the bone growth plate (24). Consistent with the tissues affected in TD, FGFR3 is primarily expressed in the central nervous system and at the cartilaginous growth plates of bones (23).

Two types of TD can be distinguished by radiological and other clinical criteria; they are referred to as type I TD (TDI) and type II TD (TDII). Several distinct mutations in FGFR3 have been demonstrated in individuals affected by TDI. Mutations to Cys at three different positions in the extracellular domain and a missense mutation in the stop codon have been reported (26, 30, 31), although the mutation(s) responsible for TDI in a number of individuals has not yet been identified. In contrast, all reported cases of TDII result from the same Lys-650→Glu point mutation in the tyrosine kinase domain of

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FGFR3 (31). This residue is in the highly conserved activation loop found in many receptor and nonreceptor tyrosine kinases. Phosphorylation of the two activation loop residues corresponding to Tyr-647 and Tyr-648 of FGFR3 has recently been shown to be essential for activation of the tyrosine kinase activity and the biological activity of the related receptor FGFR1 (17), suggesting that they would also be major sites of activating autophosphorylation in FGFR3. A change in charge two residues away from these predicted sites of activating phosphorylation, as a result of the TDII Lys-650→Glu mutation, might well be expected to have a dramatic effect on the activity of FGFR3.

In our previous work we demonstrated that the transmembrane domain Gly-380→Arg mutation found in achondroplasia results in ligand-independent activation of FGFR3 (32), in a manner analogous to the oncogenic activation of the Neu tyrosine kinase. This result suggested that unregulated signalling through the constitutively activated receptor underlies the abnormal bone development in achondroplasia and that constitutive FGFR3 activation might also be involved in TD, although the two occur via different molecular mechanisms.

In this paper we examine the effect of the TDII mutation on the tyrosine kinase activity of FGFR3. We show that receptors bearing the TDII mutation are constitutively activated and have a much higher level of activation than receptors with the achondroplasia mutation. Significantly, the clinical severity of TDII parallels the profound in vitro kinase activation described here. We also examine the amino acid requirements at and near position 650 of the activation loop for constitutive FGFR3 kinase activation. Because of the homology of the activation loop in many tyrosine kinases, our findings are relevant not only to the mechanism of FGFR3 activation in skeletal dysplasia but also to normal and abnormal signalling mechanisms through related receptor tyrosine kinases.

MATERIALS AND METHODS

Construction of FGFR3 mutants. The wild-type and achondroplasia mutant FGFR3 constructs were prepared as described previously (32) but were subcloned into the pcDNA3 expression vector (Invitrogen). The TDII construct and activation loop mutants were derived as follows. The FGFR3 sequence between the *Sca*I site at amino acid 608 and the *Sph*I site at amino acid 732 was reconstructed in the pSP64 vector (Promega) by using three long pairs of complementary oligonucleotides which were synthesized and purified as described previously (33). The first pair extended from the *Sca*I site to a novel *Eco*RV site at amino acid 641, the second pair created a silent *Age*I site at amino acid 658 and extended to a novel silent *Bam*HI site at amino acid 691, and the third pair extended from the *Bam*HI site to the *Sph*I site. The reconstructed *Sca*I-*Sph*I fragment was subcloned back into the pcDNA3 wild-type FGFR3 construct and digested with *Eco*RV and *Age*I. Pairs of annealed oligonucleotides encoding the various TDII derivative sequences were subcloned between the *Eco*RV site and the *Age*I site, such that the *Eco*RV site was destroyed. All constructs were confirmed by sequencing to be correct and by indirect immunofluorescence to be expressed at the cell surface.

Immunoprecipitation and in vitro kinase assays. NIH 3T3 cells were transfected with 10 μg of each expression construct by a modified calcium phosphate transfection method (5). Sixteen hours after the cells were refed, the medium was replaced with serum-free Dulbecco's modified Eagle medium; 12 h later, the cells were lysed in Nonidet P-40 (NP-40) lysis buffer (20 mM Tris [pH 7.5], 137 mM NaCl, 1% NP-40, 5 mM EDTA, 10% glycerol, 10 mg of aprotinin per ml, 1 mM sodium orthovanadate) and immunoprecipitated with antiserum specific for the C terminus of FGFR3 (Santa Cruz Biotechnology). Immune complexes were washed once in 20 mM Tris, pH 7.5, and subjected to in vitro kinase reactions in the presence of 40 μl of kinase buffer (20 mM Tris [pH 7.5], 10 mM $MnCl_2$, 5 mM $MgCl_2$, 5 μCi of [γ-^{32}P]ATP) for 15 min at 37°C. The products were washed extensively with NP-40 lysis buffer and then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 7.5% polyacrylamide gels) and autoradiography (32).

Immunoblotting. Immunoprecipitates prepared as described above were electrophoresed through an SDS–7.5% polyacrylamide gel and transferred to nitrocellulose membranes. The membranes were incubated with anti-FGFR3 antiserum (Santa Cruz Biotechnology) and horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G and developed by enhanced chemiluminescence (ECL; Amersham) according to the manufacturer's instructions.

Indirect immunofluorescence. To detect cell surface expression of FGFR3, transiently transfected COS-1 cells expressing each construct were fixed with 3% paraformaldehyde and incubated, without permeabilization, with antiserum SB141, directed against the extracellular domain of FGFR3 (14), and a fluorescein-conjugated goat anti-rabbit secondary antibody (Boehringer Mannheim).

RESULTS

FGFR3 is strongly activated by the TDII Lys-650→Glu mutation. The locations of known FGFR3 point mutations linked to skeletal dysplasias are diagrammed in Fig. 1. The TDII point mutation, Lys-650→Glu, lies within the second part of the split tyrosine kinase domain in the highly conserved activation loop. An alignment of the catalytic and activation loop regions of several prototypical members of different receptor and nonreceptor tyrosine kinase families is presented in Fig. 1. Lys-650, whose homolog in many but not all tyrosine kinases is a basic residue, is indicated. The two Tyr residues homologous to those identified in FGFR1 (17) and the insulin receptor (7, 34) as being essential sites of autophosphorylation are denoted by asterisks. Recently, the X-ray crystal structure of the tyrosine kinase domain of the human insulin receptor (IRK) was determined (10), which led to the development of a model for ligand-dependent kinase activation. It was proposed that in the absence of ligand, Tyr-1162 (corresponding to Tyr-647 of FGFR3) lies in the active site, blocking access to substrate and Mg-ATP. Ligand binding results in a conformational change in the kinase domain, allowing *trans* phosphorylation of this Tyr. Salt bridging between this phosphotyrosine and positively charged residues within the catalytic and activation loops (corresponding to Arg-616 and Arg-640 of FGFR3) would stabilize this noninhibiting conformation, maintaining it in the active conformation, which would result in increased tyrosine kinase activity (10).

Our prediction was that the TDII Lys-650→Glu mutation, in close proximity to the predicted sites of Tyr autophosphorylation, would have a significant effect on receptor activity. In order to assess the effect of this mutation on FGFR3 activity, constructs encoding wild-type FGFR3, the achondroplasia transmembrane domain mutation Gly-380→Arg, or the TDII mutation Lys-650→Glu were transfected into NIH 3T3 cells under the control of the cytomegalovirus promoter. FGFR3 proteins were immunoprecipitated from lysates of serum-deprived cells and subjected to in vitro kinase assays in the presence of [γ-^{32}P]ATP. Figure 2 reveals that the TDII mutant receptor exhibits at least 100-fold greater autophosphorylation than wild-type FGFR3. This activation is about 20-fold greater than is seen for the achondroplasia Gly-380→Arg mutant (Fig. 2). As a control for receptor loading, a portion of each immunoprecipitate was subjected to immunoblotting with FGFR3-specific antiserum, and by indirect immunofluorescence, each construct was shown to be expressed at the surface of transfected cells (Fig. 3). These data demonstrate that the Lys-650→Glu mutation confers pronounced constitutive activation of FGFR3 and that the extent of in vitro autophosphorylation of the TDII mutant receptor is much greater than that of the achondroplasia mutant. Although it is difficult to extrapolate directly from in vitro enzyme assays to in vivo effects, in the case of TDII and achondroplasia, the degree of severity of the dysplasia correlates well with the extent of constitutive kinase activation.

Mutational analysis of the activation loop of FGFR3 suggests specificity of charge and position for constitutive kinase activity. To begin to address the mechanism by which the TDII mutation activates the FGFR3 tyrosine kinase, we first examined the requirements at position 650 for in vitro kinase activity. In particular, we wished to establish whether the loss of the

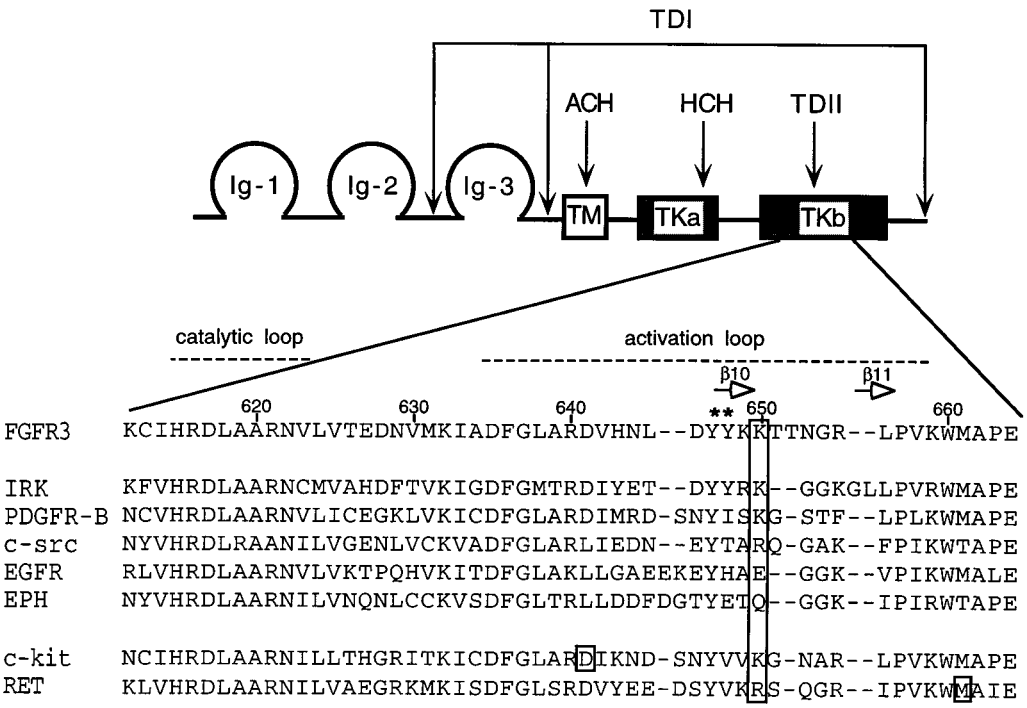

FIG. 1. (Top) Location of point mutations in FGFR3 in skeletal dysplasias. A schematic representation of the FGFR3 structure is shown, with the three immunoglobulin (Ig)-like loops of the extracellular domain, the transmembrane domain (TM), and the proximal and distal parts of the intracellular split tyrosine kinase domain (TKa and Tkb, respectively) indicated. The locations of known point mutations giving rise to TDI (26, 30, 31), achondroplasia (ACH) (25, 29), hypochondroplasia (HCH) (4), and TDII (31) are indicated by arrows. (Bottom) Sequence alignment of the catalytic and activation loop regions of various tyrosine kinase family members. The catalytic and activation loop and β-strand assignments are as for IRK (10). Sequences are as given in reference 13 (FGFR3) and reference 9 (all others). Asterisks indicate the predicted sites of ligand-induced autophosphorylation (Tyr-647 and Tyr-648) in FGFR3. The residue corresponding to Lys-650 of FGFR3, which gives rise to TDII when mutated to Glu (31), is boxed in all sequences. Asp-816 of c-*kit* and Met-918 of RET, which when mutated activate these receptors (15, 20, 27), are also boxed. PDGFR-B, platelet-derived growth factor receptor B.

basic charge, the creation of a novel acidic charge, or simply any structural alteration at position 650 mimics the Lys→Glu mutation. Several amino acid substitutions were engineered into FGFR3 at position 650, and receptors were expressed and examined for in vitro kinase activity as described above. As shown in Fig. 4, at position 650, Asp and Glu were equally activating. Receptors with either Gln or Leu at position 650 exhibited much lower levels of kinase activity, although they were above wild-type levels. This observation suggests that for strong, constitutive FGFR3 activation, an acidic residue at position 650 is beneficial, although not a strict requirement.

Because of the significant similarity between the primary sequences of IRK and FGFR3, we constructed a homology model for of FGFR3 based on the crystallographic coordinates of IRK. Our modeling studies indicate that there is little difference between IRK and FGFR3 in the expected molecular structure of the activation loop region (data not shown). Position 650 of FGFR3 lies within a subloop of the activation loop which is bounded by β-sheets 10 and 11 (residues 647 to 656) (10). We wished to examine whether changing any other position within this subloop besides Lys-650 to Glu could activate FGFR3. Each residue from Tyr-647 to Leu-656 was individually mutated to Glu, and immunoprecipitated receptors were subjected to in vitro kinase assays as described above. The data presented in Fig. 5 indicate that within this region of the activation loop, only at position 650 did a mutation to Glu activate FGFR3. Specifically, the Lys-649→Glu substitution is not activating, although the overall charge within the activation loop region would be the same in this construct as in the TDII mutant. Thus, the position of the mutant Glu residue within the loop is important for constitutive receptor activation. Furthermore, simply replacing either of the predicted sites of activating autophosphorylation, Tyr-647 or Tyr-648, with Glu, which may mimic a phosphotyrosine residue, is not sufficient to activate FGFR3. Consistent with this latter observation, mutating the analogous Tyr residues at positions 1162 and 1163 of the insulin receptor to Glu or Asp did not constitutively activate its kinase activity (34).

Tyr-647 and Tyr-648 are not required for FGFR3 kinase activity in the presence of Glu-650. Phosphorylation of the tyrosines corresponding to Tyr-647 and Tyr-648 of FGFR3 has been shown to be essential for the kinase activities of IRK (7, 34) and FGFR1 (17), as mutation of these residues to Phe significantly reduces both autophosphorylation of the kinases and their ability to transmit cellular signals in response to ligand. By analogy, it is expected that Tyr-647 and Tyr-648 should also be necessary for activation of the FGFR3 kinase domain. We wished to address whether mutation of Lys-650 to Glu is able to activate autocatalytic activity in the absence of a phosphorylatable Tyr in the activation loop. Either Tyr-647 (FYKE), Tyr-648 (YFKE), or both Tyr residues (FFKE) were changed to Phe in the presence of the Glu-650 mutation, and the ability of each receptor to autophosphorylate was examined. As shown in Fig. 6, neither Tyr-647 nor Tyr-648 is required for the autophosphorylation of FGFR3 in the presence of Glu-650, as the FYKE, YFKE, and FFKE mutant receptors all have significant kinase activity. As a control, the construct FFKK has no kinase activity above the background level observed for wild-type FGFR3. This suggests that phosphorylation within the activation loop is not required for enhanced

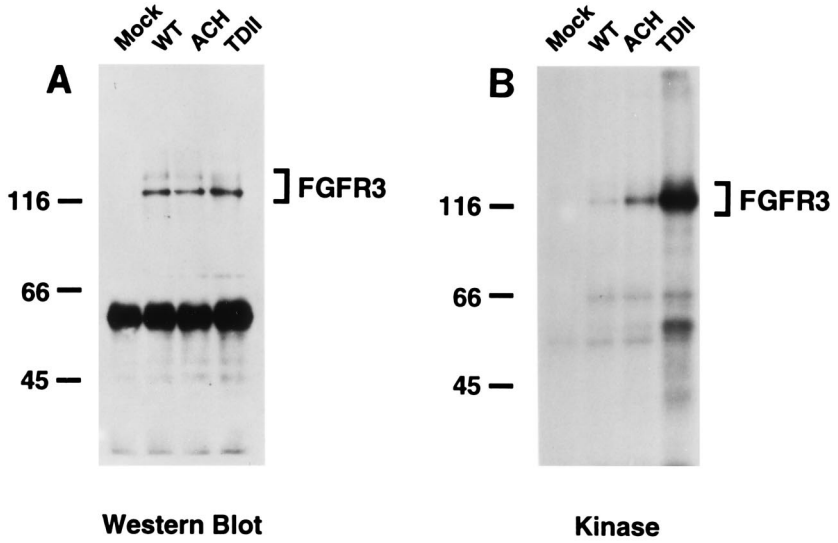

FIG. 2. The TDII mutant FGFR3 is constitutively active as a tyrosine kinase. NIH 3T3 cells transiently expressing either a vector control (Mock), wild-type FGFR3 (WT), the Gly-380→Arg mutant (ACH), or the Lys-650→Glu mutant (TDII) were lysed and immunoprecipitated with FGFR3 antiserum (Santa Cruz Biotechnology). (A) Immunoblot. Immunoprecipitates were electrophoresed through SDS–7.5% polyacrylamide gels, transferred to nitrocellulose membranes, and incubated with FGFR3 antiserum, followed by addition of horseradish peroxidase-conjugated secondary antiserum and then enhanced chemiluminescence development. Molecular mass markers (in kilodaltons) are indicated. Levels of receptor expression are similar. (B) Autophosphorylation assay. Immunoprecipitates were subjected to in vitro kinase reactions in the presence of [γ-^{32}P]ATP and analyzed by SDS-PAGE and autoradiography. Cells expressing the TDII mutant receptor construct exhibited significantly increased autophosphorylation relative to that of the achondroplasia mutant.

kinase activity in FGFR3 in the presence of the TDII Lys-650→Glu substitution. The strong autophosphorylation of the FFKE mutant, although weaker than that of the TDII YYKE mutant (possibly because the FFKE mutant has two fewer phosphorylatable Tyr residues), indicates that there must be other sites of FGFR3 autophosphorylation. This is consistent with the identification of a total of seven sites of FGFR1 autophosphorylation (17, 18), five of which lie outside the activation loop. Although beyond the scope of the present study, it will be of interest to determine whether these mutations at potential autophosphorylation sites impair ligand-dependent FGFR3 activation, as was shown for FGFR1 (17), and whether they alter substrate specificity or signalling capability.

DISCUSSION

Relevance of FGFR3 activation in TDII to other skeletal dysplasias. There are now four human skeletal disorders which map to FGFR3. TDI mutations are found in the FGFR3 extracellular domain, as well as at the termination codon (26, 30, 31); achondroplasia is due to a substitution in the transmembrane domain (3, 25, 29); in many cases of hypochondroplasia, there is a mutation in the first segment of the split kinase domain (4); and in all cases of TDII, there is a Lys-650→Glu mutation within the highly conserved activation loop of the kinase domain (31). We had previously demonstrated that the molecular mechanism underlying achondroplasia is constitutive FGFR3 tyrosine kinase activity, apparently due to stabilization of the dimeric, active conformation of the receptor because of the presence of a hydrophilic residue in the transmembrane domain, analogous to the postulated mechanism of Neu oncogenic activation (32). Because of the phenotypic similarity, we predicted that each of the skeletal-disorder mutations mapping to different domains of FGFR3 would be activating, although the mechanism of activation was not readily apparent. Results presented here demonstrate that the TDII mutation leads to considerable activation of the in vitro kinase activity of FGFR3 and that this activation is approximately 20-fold greater than that in achondroplasia.

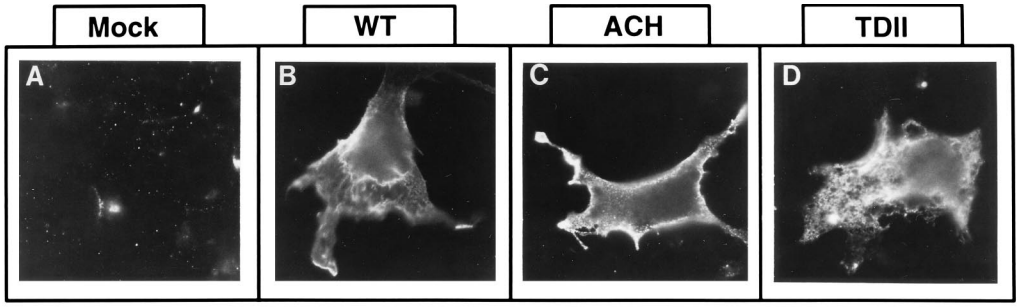

FIG. 3. The TDII mutant FGFR3 is localized to the cell surface. Nonpermeabilized COS-1 cells transiently transfected with the indicated FGFR3 constructs (Mock, vector control; WT, wild type; ACH, Gly-380→Arg mutant) were incubated with antiserum SB141 (directed against the extracellular domain of FGFR3) and a fluorescein-conjugated goat anti-rabbit polyclonal antiserum.

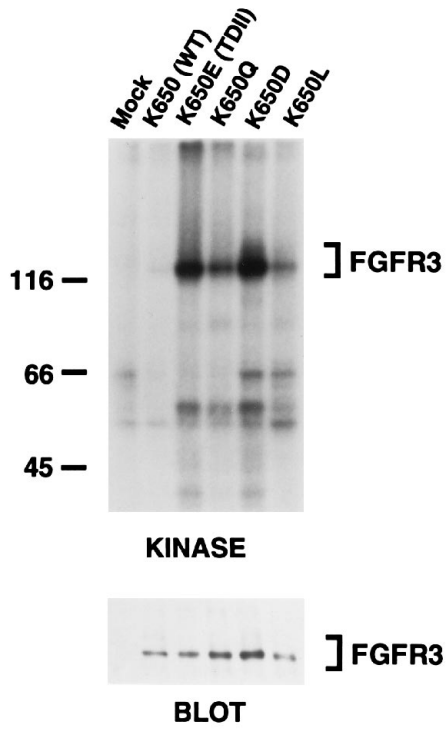

FIG. 4. The specific substitution at position 650 is important for FGFR3 activation. NIH 3T3 cells expressing receptors with the indicated amino acid substitutions at position 650 were lysed, immunoprecipitated with FGFR3-specific antibodies, and subjected to in vitro kinase and immunoblot analysis as described in the legend to Fig. 2. Mock, vector control; WT, wild type.

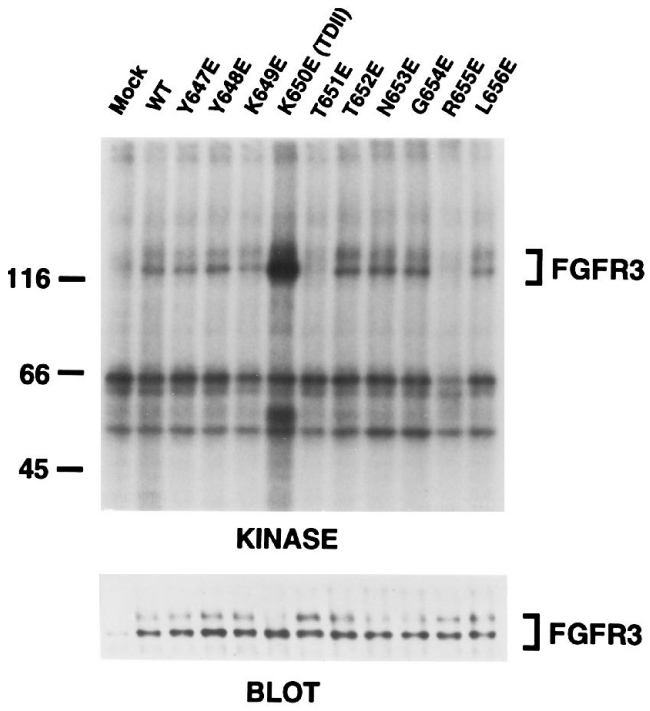

FIG. 5. The position within the activation loop of the Glu mutation is important in FGFR3 constitutive activation. NIH 3T3 cells transiently expressing receptors with the indicated Glu substitutions within the β10-β11 region of the FGFR3 activation loop were subjected to in vitro kinase and immunoblot analysis as described in the legend to Fig. 2. Mock, vector control; WT, wild type.

Homozygous achondroplasia is a neonatal lethal disorder with clinical features that strongly resemble (heterozygous) TD. In achondroplasia homozygotes, there is no normal copy of the FGFR3 gene; instead, the two copies present express receptors with in vitro kinase activity intermediate between that of wild-type and TDII receptors (Fig. 2). It therefore appears that the severity of these skeletal dysplasias is related to the degree of constitutive autophosphorylation, and presumably signalling, through FGFR3. Our prediction, then, is that TDI and TDII mutant receptors will exhibit similarly high levels of activation and that the milder hypochondroplasia mutation will confer only slightly increased FGFR3 activity. The mechanism(s) by which these mutations affect FGFR3 is currently under investigation. Mutations in the extracellular domain of FGFR3 in TDI that result in the creation of novel unpaired Cys residues might be expected to lead to intermolecular disulfide bond formation and thus ligand-independent receptor activation, in much the same way as similar mutations activate FGFR2 in the craniosynostosis disorder known as Crouzon syndrome (7a, 21).

Molecular consequences of the Lys-650→Glu mutation. Data presented here begin to address the mechanism of FGFR3 activation by the TDII activation loop mutation. We have shown that not only a Glu but also the acidic residue Asp is able to constitutively stimulate high levels of FGFR3 kinase activity, suggesting that TDII would likely also result from expression of receptors with an Asp at position 650. This is, however, unlikely to occur in nature, as such a substitution would require the mutation of more than one nucleotide, whereas a single change at nucleotide 1948 converts the Lys codon AAG to the Glu codon GAG (31). We speculate that other mutations affecting position 650 might result in less severe skeletal dysplasia, as reflected by the fact that substitution of either Gln or Leu results in levels of in vitro receptor autophosphorylation which are more similar to those induced by the achondroplasia mutation. Residue 650 is also the only residue within the β10-β11 region of FGFR3 at which mutation to Glu can cause activation of the receptor, suggesting a specificity of position for mutations which can give rise to kinase activation. Finally, receptors in which both Tyr-647 and Tyr-648 are mutated to Phe have significant kinase activity when the TDII Lys-650→Glu mutation is also present. This observation suggests that Glu-650 obviates the requirement for activation loop autophosphorylation demonstrated for other receptor tyrosine kinases (7, 17). As discussed above, phosphorylation of the conserved activation loop tyrosine, in combination with other ligand-induced conformational changes in the receptor, is proposed to maintain the availability of the active site for substrate binding and phosphorylation (10). These results suggest a model for the molecular effect of the TDII mutation whereby the Lys-650→Glu mutation promotes the conformational changes that move the activation loop out of its inhibitory position within the active site and that normally require ligand binding and autophosphorylation.

Implications of the TDII activation loop mutation for the study of other tyrosine kinases. Many receptor and nonreceptor tyrosine kinases, including some members of the Src family, the insulin receptor family, the platelet-derived growth factor receptor family, and the FGFR family (9) (Fig. 1), have a basic residue three positions C terminal to the conserved activation loop Tyr. It is therefore possible that these tyrosine kinases can also be constitutively activated by the same basic-to-acidic amino acid substitution which occurs naturally in TDII. Being

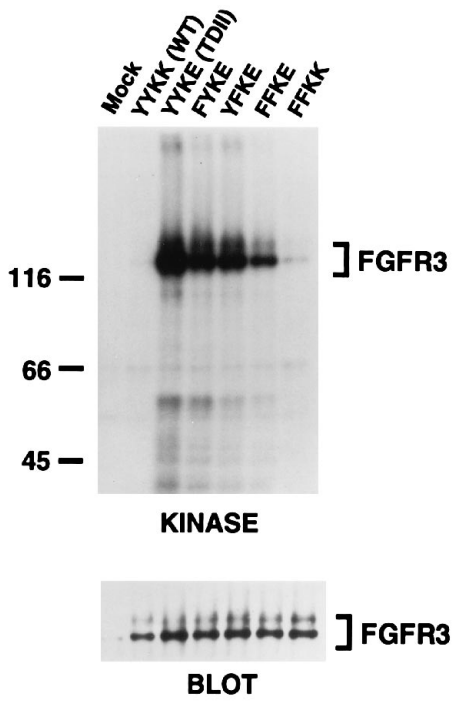

FIG. 6. Tyr-647 and Tyr-648 are not required for FGFR3 kinase activation in the presence of the TDII Glu-650 mutation. NIH 3T3 cells expressing each indicated receptor were processed for in vitro kinase and immunoblot analysis as described in the legend to Fig. 2. The one-letter code is used to represent amino acids at positions 647 through 650 of the activation loop of FGFR3. Mock, vector control; WT, wild type.

able to activate other tyrosine kinases in the absence of ligand and/or upstream modulators may be expected to advance research into signal transduction mechanisms, in much the same way that a general method for engineering kinase-dead or dominant negative kinases has been very useful for elucidating signalling pathways.

At least two classes of tyrosine kinase receptors, the Eph and the epidermal growth factor receptor (EGFR) families, provide exceptions to the Tyr-X-X-basic amino acid activation domain motif. In wild-type EGFR, for instance, there is a Glu residue at the position (Tyr-848) corresponding to the Lys-650→Glu mutation in FGFR3 of TDII, and yet EGFR normally requires ligand binding for kinase and biological activities. Interestingly, the Tyr residue corresponding to the activating site of autophosphorylation in most other tyrosine kinases, residue 845 of EGFR, is apparently not a site of phosphorylation in this receptor, and it apparently can be mutated to Phe without affecting either autophosphorylation or signalling in response to epidermal growth factor (8). This observation suggests that subtle differences between families of tyrosine kinases which share overall structural similarities will prove to be important in determining their precise mode of activation.

There are precedents for activating point mutations within the activation loops of other receptor tyrosine kinases, which also lead to serious human diseases. An Asp-816→Val substitution has been found in the catalytic domain of c-*kit* in certain patients with the hematopathologic disorder mastocytosis, and this mutation has been demonstrated to increase the ligand-independent tyrosine kinase activity of this platelet-derived growth factor receptor-related protein (15, 20). In the dominantly inherited cancer syndrome multiple endocrine neoplasia (MEN) 2B, a Met-918→Thr substitution was shown to be responsible for constitutive tyrosine kinase activity and transformation by the receptor RET (27). The locations of these mutations relative to the FGFR3 activating mutation in TDII are shown in Fig. 1, and both map to residues which are conserved in some but not all families of tyrosine kinases. Addressing the amino acid requirements at these and neighboring positions for receptor activation is expected to complement ongoing structural studies of tyrosine kinase domains and help elucidate the normal mechanism of activation of these important classes of signalling receptors.

The biologically relevant ligand(s) for FGFR3 is as yet unknown, although transgenic mice expressing high levels of basic FGF (FGF-2) primarily exhibit a phenotype of skeletal malformations similar to those of chondrodysplasia, which may suggest a normal role for basic FGF in FGFR3 regulation (6). The signalling pathway downstream of FGFR3 is also an open question. It has been extensively documented that expression of constitutively activated variants of many other receptor tyrosine kinases, including the above-mentioned c-*kit* (15), RET (27), and Neu (2), leads to oncogenic transformation of cells. Expression of highly activated FGFR3 receptors in NIH 3T3 fibroblasts, however, does not cause focus formation (data not shown), suggesting that FGFR3 may not be linked to identical mitogenic pathways. Furthermore, patients with achondroplasia, who have fairly normal life spans, and children who have survived several years with TD apparently do not exhibit an increased frequency of bone or central nervous system cancers (16). It will therefore be of interest to determine which signalling effectors are downstream of FGFR3 and which of the possible stages of chondrocyte maturation—differentiation, proliferation, hypertrophy, and death (24)—are defective because of the TDII activating mutation.

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