A novel variant of the fibroblast growth factor receptor type 1 (FGFR-1) was identified in human placental RNA. In this receptor (FGFR-1L) portions of the second and third immunoglobulin-like (Ig-like) domains are deleted. To determine whether FGFR-1L was functional, full-length variant (pSV/FGFR-1L) and wild-type (pSV/FGFR-1) receptors were stably transfected into rat L6 myoblasts cells. Transfected L6 clones expressed respective proteins and bound [125I]-labeled FGF-2 with Kd values of 99 pm (FGFR-1) and 26 pm (FGFR-1L). FGF-1 and FGF-2 competed efficiently with [125I]-FGF-2 for binding to FGFR-1 and FGFR-1L, whereas FGF-4 was less efficient. FGF-1, FGF-2, and FGF-4 enhanced mitogen-activated protein kinase (MAPK) activity, increased steady-state c-fos mRNA levels, and stimulated proliferation through either receptor, whereas KGF was without effect. FGFR-1 expressing clones exhibited ligand-induced tyrosine phosphorylation of fibroblast growth factor receptor substrate 2 (FRS2), a 90-kDa adaptor protein that links FGFR-1 activation to the MAPK cascade. In contrast, tyrosine phosphorylation of FRS2 was not evident with FGFR-1L. In addition, phospholipase C-γ was not tyrosine phosphorylated via activated FGFR-1L. These findings indicate that FGFR-1L binds FGF-1 and FGF-2 with high affinity and is capable of mitogenic signaling, but may activate MAPK to occur via non-classical signaling intermediates.

The fibroblast growth factor (FGF) family presently consists of at least 20 different members, including the well characterized acidic FGF (aFGF or FGF-1) and basic FGF (bFGF or FGF-2). These heparin-binding polypeptides share 30–70% amino acid sequence homology, are mitogenic and angiogenic, and are involved in cell differentiation and tissue development and repair (1–4). Their actions are dependent on their ability to bind and activate a family of cell surface receptors with intrinsic protein tyrosine kinase activity (5–7). Four distinct genes encoding high affinity FGF receptors designated as FGFR-1 (flg), FGFR-2 (bek), FGFR-3, and FGFR-4 have been identified (6–13). These receptors possess an extracellular ligand-binding domain that has three immunoglobulin-like (Ig-like) regions, a hydrophobic transmembrane domain, and a discontinuous intracellular tyrosine kinase domain exhibiting a short intervening sequence (6, 7).

As a consequence of alternative mRNA splicing, a number of variant FGF receptors have been described for FGFR-1, -2, -3, including some that have lost the amino-terminal Ig-like domain (domain I), resulting in the generation of 2-Ig-like forms (7, 14–16). The presence of an intron-exon boundary in the third Ig-like loop (domain III) allows for the generation of 2 alternative carboxyl-terminal domains (IIIb or IIIc) of FGFR-1, -2, and -3 (7, 10, 17–23). As a result of splice site skipping the IIIa splice form of FGFR-1 yields a secreted receptor (7). Domain III has been determined to be important in conferring ligand-binding specificity (5, 13, 18, 23, 24). The receptor for KGF (KFGF) is a splice variant of FGFR-2 exhibiting a unique IIIb domain and a high affinity for both KGF and FGF-1, while FGF-2 binds poorly to KGFR (17, 18, 22). FGFR-2 binds FGF-1 and FGF-2 with high affinity, whereas FGFR-2 does not bind KGF (8, 22, 24, 25). This region is also involved in cell-type expression. The IIIb splice form of FGFR-1 is expressed in epithelial cell types whereas expression of the IIIc splice form is restricted to mesenchymal cell types (21, 26).

Mitogenic signaling via FGFR-1 is effected following ligand-induced receptor dimerization that leads to trans-autophosphorylation on tyrosine residues in the cytoplasmic domain. FGFR-1 contains at least seven tyrosine phosphorylation sites (27). These phosphotyrosine residues in turn either stimulate the intrinsic catalytic activity of the receptor or serve as recruitment sites for downstream signaling proteins containing Src homology 2 (SH2) domains and phosphotyrosine-binding domains (27–29). Autophosphorylation of Tyr-653 and Tyr-654 is required for stimulation of kinase activity and biological function. Phosphorylation of Tyr-766 converts this residue into a high affinity binding site for PLC-γ (27). Activated FGFR-1 also tyrosine phosphorylates the docking protein FRS2 (30). FRS2 is a lipid-anchored docking protein that becomes tyrosine phosphorylated and binds to Grb2/Sos in response to FGF stimulation, thereby linking FGF receptor activation with the Ras/MAPK signaling cascade (30).

Although the FGFR-1 gene possesses an intron-exon boundary in the second Ig-like loop (domain II), splice variants based on a mRNA splicing event in this region have not been previously reported. We now report the characterization of a novel FGFR-1 variant, designated FGFR-1L, which was identified in
human placental RNA. In this variant receptor portions of the second and third Ig-like domains are deleted, resulting in a novel 2-Ig-like variant. Expression of FGFR-1L in L6 myoblasts that express exceedingly low levels of FGFRs is shown to confer mitogenic responsiveness following activation by FGF-1, FGF-2, and FGF-4, including stimulation of cell proliferation and MAPK activation. However, by comparison with FGFR-1, FGFR-1L exhibits an attenuated capacity to induce tyrosine phosphorylation of FRS2 and PLC-γ.

**EXPERIMENTAL PROCEDURES**

**Reagents**—The following were purchased: SuperSignal West Pico chemiluminescent substrate and BCA protein assay were from Pierce (Rockford, IL); PCR-dye from Sigma; [α-32P]CTP (3000 Ci/mmol; α-2,3-P(CTP) (800 Ci/mmol); and [32P]ATP (1180 Ci/mmol) IgG from Amersham Pharmacia Biotech; horseradish peroxidase-labeled anti-rabbit IgG and horseradish peroxidase-labeled anti-mouse from Bio-Rad; ribonuclease protection assay, RPA II, from Ambion (Austin, TX); LipofectAMINE and Superscript preamplification kit from Life Technologies; streptomycin.

**RNA Protection Assay**—For RNase protection mapping of FGFR-1L specific transcripts, a cDNA specific to FGFR-1L was used to generate the antisense RNA probe. A 220-bp HindIII fragment was produced by PCR amplification of pSV/FGFR-1L using P1 and P3, 5'-ctgctgaagcttcggcatctggg-3', corresponding to nt 1177–1157 (accession number X52833) and containing a HindIII site (underlined). This FGFR-1L cDNA fragment was subcloned into pBluescript IIISK+ and sequenced.

**Cell Culture**—L6 Cells were routinely propagated in a humidified incubator at 37 °C in a 5% CO2, 95% air atmosphere and grown in Dulbecco's modified Eagle's medium supplemented with 8% heat inactivated fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin.

**Reverse Transcriptase PCR Cloning**—RNA from human placenta was isolated by guanidium thiocyanate-phenol-chloroform extraction (31) and reversed transcribed using the SuperScript preamplification kit according to manufacturers directions. Two fragments (660 and 345 bp) were produced by PCR amplification of placental cDNA using sense primer (P1), 5'-gggagttgacctgctttgatc-3', corresponding to nucleotides 643–661 of FGFR-1 (accession number X52833) and containing a HindIII site at 1804. The FR1D fragment corresponding to nt 1770–2596 was ampliﬁed using the sense primer 5'-gggagttgacctgctttgatc-3' and antisense primer, 5'-ctgctgaagcttcggcatctggg-3', containing a SalI site (underlined) and a PstI site at 1804. The PstI/SalI fragment of FR1D and the HindIII/PstI fragment of FR1C were sequentially subcloned into pUC218, generating plasmid plasmid FR1CD. Two oligonucleotides were used to identify a linker that contains the XbaI EcoRI target sites: EcoRI/XbaI/HindIII/SalI/XhoI. The linker was subcloned into the pSVK3 expression vector, under the control of the simian virus 40 early promoter, at the EcoRI/XhoI sites, generating the vector, pSVK4. The HindIII/SalI fragment from FR1CD was then subcloned into pSVK4, generating the vector pSV/FRCD. A HindIII/Asp718 fragment from FR1BD (EcoRI site at 673) and the XbaI/Rfl sites of FR1A were sequentially subcloned into pSV/FRCD to yield either wild-type pSV/FGFR-1 or variant pSV/FGFR-1L, respectively. Authenticities of the constructs were confirmed by sequencing.

** establishment of Transfected Cell lines**—L6 cells were grown in 100-mm plates to semiconfluence and co-transfected with 10 μg of pSV/FGFR-1 and 0.5 μg of pRSVneo for neomycin resistance using Lipofectamine. pSV/FGFR-1L was also co-transfected into L6 cells with pRSVneo. As a control L6 cells were transfected with 1 μg of pRSVneo. Cells were split when confluent, seeded into 100-mm dishes, and cultured in the presence of 800 μg/ml G418. Multiple G418 resistant colonies were isolated and expression of FGFR-1 and FGFR-1L was confirmed by immunoblotting.

**Immunoblotting and Immunoprecipitation**—Cells were washed with cold PBS and lysed in lysing buffer consisting of 50 mm Tris (pH 7.4), 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mm NaCl, 1 mm EDTA, 1 mm phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 mm sodium fluoride, and 1 mm sodium vanadate. Extracts were clarified by centrifugation, and protein concentrations were determined by the BCA protein assay. Cell lysates were either directly analyzed (20 μg) or were immunoprecipitated (0.5–1 mg) with PY20 (2 μg), anti-flg (1 μg), anti-PLC-γ (1 μg), and FR22 antisera (10 μl) overnight at 4 °C, followed by a 2-h incubation with 50 μl of protein A-agarose. Immunoblotting was carried out with a highly specific anti-PLC-γ monoclonal antibody (1 μg/ml) and chemiluminescent detection.

**Activation of a Novel FGFR-1 Variant Receptor**

**Detection of Active MAPK**—Active MAPK was detected by immunoblotting using an anti-Active MAPK antibody (1,200,000 dilution) according to the manufacturer's instructions and a horseradish peroxidase-labeled anti-rabbit secondary antibody (1,10,000 dilution) followed by chemiluminescent detection.

**Receptor Binding Assay**—For competition binding analysis, transfected L6 cells were grown to confluency in 24-well plates, rinsed with binding buffer (Dulbecco's modified Eagle's medium, 0.2% bovine serum albumin, 25 mm Hepes, pH 7.3) and preincubated in binding buffer at

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2 M. E. Lopez and M. Kore, unpublished observations.
The initiation site (the Ig-like domain are indicated. The deleted amino acids are in black box. The cysteines in acidic region boxed. The tyrosine kinase region were run on an 8% polyacrylamide, 5M urea gel. The gel was dried and exposed to film. The FGFR-1L protected fragment (220 nt) is indicated. The FGFR-1 protected fragments are shown by heavy black arrowheads. The FGFR-1L protected fragments are shown by arrows. Molecular weight markers are indicated on the right.

**RESULTS**

PCR Analysis of the Second and Third Ig-like domains of FGFR-1—PCR amplification of placenta cDNA using FGFR-1 oligonucleotides, P1 and P2, yielded a major amplified product of the expected size of 660 bp, and a minor PCR product of 345 bp. Subcloning and sequencing of these two cDNA fragments confirmed that the 660-bp PCR product represented the wild-type FGFR-1 transcript (nt 643–1302, X52833). Sequence analysis of the 345-bp PCR product indicated that the smaller fragment was the result of a 315-bp deletion (nt 709–1023) in the 660-bp FGFR-1 transcript. This deletion of 105 amino acids is most likely the result of an alternative RNA splicing product. The sequence deletion corresponds to exons 7 and 8 (35, 36) which encode the carboxyl-terminal of the Ig-like domain II, the intervening sequence between Ig-like loops 2 and 3, and the amino-terminal of the Ig-like domain III (Fig. 1A). A second set of primers, described previously (37), confirmed that a 315-bp deletion does exist (data not shown). These primers amplified the entire extracellular domain. Several cDNA fragments were generated and sequenced. The large fragment (1.1 kilobases) was the 3-Ig loop FGFR-1. There were two fragments (0.8 kilobase) that were approximately the same size. One of these 0.8-kilobase fragments was the commonly described 2-Ig loop FGFR-1—that is most likely the result of an alternative RNA splicing product. A highly specific RNase protection assay using human placenta RNA revealed the presence of low levels of a protected fragment of 220 nt corresponding to FGFR-1L (Fig. 1, C and D). Furthermore, a hybrid between the probe and FGFR-1 mRNA could yield four possible protected fragments, 157 and 63 nt, and/or 154 and 67 nt (Fig. 1C). As can be seen in Fig. 1D, the 157- and 154-nt fragments appear as a single band whereas the 66- and 63-nt fragments are clearly visible. In contrast, there was no signal when yeast tRNA was used as a negative control.

**FGFR-1 and FGFR-1L Expression in L6 Cells—**To determine whether FGFR-1L could mediate the biological actions of FGF-1 and FGF-2 on c-fos Induction—Cells were cultured for 18 h in low serum medium and then incubated in the absence or presence of 0.5 nM FGF-1 and FGF-2 for 1 h. Total RNA was size fractionated, and transferred onto GeneScreen membranes. A c-fos cDNA probe was synthesized in the presence of [α-32P]dCTP and used to carry out hybridization under high stringency conditions (33). The blots were then exposed at −80 °C to film with intensifying screens.

15 °C for 20 min. 125I-FGF-2 was then added (50,000 cpm/ml/well) with various concentrations of unlabeled ligand (0–300 ng) in a final volume of 0.5 ml. After 2.5 h the cells were washed twice with cold PBS and twice with PBS, 1.5 % NaCl. The cells were then lysed with 0.1 % NaOH, 1% SDS and the radioactivity in the lysates was measured by counting. Specific binding was determined by subtracting nonspecific binding of samples incubated with 400-fold excess unlabeled ligand. For Scatchard analysis cells were grown to confluency in 24-well plates and binding was performed as above except that increasing concentrations of 125I-FGF-2 were added. Estimates of receptor affinity and total binding capacity were made using the Ligand software (34).

**Effects of FGF-1 and FGF-2 on c-fos Induction—**Cells were cultured for 18 h in low serum medium and then incubated in the absence or presence of 0.5 nM FGF-1 and FGF-2 for 1 h. Total RNA was size fractionated, and transferred onto GeneScreen membranes. A c-fos cDNA probe was synthesized in the presence of [α-32P]dCTP and used to carry out hybridization under high stringency conditions (33). The blots were then exposed at −80 °C to film with intensifying screens.

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**FGFR-1 and FGFR-1L Expression in L6 Cells—**To determine whether FGFR-1L could mediate the biological actions of...
FGFs, a full-length FGFR-1L receptor cDNA incorporating this deletion was constructed by ligating the extracellular and transmembrane FGFR-1L cDNA sequence to FGFR-1 cytoplasmic tyrosine kinase domain followed by subcloning into the pSVK4 expression vector. L6 rat skeletal muscle myoblasts were stably transfected with either FGFR-1L or FGFR-1. Expression of the transfected receptors was confirmed by immunoblotting with an anti-FGFR-1 antibody that recognizes the first Ig-like domain (Fig. 2A). As anticipated, L6 cells expressing FGFR-1 exhibited a 150-kDa protein whereas FGFR-1L expressing cells exhibited a 100-kDa protein. Parental L6 cells did not exhibit either protein, even following immunoprecipitation with an anti-flg antibody (data not shown). The larger translated size can be accounted for the presence of potential glycosylation sites. FGFR-1 has 9 and FGFR-1L has 5 potential glycosylation sites. Treatment with tunicamycin, an inhibitor of N-linked glycosylation synthesis, reduced the mass of FGFR-1 from 150 kDa to approximately 140 kDa and FGFR-1L from 100 kDa to approximately 89 kDa. These observations are consistent with the predicted molecular mass for FGFR-1 (100 kDa) and FGFR-1L (86 kDa) based on their deduced amino acid sequence. Two FGFR-1L clones expressing high and low levels (clones 40 and 47, respectively) of FGFR-1L protein and a FGFR-1L clone with a high level of receptor expression were selected for further characterization.

**125I-FGF-2 Binding in L6 Cells**—To characterize 125I-FGF-2 binding, cells transfected with FGFR-1, FGFR-1L, and the neomycin resistance vector (Neo), a full-length human wild-type FGFR-1 cDNA, or the variant FGFR-1L cDNA. Cell lysates were electrophoresed on 7.5% SDS-PAGE, transferred, and immunoblotted with anti-FGFR-1 antibody. FGFR-1L clone 47 expressed low levels of the receptor. Therefore, clone 47 homogenates were also immunoprecipitated with the anti-flg antibody and then subjected to immunoblotting with anti-FGFR-1 antibody (extreme right). B, cells were treated with 5 µg/ml tunicamycin overnight. Lysates were electrophoresed on 7.5% SDS-PAGE, transferred, and immunoblotted with anti-FGFR-1 antibody. The FGFR-1 (RI) and FGFR-1L (RIL), clone 40, are indicated as closed arrowheads and asterisks, respectively. Molecular weight standards are indicated on the left.

**FIG. 2. Immunoblot analysis of FGFR-1 and FGFR-1L expression in transfected L6 cells.** A, L6 cells were stably transfected with the neomycin resistance vector (Neo), a full-length human wild-type FGFR-1 cDNA, or the variant FGFR-1L cDNA. Cell lysates were electrophoresed on 7.5% SDS-PAGE, transferred, and immunoblotted with anti-FGFR-1 antibody. FGFR-1L clone 47 expressed low levels of the receptor. Therefore, clone 47 homogenates were also immunoprecipitated with the anti-flg antibody and then subjected to immunoblotting with anti-FGFR-1 antibody (extreme right). B, cells were treated with 5 µg/ml tunicamycin overnight. Lysates were electrophoresed on 7.5% SDS-PAGE, transferred, and immunoblotted with anti-FGFR-1 antibody. The FGFR-1 (RI) and FGFR-1L (RIL), clone 40, are indicated as closed arrowheads and asterisks, respectively. Molecular weight standards are indicated on the left.

**Receptor Activation**—To determine whether FGF-2 could induce tyrosine phosphorylation of transfected L6 cells, the cells were incubated in the absence or presence of 1 nM FGF-2 for 5 min. Cell lysates from these cells were immunoprecipitated with anti-phosphotyrosine antibodies and immunoblotted with anti-FGFR-1 antibodies (Fig. 5). In the absence of FGF-2, neither receptor was tyrosine phosphorylated. Following addition of FGF-2, both FGFR-1 and FGFR-1L were clearly tyrosine phosphorylated. In contrast, FGF-2 was without effect in sham transfected L6 cells. FGFR-1 and FGFR-1L expressing cells exhibited similar increases in cell growth in response to FGF-1, FGF-2, and FGF-4 (Fig. 6). However, FGF-2 (0.5 and 5 nM) had a slightly greater overall effect on cell growth than FGF-1 or FGF-4 in FGFR-1 expressing cells. In contrast, FGFR-1, FGF-2, and FGF-4 exerted similar effects on growth in FGFR-1L expressing cells. There was no response to ligand in L6 cells transfected with pRSVneo alone.

FGFR-1, FGF-2 and FGF-4 also activated MAPK in L6 cells expressing FGFR-1 (Fig. 7A), but had no effect in L6 cells transfected with pRSVneo (data not shown). All three ligands binding. Scatchard analyses revealed that FGFR-1 expressing cells have 20,000 binding sites per cell with a dissociation constant (K_d) of 9.9 pM. In contrast, FGFR-1L expressing clones (40 and 47) were calculated to have 3,000 binding sites per cell with a K_d of 26 pM. Thus, the affinity of 125I-FGF-2 for FGFR-1L was 4-fold higher than the affinity of 125I-FGF-2 for FGFR-1. To analyze the ligand binding specificity of FGFR-1L, competition-inhibition binding studies were carried out using 125I-FGF-2 and unlabeled FGF-1, FGF-2, FGF-4, and KGF. Analysis of the competition-inhibition binding data indicated that FGF-1 (Fig. 4A), FGF-2 (Fig. 4B) and FGF-4 (Fig. 4C) were relatively equipotent at displacing 125I-FGF-2 in FGFR-1 and FGFR-1L expressing cells. In contrast, no measurable effect on 125I-FGF-2 binding by KGF could be demonstrated (data not shown).

**FIG. 3. Scatchard analyses of 125I-FGF-2 binding to FGFR-1 and FGFR-1L.** L6 cells transfected with FGFR-1 (square) or clone 47 expressing FGFR-1L (circle) were incubated with various concentrations of 125I-FGF-2 at 15 °C. Specific binding to FGFR-1 or FGFR-1L was calculated by subtracting nonspecific binding of samples incubated with a 400-fold excess of unlabeled FGF-2. Dissociation constants (K_d) and the number of receptors per cell were determined by the Ligand program (34). Values shown are the means of duplicate samples and are representative of three or more experiments.
also induced MAPK activation in FGFR-1L-transfected cells (Fig. 7A), although to a slightly lesser extent than FGFR-1. In contrast, KGF did not activate MAPK in any of the cells (Fig. 7A). Despite the differences in the level of FGFR-1L in clones 40 and 47, FGF-2 enhanced MAPK activation to the same extent in both clones (Fig. 7B). Furthermore, FG-1 and FG-2 enhanced the steady state levels of c-fos mRNA in FGFR-1 and FGFR-1L expressing cells, but not in L6 cells transfected with pRSVneo (Fig. 8).

**Attenuated Tyrosine Phosphorylation of FRS2 and PLC-γ**

FRS2 is identical to suc1-associated neurotrophic factor target which was identified as a tyrosine-phosphorylated protein that binds to the SH2 domain of Grb2 and which can also bind to p13suc1 protein conjugated to agarose (38). To determine whether FRS2 contributes to FGFR-1L mediated signaling, cells were stimulated with 1 nM FGF-2 for 5 min before lysis. Cell lysates were then either incubated with p13suc1-agarose beads or immunoprecipitated with the FRS2 antibody prior to immunoblotting with the anti-phosphotyrosine antibody. FGF-2 induced FRS2 phosphorylation in FGFR-1 expressing cells in both p13suc1 precipitates and FRS2 immunoprecipitates (Fig. 9). In contrast, there was no evidence of a tyrosine-phosphorylated 90-kDa/FRS2 protein in FGFR-1L transfected cells in either the p13suc1 precipitates or FRS2 immunoprecipitates.

PLC-γ has been reported to be a major substrate for FGFR-1 (39). Therefore, we next examined tyrosine phosphorylation of PLC-γ in FGFR-1 and FGFR-1L expressing cells. Anti-phosphotyrosine immunoblotting of PLC-γ immunoprecipitates indicated that FGF-2 induced tyrosine phosphorylation of PLC-γ in FGFR-1 expressing cells (Fig. 10). In contrast, tyrosine phosphorylation of PLC-γ was not detectable in FGFR-1L expressing cells or in neo controls. Immunoblotting with PLC-γ showed that this difference was not due to different amounts of PLC-γ protein in the two FGFR-1L clones.

**DISCUSSION**

Ig-like domains are characterized by two cysteine residues in each domain, a tryptophan residue 11 or 12 residues downstream...
stream of the first cysteine residue, and the presence of a consensus sequence Asp-Xaa-Gly-Xaa-Tyr-Xaa-Cys 50–70 residues further downstream. In the present study we identified a novel variant of FGFR-1, termed FGFR-1L, in which portions of domain II, the linker between domain II and domain III, and domain III are deleted. FGFR-1L is expressed at low levels in human placenta and appears to form as a consequence of an alternative splicing event. Thus, removal of exons 7 and 8 leads to a unique Ig-like domain (II:III) encoded by exons 6 and 10 (exon 9 encodes IIIb, Refs. 35 and 36). These two exons meet the criteria of a constant-type Ig-like domain (40). Therefore, FGFR-1L exhibits a novel 2 Ig-like extracellular domain, consisting of domain I and domain II:III. The latter domain constitutes a fusion of the amino-terminal of domain II and the carboxyl-terminal of domain III.

Several lines of evidence in the present study suggest that FGFR-1L is a functional high affinity FGF receptor. First, when introduced into L6 cells, this novel splice variant bound FGF-2 with a high affinity. Indeed, the dissociation constant of I25F-2 for FGFR-1L, 26 pM, was about 4-fold higher than for FGFR-1. Furthermore, FGF-1, FGF-2, and FGF-4 displaced the binding of I25F-2 to the same extent in FGFR-1 and FGFR-1L expressing cells. Second, FGF-1, FGF-2, and FGF-4 enhanced the proliferation of FGFR-1L expressing cells, but had no effect on the growth of parental cells. Third, all three FGFs activated MAPK in FGFR-1L expressing cells. In contrast L6 neo cells did not exhibit an increase in MAPK activity in response to FGFs. Fourth, FGFs enhanced steady state c-fos mRNA levels in FGFR-1 and FGFR-1L expressing clones. Taken together, these observations indicate that FGFR-1L is capable of activating mitogenic signaling pathways.

The naturally occurring 2 Ig-like splice variant of FGFR-1 retains full ligand binding capacity in comparison with the 3 Ig-like FGFR-1 (41). In contrast, the secreted form of FGFR-1 consisting of domain I is incapable of ligand binding (42). Similarly, a cDNA encoding a 2-Ig-like isoform consisting of do-

Fig. 7. Activation of MAP kinase. A, quiescent Neo, FGFR-1, and FGFR-1L (clone 47) transfected L6 cells were incubated for 5 min in the absence (−) or presence (+) of 0.5 nM FGF-1, FGF-2, FGF-4, or KGF. Cell lysates were prepared as described under “Experimental Procedures” and were analyzed by immunoblotting with an anti-active MAPK antibody. B, MAP kinase activation of FGFR-1L clones 40 and 47. The indicated clones were incubated for 5 min in the absence (−) or presence (+) of 0.5 nM FGF-2.

Fig. 8. Effects of FGF-1 and FGF-2 on c-fos expression. Cells were incubated with 0.5 nM FGF-1 or FGF-2 for 1 h. Northern blots (20 μg of RNA/lane) were hybridized with a 32P-labeled c-fos cDNA probe. The filter was hybridized with a 32P-labeled 7s cDNA probe as an internal control.

Fig. 9. FRS2 tyrosine phosphorylation by FGFR-1 and FGFR-1L. Cell lysates from Neo, FGFR-1, and FGFR-1L (clone 40) cells were stimulated for 5 min in the absence (−) or presence (+) of 1 nM FGF-2 and were precipitated (P) with (A) p13Suc1 followed by immunoblotting (IB) with anti-phosphotyrosine antibody (PY20). FRS2 is indicated (arrowhead). B, the same lysates were also immunoprecipitated (IP) with FRS2 antiserum followed by immunoblotting with anti-phosphotyrosine antibody (neo not shown). FRS2 is indicated (arrowhead).

Fig. 10. Effects of FGF-2 on PLC-γ phosphorylation. Cells transfected with the neo resistance plasmid (Neo), FGFR-1 (R1), or FGFR-1L (R1L) were incubated for 5 min in the presence (1) or absence (2) of 1 nM FGF-2, lysed, and immunoprecipitated (IP) with anti-PLC-γ. The samples were separated by 7.5% SDS-PAGE, transferred to Immobilon-P, and immunoblotted (IB) with anti-phosphotyrosine antibody (PY20, top). To verify equal loading, aliquots of the immunoprecipitates were also blotted with anti-PLC-γ (bottom). Two FGFR-1L clones (40 and 47) are shown.
main I, the interlinker III/II sequence, and domain III does not display ligand binding activity (16). Also, when domains I and II are adjacent to a defective domain III ligand binding is abolished (16). Thus, the concomitant presence of domains II and III of FGFR-1 is generally believed to be an essential prerequisite for ligand binding. To our knowledge, the present study is the first to demonstrate that a variant of FGFR-1 that is missing significant portions of domains II and III is able to bind FGF ligands and activate mitogenic signaling.

The crystal structure of the extracellular domain of the 2 Ig-like form of FGFR-1 in complex with FGF-2 has been recently elucidated (43). Two FGF-2-FGFR-1 complexes were shown to form a dimer that is stabilized by interactions between FGF-2 and domain II of the adjoining receptor and by a direct interaction between domain II of each receptor (43). FGFR-2 was suggested to interact with residues in domains II and III as well as with the linker region between these domains. It is noteworthy that FGFR-1L retains the majority of the residues that interact with FGF-2 as well as all the heparin-binding sites (lysine residues) in domain II (44). There are three areas of interaction between FGF-2 and FGFR-1L in domain III, and two of these regions are located in the carboxy-terminal of domain III. Furthermore, almost all the residues involved in ligand-receptor and receptor-receptor interactions that stabilize dimerization are in the amino-terminal region of domain II, which are retained in FGFR-1L. Together, these observations suggest that the residues that FGFR-1L retains are sufficient for dimerization and FGF-1, FGF-2, and FGF-4 binding.

Despite the similarities between FGFR-1 and FGFR-1L with respect to ligand binding and MAPK activation, there were several important differences between the two receptors. Thus, tyrosine phosphorylation of FRS2 could not be detected in FGFR-1L expressing cells in response to ligand stimulation. Although FRS2 lacks an SH2 domain, it contains a phosphotyrosine-binding domain that allows for phosphotyrosine-independent activation by FGFR-1L. Activation of FGFR-1L leads to attenuated tyrosine phosphorylation of FRS2, our findings suggest that a FRS2 independent pathway may lead to MAPK activation by FGFR-1L. Alternatively, it is possible that activation of FGFR-1L leads to attenuated tyrosine phosphorylation of FRS2 that cannot be detected in our antibody immunoprecipitation and p130cbl pulldown experiments, but that allows for Grb2/Sos-mediated MAPK activation.

FGFR-1L activation also failed to induce tyrosine phosphorylation of PLC-γ, indicating that FGFR-1L may also not be able to induce tyrosine phosphorylation of this key signaling molecule. Mutagenesis experiments have indicated that phosphorylation of Tyr-766 on the FGFR-1 is essential for phosphorylation of PLC-γ and for phosphatidylinositol hydrolysis but is not essential for FGF-induced mitogenic and differentiation response (27, 46). Our findings suggest, therefore, that a naturally occurring variant of FGFR-1 can effect mitogenic signaling without inducing phosphorylation of Tyr-766 of PLC-γ. Since it is not established that FGFR-1 activates mitogenesis via FRS2 in L6 cells, it is also conceivable that both FGFR-1 and FGFR-1L activate mitogenic signaling via a common mechanism in these cells. However, it is highly unlikely that the observed differences between FGFR-1 and FGFR-1L were due to differences in the level of expression of these two receptors since one of the two FGFR-1L clones (clone 40) had similar receptor protein expression as the FGFR-1 clone. Instead, it is clear that FGFR-1L does not detectably activate FRS2 or PLC-γ. Taken together, our findings suggest that alterations in the extracellular domain of FGFR-1 can lead to significant and diverse qualitative differences in ligand-induced intracellular signaling pathways. Such differences have potential to add to the complexity of signaling pathways that are activated following ligand binding to multiple FGF receptors.