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# Signal transduction by fibroblast growth factor receptors

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## 1. ABSTRACT

The fibroblast growth factor family, with its prototype members acidic FGF (FGF-1) and basic FGF (FGF-2), binds to four related receptor tyrosine kinases, expressed on most types of cells in tissue culture. In many respects, the FGF receptors appear similar to other growth factor receptors. Thus, dimerization of receptor monomers upon ligand binding is likely to be a requisite for activation of the kinase domains, leading to receptor trans phosphorylation. FGF receptor-1 (FGFR-1), which shows the broadest expression pattern of the four FGF receptors contains at least seven tyrosine phosphorylation sites. A number of signal transduction molecules are affected by binding with different affinities to these phosphorylation sites. The potential roles of these signal transduction molecules in FGF-induced biological responses and in pathological processes are discussed.

## 2. INTRODUCTION

The fibroblast growth factor family encompasses at present about twenty factors, which are 30-70% identical in their primary sequences (1; see 2 for a review). FGF-1 and FGF-2 lack signal sequences for export out of the producer cell. Most other FGF members possess signal sequences and several of them have been identified as transforming proteins (3). FGF's are potent mitogens for a wide variety of different cell types in tissue culture and *in vivo* and are implicated in differentiation of endothelial cells and neuronal cells. FGF's are expressed in a strict temporal and spatial pattern during development and have important roles in patterning and limb formation. The FGF's bind in an overlapping pattern to four structurally related receptor tyrosine kinases (4). The high sequence similarity between the receptors together with the overlapping pattern of FGF binding, *i.e.*, most FGF's bind to all four receptors, implies redundancy within this growth factor - receptor family. In spite of this redundancy, targeted gene-inactivation of different FGF members yields a specific phenotype for each factor.

The biological effects of FGF are established as a result of intracellular signal transduction initiated by the growth factor-bound, activated FGF receptors. Different FGF family members will activate the FGF receptor subtypes to different extents depending on their abilities to bind with high affinity to each receptor type (5). Receptor activation leads to tyrosine autophosphorylation of the receptors. Tyrosine phosphorylation sites serve as high affinity binding sites for Src Homology 2 (SH2) domain-containing signal transduction molecules (6). These molecules transduce signals from the receptor, in signaling chains or cascades, which eventually result in biological responses, often involving changes in gene transcription. This review will discuss the current knowledge of signal transduction initiated by FGF receptors and the role of the different signal transduction molecules in FGF-induced cellular responses.

## 3. FGF RECEPTOR STRUCTURE

### 3.1. FGF receptor primary and schematic structures

Lee *et al.* (7) used immobilized FGF in affinity chromatography to purify a receptor tyrosine kinase, later denoted FGFR-1. The availability of the FGFR-1 primary structure led to a very rapid development in the

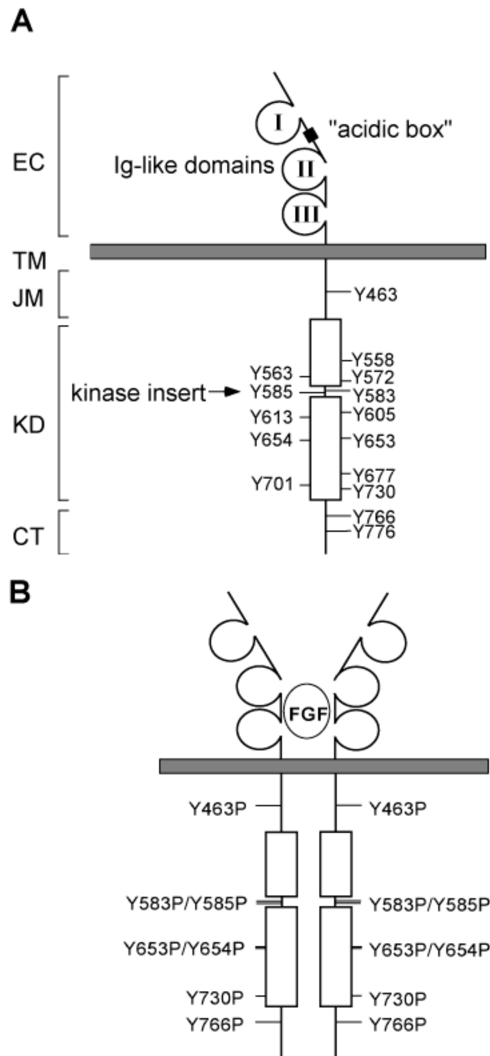


**Figure 1.** Schematic structure of FGFR-1. A. The overall structural organization is similar for the four FGF receptors. The extracellular domain (EC) contains two (II and III) or three (I, II, III) immunoglobulin (Ig)-like domains, followed by the transmembrane (TM) stretch, the juxtamembrane (JM) domain, the kinase domain (KD) interrupted by a short kinase insert, and a C-terminal tail (CT). The positions of tyrosine residues in the intracellular domain are indicated. The acidic box indicated in the intracellular domain is a specific feature of FGF receptors. B. The positions of identified phosphorylatable tyrosine residues are indicated in the ligand-bound, dimerized FGFR-1. Tyrosine phosphorylation sites in other FGF receptors remain to be determined; however, FGFR-2, -3 and -4 all contain tyrosine residues corresponding to Tyr653, Tyr654, Tyr730 and Tyr766 in FGFR-1.

field, and soon, FGFR-1 as well as several related receptors, now denoted FGFR-2, -3 and -4 were identified in a number of different species. Subsequently the FGF receptors were shown to exist in a variety of splice variants, creating soluble or membrane-anchored receptors differing with regard to a number of structural motifs in the extra- and intracellular domains (8).

The FGF receptors are very similar in their overall structural organization. Alternative splicing of the extracellular domain creates variants composed of two or three immunoglobulin-like folds (figure 1A). A row of eight consecutive acidic residues (the "acidic box" ) is situated between the first and the second immunoglobulin-like fold in all FGF receptor types; the acidic box is a unique feature of the FGF receptors (8) and appears to be important for FGF receptor function (9). A single transmembrane stretch connects the extracellular part, with the intracellular juxtamembrane (JM) domain. The juxtamembrane domain is considerably longer in the FGF receptors than in other receptors; it is not known whether this feature has functional implications. The JM domains of FGFR-1 and -2 contain one phosphorylatable tyrosine residue. FGFR-3 and -4 lack tyrosine residues in their JM domains. The tyrosine kinase domain of FGF receptors contains the classical conserved tyrosine kinase motifs (10). The tyrosine kinase domain is split in two parts by a short non-catalytic insert of about 15 amino acid residues, which contains two phosphorylatable tyrosine residues in FGFR-1 and -2, one in FGFR-3 and none in FGFR-4. The mitogenic potential appears to be lower for FGFR-4 than the other FGF receptors, which in part is due to the lack of the kinase insert tyrosine residues (11). The kinase insert tyrosine residues appear however to be dispensable for FGFR-1 function (12). The C-terminal tails of the FGF receptors contain a number of tyrosine residues of which some are located at identical positions in the receptors.

### 3.2. Crystal structure of FGFR-1

Mohammadi *et al.* (13) expressed an FGFR-1 fragment encompassing residues 456-765 in the baculovirus system and used the purified unphosphorylated receptor intracellular domain, in complex with an ATP analogue, for crystalization. Each crystal contained two FGFR-1 intracellular domains organized in two lobes, similar to what has been described earlier for the insulin receptor (14) and protein serine/threonine kinases. There are important differences between the FGFR-1 and insulin receptor structures, however. Thus, residues in the FGFR-1 activation loop appear to interfere with substrate peptide binding, but not with ATP-binding, indicating a mechanism for autoinhibition different from that proposed for the insulin receptor. Six of the seven identified, potentially phosphorylated tyrosine residues in FGFR-1 (figure 1B) are present in the crystallized fragment, and of these six residues, five are found in flexible segments of the molecule. The sixth residue, Y730 is buried and conformational changes are required for this residue to be exposed on the surface.

### 3.3. Receptor splice variants

Alternative splicing of the FGF receptor mRNA's generates several different variants of the receptors (8). A functionally critical splicing event is

created by differential exon usage in the third immunoglobulin-like loop (see figure 1A for nomenclature). The genomic sequence of this region in FGFR-1 to -3 contains two alternative exons (IIIb and IIIc) for the second half of the third Ig-like loop (15-17). Dependent on the exon usage, FGFR proteins with different ligand-binding properties are created. Moreover, differential polyadenylation sites within the intron sequences preceding these alternative exons result in the production of soluble receptors (denoted IIIa splice variants). FGFR-4 differs from the other FGF receptors in that it has a single exon in the C-terminal half of the third Ig-like loop (18).

### 3.4. Expression patterns of FGF receptors

Most cells in tissue culture express FGF receptors. It is unclear whether the general expression *in vitro* is a reflection of the *in vivo* expression pattern, or if it is irrelevantly induced in conjunction with explantation. Analogous situations exist for other growth factor receptors, like the PDGF receptors, which are expressed on fibroblasts in tissue culture, but not on normal fibroblasts in unharmed skin. PDGF receptors are however expressed on explanted fibroblasts and in tissues undergoing inflammation (19). It remains to be shown which cells express the different FGF receptors *in vivo* under normal and pathological conditions. The expression patterns of the different FGF receptors during embryonal development are distinct but overlapping. Thus, in the mouse embryo, FGFR-1 is expressed in the mesenchyme, whereas FGFR-2 is expressed in several epithelial tissues (20-21). FGFR-3 expression is predominant in brain, spinal chord and cartilage rudiments of the developing bone (22). FGFR-4 is expressed in several tissues of endodermal and mesodermal origin (22-23). For details on FGF receptor expression, see ref. 8.

## 4. HEPARAN SULFATE PROTEOGLYCANS IN FGF SIGNALING

Proteoglycans are cell membrane attached or extracellular matrix proteins carrying carbohydrate side chains denoted glycosaminoglycans. The glycosaminoglycans are composed of repeating units of disaccharides, which are negatively charged due to the presence of sulfate and carboxyl groups. The highly complex glycosaminoglycan heparan sulfate has been shown to be critical for FGF receptor function (24-25). FGF's fail to bind and activate FGF receptors in cells lacking endogenous heparan sulfate, and the FGF responsiveness is restored by addition of exogenous heparin. Different models have been proposed to explain the role of heparan sulfate in FGF signaling. Thus, heparan sulfate may present the monomeric FGF as repeating units to the FGF receptors, thereby facilitating dimerization and activation of receptors (26). However, the minimal FGF-binding heparan sulfated oligosaccharides are unable to support FGF-mediated receptor activation (27). For this to occur, longer oligosaccharide units with a distinct sulfation pattern are required. These observations have led to the proposal that heparan sulfate allows formation of ternary heparan sulfate/FGF/receptor complexes by interacting not only with the growth factor but also with the receptor (28).

## 5. ACTIVATION OF FGF RECEPTORS AND INITIATION OF SIGNAL TRANSDUCTION

### 5.1. Mode of activation and phosphorylation of FGF receptors

Binding of FGF leads to dimerization of FGF receptors, following the by now well established consensus model for receptor tyrosine kinases (29). Dimerization occurs both between FGF receptors of the same type, homodimerization, and between different FGF receptor types, heterodimerization (30). The mechanisms for FGF receptor dimerization are not yet well defined, compared with other growth factor/receptor models. Thus, PDGF is a dimeric factor, which presents two binding sites, each binding one receptor molecule. FGF's on the other hand are monomeric factors, although there are reports on the presence of two distinct receptor-binding sites in FGF, which might facilitate receptor dimerization (31). Moreover, the fourth immunoglobulin-like loop in the PDGF receptor and in c-kit, the stem cell factor receptor, have been shown to stabilize receptor dimerization (32). Such an element has not yet been identified in FGF receptors.

Dimerization of receptor tyrosine kinases appears to be a prerequisite for activation of the tyrosine kinase. It is probable that the close proximity allows phosphorylation *in trans* between the receptor monomers (see 13). Dimerization has for other receptor tyrosine kinases been shown to be accompanied by conformational changes in the intracellular domain (see *e.g.* refs. 33-34) which probably is critical in activation of the kinase. FGFR-1 has been examined in detail for the position of phosphorylation sites (figure 1B). Of the five tyrosine residues located in the cytoplasmic part of the receptors, not including the kinase domain, Y776 appears not to be phosphorylated (35), whereas Y463, Y583, Y585 and Y766 are potential phosphorylation sites (12). By analogy with other receptor tyrosine kinases, these sites together with surrounding amino acid residues present binding sites for SH2 domain-containing signal transduction molecules (for a review, see 6). This far, only one site, Y766, have been shown to participate in such interactions; phosphorylated Y766 binds phospholipase C$\gamma$ (36). Three phosphorylation sites have been identified in the FGFR-1 kinase domain, Y653, Y654 and Y730. Y653 and Y654 appear to be involved in regulation of the kinase, since mutation of Y653 and Y654 to phenylalanine residues leads to loss of kinase activity (12).

Potential tyrosine phosphorylation sites in other FGF receptors have not yet been identified. The phosphorylatable tyrosine residues in FGFR-1 show varying degrees of conservation in FGFR-2 to -4. Thus, tyrosine residues corresponding to Y463, Y583 and Y585 are found in FGFR-2, Y583 is found in FGFR-3, and Y653, Y654, Y730 and Y766 are found in all FGF

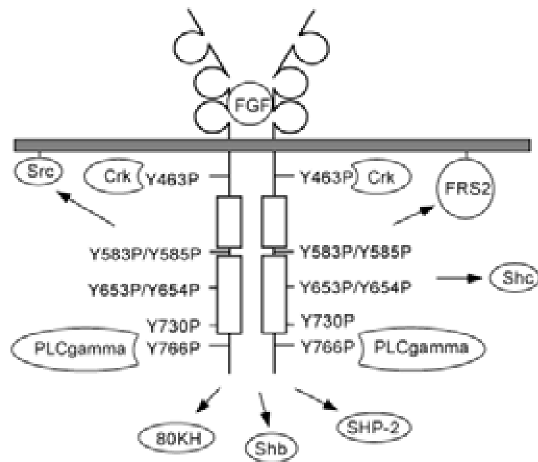


**Figure 2.** Schematic outline of FGFR-1 signal transduction molecules. Crk tyrosine phosphorylation and downstream signal transduction dependes on Tyr463 in FGFR-1. The activated FGFR-1 dimer strongly binds PLC-gamma, to Tyr766 in the receptor. All other indicated signal transduction molecules do not interact with any one single tyrosine phosphorylation site in the receptor. FRS2 and the Src family tyrosine kinases are anchored in the membrane, whereas Crk, PLCgamma, Shc, SHP-2, Shb and 80 KH are not. Signal propagation from FGFR-1 to these downstream signaling molecules depend on their tyrosine phosphorylation, in most cases probably directly by the FGFR.

receptor types. Moreover, FGFR-1 as well as FGFR-4 contain a number of phosphoserine residues; some are induced as a consequence of activation of the receptors, whereas others are independent of ligand-stimulation (see 37; Klint and Claesson-Welsh, unpublished).

**5.2 SH2-domain protein interactions with FGF receptors**

SH2 domains are 100 amino acid residue long conserved motifs that are found in different enzymes (such as lipid kinases, GTPases, phospholipases, phosphatases) or in proteins that lack enzymatic activities, denoted adaptors, which indirectly affect enzymatic activities. SH2-domains mediate binding to phosphotyrosine residues with specificity for the 3-6 amino acid residues C-terminal of the tyrosine (38). Both SH2 domain-containing enzymes and adaptors are affected by binding to the receptor, by tyrosine phosphorylation, by conformational changes or by increased availability of their substrates (for a review, see 39). The ensuing change in enzymatic activity is propagated further in signaling chains, eventually giving rise to cellular responses (see 6). Below is an outline of SH2 domain-containing enzymes and adaptors that participate in FGF receptor signal transduction (see figure 2 for a schematic summary ).

PLCgamma: Phosphorylation of Y766 in FGFR-1 allows binding and subsequently, tyrosine phosphorylation, of PLCgamma. Tyrosine phosphorylation of PLCgamma is known to be accompanied by its activation, leading to hydrolysis of phosphatidylinositol 4,5 bisphosphate to inositol 1,4,5 trisphosphate and diacylglycerol. Ins 1,4,5 $P_3$ generation leads in turn to release of $Ca^{2+}$ from internal stores, whereas diacylglycerol accumulation activates members of the protein kinase C family (for a review, see ref. 40). Although PLCgamma has been implicated in mitogenic as well as motogenic responses to other growth factors, the role of this phospholipase in FGF-induced cellular responses is unclear. Thus, cells expressing a mutant FGFR-1, lacking Y766, still respond to FGF stimulation with increased mitogenicity (41-42), although not as efficiently as cells expressing the wild type receptor (43). In agreement, Huang *et al.* (44), showed that the level of Raf and MAPK activities were reduced in FGF-stimulated L6 myoblasts expressing the Y766F mutant FGFR-1, indicating that PLCgamma via PKC modifies the phosphorylation status and thereby activity of Raf. Removal of the PLCgamma binding site in FGFR-1 does not affect migration towards FGF (35), or induction of urokinase (45), neuronal differentiation (46) and mesoderm induction in Xenopus oocytes (47) in response to FGF. Internalization of the Y766F mutant FGFR-1 expressed in rat myoblasts and in hematopoietic Ba/F3 cells appears however to be decreased (48). FGFR-4 induces only a weak tyrosine phosphorylation and activation of PLCgamma (18), but the implication for FGFR-4-mediated biological responses is unclear.

Src family kinases: The Src family of cytoplasmic kinases are potential substrates for FGF receptors, but there are conflicting reports on whether there is a direct association between Src and FGFR receptors. Zhan *et al.* (49) identified binding of Src to FGFR-1 and suggested that Src may be the responsible kinase mediating phosphorylation of cortactin in FGF-stimulated cells. Other attempts to identify such interactions failed (50) and moreover, dependent on the cell type, the outcome of FGF stimulation on Src kinase activity was shown to be stimulatory or inhibitory, in a PKC-dependent manner. Therefore, positively and negatively regulating serine and tyrosine phosphorylation of Src allows fine-tuning of kinase activity. Src is potentially involved in a variety of signal transduction cascades in FGF-stimulated cells. Thus, Src probably plays an important role in FGF-stimulated mitogenesis (51) and the transforming potential of v-Src is likely to be a subversion of the normal function of Src kinase activity in proliferation. Moreover, Src is a substrate for focal adhesion kinase (52) and could thereby play a role in migratory events. Src kinase activity appears to be critical in differentiation of neuronal cells (53) and endothelial cells (54). Such data are based both on the ectopic expression of dominant-negative Src, and of the transforming variant v-Src, as well as on the use of an apparently specific Src kinase inhibitor, PP1 (55).

FRS2: The FGF receptor substrate 2 (FRS2) is a recently characterized adaptor molecule of 90 kDa

(56). It is a substrate for FGFR-1 (43) as well as for the other FGF receptors (57). A family of FRS2 (also denoted SNT) related proteins has been described (58). The structure of FRS2 is unique, but its function mimics that of the insulin receptor substrate-1, which associates with the activated insulin receptor and becomes phosphorylated on multiple tyrosine residues, thereby presenting binding sites for other signal transduction molecules (for a review, see ref. 59). FRS2 lacks an SH2 domain, but contains a phosphotyrosine binding domain (PTB; 56) which mediates phosphotyrosine-independent interaction with amino acid residues 407-433 in FGFR-1, as examined in a yeast two-hybrid assay (60). Activation of FGFR-1 leads to tyrosine phosphorylation of FRS2 at several sites (56). Phosphorylation of Y196, Y306, Y349 and Y932 allow binding of Grb2, which is a small adaptor molecule involved in activation of the GTP-binding protein Ras (see below). In addition, phosphorylation of FRS2 at Y436 potentially allows binding of the tyrosine phosphatase SHP-2 (61). It is possible that FRS2 also mediates other types of interactions with signal transduction molecules downstream of FGF receptors. This far, FRS2 has been shown to be utilized by FGF and NGF receptors (56, 62-63), but not by other types of receptor tyrosine kinases, such as the PDGF and EGF receptors (43). This would possibly imply that FRS2 has a unique signaling function, however, Grb2 and SHP-2 operate downstream of both PDGF and EGF receptors. Whether FRS2 has a particular role in specific FGF receptor-mediated biological responses remain to be shown. FRS2 has been implicated in differentiation, *i.e.* neurite outgrowth of the rat phaeochromocytoma cell line PC12 (56) and in proliferation, but not in differentiation, i.e. formation of vessel-like structures in three-dimensional collagen gels, of a murine brain endothelial cell line (54). FRS2 has been proposed to correspond to the classical major FGFR-1 substrate denoted p90. Structurally distinct FGF receptor substrates with similar molecular masses have however been identified. One such example is a protein denoted 80K-H (64) the biological function of which remains to be determined.

Grb2: Growth factor receptors are known to couple to the Ras pathway via the small adaptor molecule Grb2, which exists in complex with the nucleotide exchange factor Sos. Sos catalyzes the exchange of GDP for GTP on Ras, thereby promoting Ras activation (6). All identified FGF receptors lack direct binding sites for Grb2 (Y-X-N; 65). Instead FGF-stimulation leads the association of the Grb2-Sos complex with either FRS2 or the adaptor protein Shc, both of which are phosphorylated on tyrosine by FGFR-1, and thereby present binding sites for Grb2 (see FRS2 paragraph above). The significance of the two alternative routes for Grb2-Sos mediated Ras activation in cells expressing FGFR-1 remains to be shown. Recent development within the field has shown that the Ras pathway branches out into several parallell signal transduction cascades, which might be differentially utilized in different biological responses. FGF induces sustained activation of the MAP kinases, Erk1 and Erk2, which are positioned downstream of Ras in the pathway. Sustained Erk activation has been implicated in differentiation, i.e. neurite outgrowth, of different types of neuronal cell lines (66).

PI3-K: Phosphatidylinositol 3' kinase (PI3-kinase), which phosphorylates the inositol ring in phosphatidylinositol at the 3' position, has been shown to be involved in signal transduction downstream of most if not all tyrosine kinases (see ref. 67 for a review). The FGF receptors lack optimal binding motifs for PI3-kinase and FGF-induced PI3-kinase activity is difficult to detect *in vitro*. *In vivo*, no or very little accumulation of the PI3-kinase metabolite, *e.g.* phosphatidylinositol 3,4,5 trisphosphate, is detected (68-69). PI3-kinase activity has been implicated in both mitogenesis and migration stimulated by a range of other growth factors. FGF receptors are capable of transducing both these responses, although migration towards FGF is less efficient than towards PDGF of cells expressing both types of receptors (70). In agreement, FGF stimulation fails to induce membrane ruffling, which appears to be an integral part of the migratory response (69-70).

SHP-2: The SH2 domain-containing phosphotyrosine phosphatase SHP-2 is critically involved in regulation of signal transduction downstream of tyrosine kinases (for a review on tyrosine phosphatases, see ref. 71). Several growth factor receptors mediate tyrosine phosphorylation and activation of SHP-2. In FGF-stimulated cells, SHP-2 is not detectably tyrosine phosphorylated, as compared with PDGF or EGF stimulation of the same cell type (43), but SHP-2 has been shown to associate with FRS2 in FGF stimulated cells (61, 72). Interestingly, a targeted deletion of the mouse SHP-2 gene, removing most of the N-terminal SH2 domain, showed that the kinetics of FGF-stimulation of the Ras pathway was dependent on SHP-2 (73). In agreement, interruption of complex-formation between FRS2 and SHP-2, by expressing a Y436F mutant FRS2 in PC12 cells (see above) allows transient but not sustained activation of MAP kinases Erk1 and 2, and leads to a decreased FGF-induced neurite outgrowth (61).

FAK: The cytoplasmic tyrosine kinase FAK is activated both in response to growth factor stimulation, such as FGF and PDGF, and in response to extracellular matrix (ECM)-mediated ligation of integrins (74). Interestingly, focal adhesions, which are focal points of contact between integrins and the extracellular matrix, appear to be signal transduction hot spots and contain not only FAK and a number of other signal transduction molecules, such as Src, but also FGF receptors (75). Thus, FGF receptors may have a particular role in integration of growth factor and integrin-mediated signal transduction.

Crk: The adaptor protein Crk is tyrosine phosphorylated by FGFR-1; a mutant FGFR-1 lacking the juxtamembrane tyrosine residue 463 fails to mediate Crk tyrosine phosphorylation. Cells expressing the Y463F FGFR-1 mutant are unable to proliferate in

**Table 1.** Summary of FGFR-1 substrates, different FGFR-1 sites required for their phosphorylation, and their potential roles in cellular responses to FGF.

| FGFR-1 substrate | FGFR-1 sites | Cellular response |
|---|---|---|
| Crk | Y463P | Proliferation* |
| PLCgamma | Y766P | Proliferation |
| FRS2 | JM | Proliferation, differentiation |
| Shc | Multiple | Proliferation, differentiation |
| Src | ? | Proliferation, differentiation, migration |
| 80KH | ? | ? |
| Shb | ? | Apoptosis |
| SHP-2 | via FRS2 | ? |
| ? | a.a. 759-774 | Migration |

*for references, see text

response to FGF-2, in agreement, overexpression of Crk or an SH2 domain mutant of Crk, abrogates FGF-2-induced DNA synthesis (76). Whether Crk is critical also for other FGF-mediated cellular responses remains to be shown.

Nck: The potential role of the adaptor protein Nck in FGF function remains to be established, but in a recent report, Nck was shown to participate in FGFR-1 signal transduction during mesoderm induction in Xenopus (77). The Nck SH2 domain binds to the same phosphotyrosine-containing motif as Crk, and therefore, Y463 is a potential binding site for Nck as well as for Crk. There is no detectable increase in Nck tyrosine phosphorylation in FGF-treated mammalian cells (76).

Shb: The adaptor protein Shb (78) is tyrosine phosphorylated in FGF stimulated endothelial cells (79). Shb has been implicated in apoptosis in a pathway that appears to also involve FAK.

Structurally unidentified: An 85 kDa serine kinase is tyrosine phosphorylated via FGFR-4, but apparently not via FGFR-1 or other growth factor receptors. p85 has been implicated in regulation of FGFR-4 kinase activity (37). Moreover, a novel 66-kDa phosphoprotein has been identified downstream of FGFR-3 (80).

Other signal transduction components: A number of signal transduction proteins and pathways are not efficiently utilized by FGF receptors. These include PI3-kinase, as discussed above, with consequences for the Akt/PKB and pp70 S6 kinase pathway which is regulated by PI3-kinase (for a review, see ref. 81). The Jak (Janus kinase)/Stat (Signal transducers and activators of transcription) pathway was originally identified in cytokine-treated cells, but has later been shown to operate in growth factor signal transduction as well. Jak's are cytoplasmic tyrosine kinases, which in cytokine-treated cells mediate phosphorylation of Stat molecules, which are a family of SH2 domain-containing transcription factors (for a review, see ref. 82). Stat tyrosine phosphorylation cannot be detected as a consequence of activation of FGFR-1 (Valgeirsdottir and Claesson-Welsh, unpublished) but activation of Stat1 has been implicated in FGFR-3 signal transduction (83). For MCF-7 breast cancer cells, FGF treatment is growth inhibitory under circumstances where Stat1 becomes tyrosine phosphorylated (84). The MCF-7 cells express several types of FGF receptors. The SH2 domain-containing GTPase activating protein of Ras (RasGAP) is differentially utilized by growth factor receptors and RasGAP appears not to be a substrate for FGF receptors (85).

### 5.3. FGF receptor signal transduction in biological responses

Mitogenesis: FGF receptors stimulate cell proliferation and they are implicated in cellular transformation in pathological processes (see below). It is likely that a number of parallell pathways contribute to FGFR-mediated mitogenic signal transduction. Thus, Crk, FRS2, Shc, PLCgamma and Src are all potentially contributing to FGFR-1 mediated mitogenesis (table 1). The degree of mitogenic signal transduction through FGFR-4 appears to be dependent on the cell type. Thus, Wang *et al.* (86), reported that FGFR-1, but not FGFR-4, ectopically expressed in BaF3 cells was capable of mediating proliferation in response to FGF. Wang and Goldfarb (11) later showed that a number of key amino acid residues present in FGFR-1, but not in FGFR-4, allowed the transduction of a strong mitogenic signal. The two tyrosine residues present in the kinase insert of FGFR-1, but absent in FGFR-4, belong to these key residues, as do FGFR-1-specific residues within the kinase domain.

FGF's as well as FGF receptors are known to be translocated to the nucleus, which appears to be a prerequisite for the mitogenic response (for further aspects on the potential function of FGF receptors in the nucleus, see see ref. 87).

Migration: FGFR-1 has been shown to be critical for cell migration *in vivo*. A dominant-negative version of FGF receptor-1 was introduced into oligodendrocyte progenitors *in vitro*. When transplanted into the brains of neonatal rats, mutant cells were unable to migrate and remained within the ventricles (88). Genetic approaches have also implicated an FGF receptor homolog in Drosophila melanogaster as critical for migration of cells to form tracheal branches during the development (89, 90; for a review, see 91). Furthermore, cell migration of sex myoblasts in C. elegans involves an FGF receptor-like tyrosine kinase (92). FGFR-1-mediated migration appears to be regulated by other signal transduction pathways than in cells migrating *e.g.* towards EGF or PDGF (70, 93-94), which require PI3-kinase and PLCgamma activities. Thus, activation of the Src pathway has been implicated in FGF-induced migration (95). None of the individual tyrosine phosphorylation sites in FGFR-1, such as the PLCgamma binding site Y766, are required for

migration (35, 96). Although PI3-kinase activation is very weakly or not at all stimulated via FGFR-1, FGF-driven migration can be abrogated using PI3-kinase inhibitors, such as wortmannin and Ly 294002 (35). It is likely that these inhibitors act also on other enzymes, such as $PLA_2$ (97) and lipid kinases other than PI3-kinase. A stretch of 15 amino acid residues C-terminal of the second tyrosine kinase domain is critical for FGFR-1-mediated migration (35). This stretch contains Y766, which however is dispensable. The role of this 15-amino acid residue stretch in regulation of migration via FGFR-1 remains to be determined.

Differentiation: FGF is a potent regulator of CNS development (98) and in tissue culture, FGF efficiently stimulates neurite outgrowth. There is as yet no consensus on a critical signal transduction pathway induced by FGF in neuronal cell differentiation and it is likely that several pathways are involved. Lin *et al.* (99) showed that neurite outgrowth of PC12 cells is dependent on the FGFR-1 juxtamembrane domain, which in a phosphotyrosine-independent manner mediates coupling to the Grb2 and SHP-2-binding adaptor FRS2 (see above). These data conform with the "whisper and shout" model, according to which neuronal differentiation depends on a potent, sustained activation of a family of serine/threonine kinases downstream of Ras, *i.e.* the MAP kinases (see 66). Signal transduction via FRS2 has been coupled to sustained MAP kinase activation (61). Dependent on the cell model, however, abrogation of FGF-stimulated sustained MAP kinase activity fails to affect neuronal cell differentiation *in vitro* (53). Similar data exist for endothelial cell differentiation; treatment of endothelial cells with the specific MEK inhibitor PD98059 attenuates acute and sustained MAP kinase activity, but still allows tube formation of the cells (54). Instead, activation of the serine/threonine kinase Raf, which is positioned immediately downstream of Ras, and activation of the cytoplasmic tyrosine kinase Src, appears to be necessary for differentiation in at least certain neuronal and endothelial cell models (53, 54). Axonal growth of neuronal cells has moreover been shown to be stimulated by cell adhesion, via cell adhesion molecule-mediated activation of FGF receptors (100, 101).

Epithelial cell differentiation is influenced by binding of an FGF family member (FGF-7; keratinocyte growth factor) produced by mesenchymal cells, to a splice variant of FGFR-2 expressed on epithelial cells of different kinds (102). The growth and differentiation of the skeleton is regulated by FGF's, and activating mutations in FGF receptors (103) leads to skeletal anomalies (see below). Myoblast differentiation on the other hand is inhibited by FGF (104) which has been described to involve a pertussis toxin-sensitive trimeric G-protein (105).

**5.4. FGF receptor function during embryonic development**

The FGF ligand and receptor family has a broad range of effects on cells *in vitro* and *in vivo*, as outlined above. During embryonal development, FGF receptor signal transduction appears to be critical for the development of a wide range of organs and for patterning of the embryo. Targeted inactivation of the FGFR-1 gene leads to embryonal death prior to gastrulation and the embryos display severe growth retardation (106). Yamaguchi *et al.* (107), generated FGFR-1-deficient embryos that remained capable of gastrulating and generating mesoderm, however, mesodermal patterning was aberrant. Thus, both Deng *et al.* (106) and Yamaguchi *et al.* (107) conclude that FGFR-1 is not needed for mesoderm formation per se. The early lethality of the FGFR-1 deficient embryos has prevented conclusions on FGFR-1 function during later stages of development and in the adult stage. To circumvent this problem, Deng *et al.* (108), created chimeric mouse embryos by injecting FGFR-1-deficient (R1-/-) ES cells into wild-type blastocysts. Embryos with a low contribution of R1-/- cells completed gastrulation and displayed malformations of the limb buds, partial duplication of the neural tube, tail distortion, and spina bifida, indicating that FGFR-1 plays a role in neurulation.

Targeted inactivation of the FGFR-2 gene leads to failure of early postimplantation development (109), by affecting visceral endoderm differentiation and the growth and maintenance of the inner cell mass. Werner *et al.* (110) examined the *in vivo* function of FGFR-2 by expressing a dominant-negative form of the receptor under the control of the keratin 10 promoter. The transgene was expressed specifically in the skin and disrupted the organization of epidermal keratinocytes, induced aberrant expression of keratin 6 and epidermal hyperthickening. This suggests that FGFR-2 is essential for keratinocyte differentiation. In a similar approach (111), dominant-negative FGFR-2 was expressed under control of the human surfactant protein C promoter, allowing expression of the transgene only in lung bud epithelium. Newborn mice expressing the transgene lacked normally developed lungs; instead they had two undifferentiated epithelial tubes that extended from the bifurcation of the trachea down to the diaphragm, a defect that resulted in perinatal death. Thus, the dominant-negative FGF receptor completely blocked airway branching and epithelial differentiation, without prohibiting outgrowth, establishing a specific role for FGF's in branching morphogenesis of the mammalian lung.

Targeted inactivation of the FGFR-3 gene (112) leads to severe and progressive bone dysplasia with enhanced and prolonged endochondral bone growth, indicating that FGFR-3 exerts negative regulation on chondrocyte proliferation. Celli *et al.* (113), expressed a secreted FGF receptor extracellular domain during mid-gestation in the mouse, which caused agenesis or severe dysgenesis of multiple organs and limb abnormalities reminiscent of human skeletal disorders associated with FGF receptor mutations (see below). These studies show the critical roles that FGF receptors have during skeletal development. Due to the

severe phenotypes of transgenic animals, it is plausible that FGF receptor functions in many cell types and processes are clouded.

## 6. FGF receptors in pathological processes

Tumorigenesis: FGF's have been shown to promote endothelial cell migration, proliferation and differentiation *in vitro* and FGF's appear to play a major role *in vivo* in regulation of blood vessel formation, angiogenesis (114). Angiogenesis is critical in different physiological processes, such as embryonal development, wound healing and ovulation. On the other hand, many pathological conditions depend on deregulated angiogenesis. One striking example is tumor progression and growth of metastases, which for many different forms of cancer is dependent on angiogenesis (see ref. 115). FGF's may also have a direct stimulatory effect on tumor cells and a number of FGF family members were identified as oncogenes (see ref. 3, for a review). It is probable that the mechanisms of action of various FGF family members in tumorigenesis differ. One mechanism of action includes overproduction of the factor resulting from activating viral integrations, leading to chronic receptor activation. Another potential molecular mechanism would involve mutations or gene rearrangements creating constitutively active receptors. Lorenzi *et al.* (116) have described a rearrangement of the FGFR-2 gene in rat osteosarcoma, in which the C-terminus of the receptors is replaced by a novel gene product, denoted FGFR activating gene, whose presence leads to increased receptor kinase activity and transformation.

Skeletal disorders: A number of skeletal disorders have been coupled to mutations in FGF receptor genes. Achondroplasia (dwarfism), the most common non-lethal skeletal disorder (117), depends on activating mutations in the FGFR-3 gene (118). Most people affected by achondroplasia carries mutation of Gly 380, located in the transmembrane domain, to Arg (119), which results in ligand-independent activation of the receptor tyrosine kinase (120). Hypochondroplasia, on the other hand has been coupled to a mutation of Asn540, located in the first tyrosine kinase domain, to Lys in FGFR-3 (121). A number of disorders have been described in which mutations in the extracellular domains of different FGF receptors result in premature closure of the skull sutures. In Crouzon syndrome anomalities are limited to the skull and face, which depend on any of multiple mutations in the extracellular domain of FGFR-2 (see ref. 117 for references). In Apert syndrome, FGFR-2 is mutated at a restricted number of sites; the extracellularly located Pro253 to Arg and Ser252 to Trp, which result in defect bone formation involving both the skull and the digits. More rarely occurring disorders involving premature closure of skull sutures, such as the Pfeiffer syndrome and the Jackson-Weiss syndrome, depend on mutations in the extracellular domain of FGFR-2, and occasionally, FGFR-1. The molecular basis for the anomalities resulting from different mutations in FGF receptor extracellular and intracellular domains is believed to be the creation of constitutively active receptors, perhaps through ligand-independent dimerization of mutated receptors (122; for a review, see ref. 123).

## 7. PERSPECTIVES

The FGF receptors clearly play important roles in the development and growth of the organism. Due to their apparent general expression in many different cell types, the central role of these receptors in differentiation of *e.g.* endothelial cells, neuronal cells, and osteogenic cells may not have been appreciated. For other receptor tyrosine kinases, a wealth of information about their *in vivo* function, at least during development, stems from analyses of targeted inactivation of their respective genes. Due to the very early lethality of mice in which *e.g.* the FGFR-1 gene has been inactivated, this approach has not been advantageous. Since the FGF receptors appear to be fundamental in so many different processes, the phenotypes of mice with more discrete inactivations of *e.g.* only parts of the receptor domains, could still be overwhelmingly difficult to sort out. To further analyze the role of the *in vivo* function of the FGF receptors during development and after birth, it will probably be necessary to combine discrete mutations of the FGF receptor tyrosine phosphorylation sites, with directed expression in specific tissues. With regard to the function of FGF receptors after birth, one should remember that the spectrum of expression and regulation of expression of the FGF receptors *in vivo*, in healthy tissues and in diseases, largely remain to be determined.

It is interesting to note that, in spite of the high degree of structural conservation between the FGF receptors (see 8, for a comparison of the primary sequences of the human FGF receptors) and the overlapping expression pattern, that the receptors do not appear to complement each other in mice in which one of the receptor genes has been inactivated. It is possible that expression is tightly regulated in a temporal and spatial pattern, which is not apparent in the types of analyses that have been performed this far. It is also possible that in spite of their high degree of sequence similarity, that receptors differ in their signal transduction properties. The adaptor FRS2 transduces signals from all four FGF receptors, but it is still possible that there are FGFR subtype specific adaptor proteins. It is also possible that the FGF receptors couple to signal transduction proteins expressed in a restricted manner, which allows transduction of cell specific functions, such as differentiation. Currently, the FGF/FGF receptor field is in a rapidly developing phase, since the quality and number of tissue culture and *in vivo* models are increasing. The techniques to identify and structurally characterize minute quantities of proteins expressed in specific cells, such as two-dimensional mapping of cellular proteins and mass spectrometry to deduce primary structures are developing rapidly and offer exciting perspectives for detailed identification of signal transduction molecules operating in the FGF receptor signaling cascades.

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