The canonical FGF-FGFR signaling system at the molecular level

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Abstract

Extracellular signaling molecules, among them the fibroblast growth factors (FGFs), enable cells to communicate with neighboring cells. Such signaling molecules that receive and transmit a signal require specific tyrosine kinase receptors located at the cell surface (fibroblast growth factor receptors, FGFRs). The binding of a signaling molecule to its specific receptor results in receptor dimerization and conformational changes in the cytoplasmic part of the receptor. The conformational changes lead to trans-autophosphorylation of the tyrosine kinase domains of the receptors and subsequently to induction of several downstream signaling pathways and expression of appropriate genes. The signaling pathways activated by FGFs control and coordinate cell behaviors such as cell division, migration, differentiation, and cell death. FGFs and their transmembrane receptors are widely distributed in different tissues and participate in fundamental processes during embryonic, fetal, and adult human life. The human FGF/FGFR family comprises 22 ligands and 4 high affinity receptors. In addition, FGFs bind to low affinity receptors, heparan sulfate proteoglycans at the cell surface. The availability of appropriate ligand/receptor pair, combined with the co-receptor, initiates signaling. Inappropriate FGF/FGFR signaling can cause skeletal disorders, primarily dwarfism, craniofacial malformation syndromes, mood disorders, metabolic disorders, and Kallman syndrome. In addition, aberrations in FGF/FGFR signaling have already been reported in several types of malignant diseases. Knowledge about the molecular mechanisms of FGF/FGFR activation and signaling is necessary to understand the basis of these diseases.

Keywords

Fibroblast growth factors • fibroblast growth factor receptors • cellular signaling • tissue homeostasis

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Introduction

The sophisticated organization of tissues in multicellular organisms requires communication between cells. They do it by producing regulatory proteins, so-called growth factors that are sent out to the cell exterior to instruct neighboring cells in a tissue. The receiver cells present specific tyrosine kinase receptors at their cell surface that recognize specific ligands. The fibroblast growth factors (FGFs) and FGF-receptors (FGFRs) constitute such a communication system.

FGFs and FGFRs have evolved by gene amplification and differentiation as a regulatory signaling system that is operative in invertebrates as well as in vertebrates [1, 2]. Their phylogenesis goes back to the time and events called the Cambrian Explosion, half a billion years ago [3, 4]. The long evolution of this growth factor/receptor system implies

that FGF-induced signaling controls fundamental processes during embryonic, fetal, and adult life [5, 6, 7, 8]. The FGF-FGFR axis of signaling is a key regulator of mesenchymal-epithelial communication and is thus required for organogenesis. The FGF-FGFR signaling also plays crucial roles in tissue homeostasis [9]. They represent fundamental mediators of such cell-to-cell communication. Therefore, imbalances in FGF-FGFR signaling cause a wide spectrum of pathological conditions, including different types of developmental and metabolic disorders as well as malignant diseases [10].

The pleiotropic biological action of FGFs is exerted through binding to and activation of four high affinity, tyrosine kinase, cell surface FGF receptors (FGFR1-4) and splicing variants for three of them (FGFR1-3). The active receptor further initiates various intracellular, downstream signaling pathways leading to positive and negative regulation of cell differentiation, proliferation, migration, and survival. The different biological responses

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induced by FGF-FGFR signaling seem to depend on the type of receiver cell.

Ligands for Fibroblast Growth Factor Receptors

The FGFRs are mainly activated by FGFs. In humans, the FGF family comprises 22 structurally related polypeptides (FGF1-23) [11]. Since human FGF15 and mouse FGF19 have not been identified and they have 15% amino acid identity, they have been suggested to be orthologs [12]. All FGFs are recognized by a highly conserved core of around 140 amino acids [13]. They exhibit a beta-trefoil structure [14, 15]. The beta-trefoil protein fold belongs to the evolutionary oldest protein folds and contains 12 beta-strands, which form six two-stranded beta-hairpins [16]. The FGF protein family represents one of the largest signaling families in vertebrates.

The mammalian FGFs can be divided into three subgroups based on their way of action: the paracrine (canonical) FGFs, the endocrine FGFs, and the intracrine (intracellular) FGFs (Fig. 1).

The paracrine (canonical) FGFs can additionally be divided into 5 subfamilies based on their evolutionary relationship: FGF1 subfamily (FGF1, FGF2), FGF4 subfamily

(FGF4, FGF5, FGF6), FGF7 subfamily (FGF3, FGF7, FGF10, FGF22), FGF8 subfamily (FGF8, FGF17, FGF18) and FGF9 subfamily (FGF9, FGF16, FGF20) [1]. FGF1 and FGF2 were the first FGFs discovered and their original names – acidic and basic FGFs (aFGF and bFGF) – are used in earlier publications [17]. FGF1 is the only FGF that activates all FGFRs and their splicing variants similarly [11]. Both FGF1 and FGF2 lack the classical secretory signal peptides and are released through a nonclassical ER-Golgi independent exocytic pathway as well as from damaged cells [18, 19]. The canonical FGFs also have relatively high affinity to heparan sulfate (HS) and heparin (Hp) [20]. Upon secretion they are trapped by binding to HS in the extracellular matrix and thus usually act in a paracrine and/or autocrine manner. Moreover, binding of paracrine FGFs to HS stabilizes the structure of the ligand as well as the receptor-ligand complex and enhances resistance to proteolysis [21, 22, 23].

FGF15/19, FGF21, and FGF23 have evolved as the endocrine FGF subgroup, and are specific to vertebrates [24]. These FGFs have lost their HS/Hp binding affinity. Therefore, the endocrine FGFs are not retained in the location where they are produced and can act as secreted hormones. Instead of HS, they require the presence of Klotho proteins (α Klotho and β Klotho) or Klotho-related proteins (KLPH) that act as co-receptors to form the active complex with FGFR. Endocrine FGF knockout mice have proved the important roles of these FGFs in regulating metabolism [25, 26].

The intracrine (intracellular) subgroup includes FGF11, FGF12, FGF13, and FGF14. Since they have a genomic structure and protein fold similar to the other FGFs, but lack the ability to bind and activate FGFRs, they have been called the homologue FGFs [17, 27, 28]. The intracellular FGFs lack signal sequence, so they are not secreted and remain inside the cell, mainly in the nucleus, and are able to interact with a separate set of target proteins than canonical and endocrine FGFs [12]. The biological function of the intracellular FGFs is not very well known, but some have been implicated in the regulation of voltage-gated sodium channels [29, 30]. The intracellular FGFs also interact with other intracellular proteins, such as, for example, FGF13, which interacts with microtubules [31].

It should be mentioned that although FGF1, FGF2, and FGF3 act in a paracrine or autocrine fashion, they have also been found in the nucleus as endogenous as well as exogenous proteins [32, 33, 34, 35, 36]. The nuclear function of FGF1 and 2 is not fully understood but nuclear FGF1 might play a role in regulation of the cell cycle, cell differentiation, survival, and apoptosis [37].

Figure 1. The FGF family. The mammalian FGFs can be divided into three subgroups based on their way of action. The intracrine FGFs are not secreted and remain inside the signaling cell. The paracrine and endocrine FGFs are secreted and bind to receiver cells but the endocrine FGFs mainly play roles in an endocrine manner via the blood circulation system.

Fibroblast growth factor receptors

In humans, there are four high affinity FGFRs. They belong to the receptor tyrosine kinase family. They are single-pass membrane receptors localized at the cell surface and are comprised of an extracellular, a transmembrane, and a cytosolic domain [38, 39].

In addition to the four high affinity FGF receptors at the cell surface, the paracrine FGFs also bind to heparan sulfate proteoglycans (HSPGs) that act as low affinity receptors at the cell surface. HSPGs exist as transmembrane or lipid-anchored proteins at the cell surface or as diffusible proteins in the extracellular environment. HSPGs are composed of a core protein covalently attached to one or more heparan sulfate chains. The heparan sulfate chains are linear polysaccharides of variably sulfated repeating disaccharide units [40]. HS/Hp act as powerful cofactors/receptors, as they are required for most secreted FGFs for signaling (see text below) [20, 41]. Even though FGFs have around 100-fold lower affinity for HSPGs than for FGFRs, HSPGs play important roles in FGF signaling, as co-receptors but also as storage reservoirs for the growth factors [21].

FGFR activation

FGFRs consist of two or three extracellular immunoglobulin (Ig)-like domains (Ig-like domain I-III), a transmembrane domain and an intracellular split tyrosine kinase domain (Fig. 2a). The Ig-like I domain and the serine-rich sequence located in the linker between Ig-like I and II domains, also called the acid box, play a role in autoinhibition of the receptor [38]. Ig-like II domain contains a positively charged HSPG binding site. Ig-like II and III domains and the region between these domains are forming the binding sites for FGFs [42]. FGFs differ in their FGFR binding affinities. FGFR1-3 are alternatively spliced in Ig-like domain III to generate FGFR1Ib or FGFR1Ic variants that determines FGF binding specificity [9]. FGFR1Ib splice variants are more likely to be expressed in epithelial tissue while FGFR1Ic splice variants are expressed in the mesenchymal tissue [9]. On the other hand, the ligands for FGFR1Ib isoforms are often secreted from mesenchymal cells whereas the ligands for FGFR1Ic isoforms are often secreted from epithelial cells. In this way FGFs and FGFRs enable an epithelial-mesenchymal crosstalk [43].

The FGF-FGFR interaction triggers receptor dimerization and activation of the tyrosine kinase domain of the receptors (Fig. 2b). The molecular mechanism that leads to FGFR activation is still somewhat unclear but several models have been proposed. Two ternary complex models of FGF, FGFR, and HS/Hp, the asymmetric and the symmetric model, are now recognized as the most probable. Both models are

based on crystallographic studies. In the asymmetric model, FGF:FGFR:Hp are thought to associate in a 2:2:1 complex. Hp facilitates FGFs dimerization bringing together the two FGFRs. In addition, HS/Hp binds to Ig-like domain II of only one of the FGFRs in the complex [44]. In the symmetric model, a FGF:FGFR:Hp 1:1:1 ternary complex recruits a second similar 1:1:1 complex via receptor dimerization forming a dimeric 2:2:2 complex. FGF has two receptor-binding sites (a primary and a secondary) for interaction with both FGFRs in the dimer. Each receptor binds to both ligands through the complementary primary and secondary ligand-binding sites located within Ig-like domains II and III and the linker between them. In addition, the two receptors interact with each other directly [45]. The role of HS/Hp in FGF/FGFR assembly varies between the two models. In the asymmetric model HS/Hp bridges the ligand-receptor complex by mediating growth factor dimerization while binding one of the two receptors. In the symmetric model, HS/Hp increases stability of the ligand-receptor complex and thus helps the dimerization but is not required for dimerization [46].

At present, most studies support the symmetric model of assembly, since the symmetric model fits with the finding that receptors can dimerize in the absence of ligand [47, 48]. The asymmetric model is still not excluded, and it has been proposed that both models might coexist [49]. The two models

Figure 2. A schematic presentation of the structure and the activation of FGFRs. (A) FGFRs consist of two or three extracellular immunoglobulin (Ig)-like domains (IgI-IgIII), a transmembrane domain and an intracellular split tyrosine kinase domain (TKI and TKII). An acid box is located between IgI and IgII. The IgII contains also a heparin binding domain. IgIII in FGFR1-3 is alternatively spliced to IgIIIb or IgIIIc variants. (B) Activation of FGFRs. FGF-FGFR interaction in the presence of heparan sulfate triggers receptor dimerization and activation of the tyrosine kinase domain of the receptors.

are based on crystallographic studies of different ligands/receptors. It is possible that differences in specificity of ligand binding motifs, distinct HS/Hp-binding sites for FGFs and FGFRs, and structural changes in FGFRs after ligand binding might explain the discrepancies between the two models [50].

Activation of FGFR signaling by the paracrine FGFs is regulated by the pattern of sulfation and length of the HS chains. Generally, the higher level of sulfation of HS chains and the longer chains facilitate the formation of the FGF:FGFR:HS/Hp complex. Shorter HS chains and lower sulfation levels have the same capability but with less efficiency [51].

Ligand binding and receptor dimerization lead to the activation of the tyrosine kinase domain in the intracellular part of the receptor. Each kinase domain phosphorylates tyrosine residues present in the intracellular part of the other receptor [48]. This process is called trans-autophosphorylation [52]. FGFR1 has seven tyrosine residues that are phosphorylated in a specific order (Y463, Y583, Y485, Y653, Y654, Y730, and Y766) [53, 54]. At the end of this strictly ordered trans-autophosphorylation cascade, the tyrosine kinase activity of the receptor is increased up to 1,000-fold [55].

Canonical FGFR signaling

The four classical, major signaling pathways activated by FGF/FGFRs include the RAS-MAPK (mitogen-activated protein kinase) pathway, the PI3K (phosphatidylinositol-4,5-bisphosphate 3-kinase)-AKT pathway, the PLC γ (phospholipase C γ)/PKC (protein kinase C) pathway, and the STAT (signal transducer and activator of transcription) pathway [11]. Direct recruitment of signaling proteins or adaptor proteins to the phosphorylated tyrosines in the kinase domain of the receptor mediates FGFR signaling. Proteins bind directly to the phosphorylated tyrosines via an SH2 (Src homology 2) domain or a PTB (phosphotyrosine binding) domain. Activated FGFR phosphorylates bound proteins, resulting in recruitment of additional signaling molecules. FGFR substrate 2 α (FRS2 α) is constitutively bound to FGFRs and is phosphorylated on tyrosine residues by the activated FGFR kinase leading to activation of RAS-MAPK and PI3K-AKT signaling pathways [11]. The phosphorylated tyrosines in FRS2 serve as docking sites, recruiting the SH2 domain-containing adaptors GRB2 (growth factor receptor-bound 2) and SHP2 (Src homology region 2 domain-containing phosphatase-2, also known as PTPN11; tyrosine-protein phosphatase non-receptor type 11) [56, 57]. This again leads to recruitment of the guanine nucleotide exchange factor, SOS (son of sevenless homology) [58]. SOS exchanges GDP for GTP in RAS, resulting in activation of RAF and distinct MAPKs including ERK1/2, p38, and JNK (c-Jun N-terminal kinase) [11]. Activation of the RAS-MAPK pathway leads to

phosphorylation and activation of E26 transformation-specific (ETS) transcription factors that regulate expression of target genes [11].

PI3K-AKT activation requires recruitment of the docking protein GAB1 (GRB2-associated-binding protein1) by GRB2 to the signaling complex at the plasma membrane [59]. PI3K then phosphorylates PIP2 (phosphatidylinositol-4,5-bisphosphate) generating PIP3 (phosphatidylinositol-3,4,5-trisphosphate) in the plasma membrane. This leads to translocation of PDK1 (phosphoinositide-dependent kinase 1) and AKT (also known as protein kinase B (PKB)) to the plasma membrane. Both PDK1 and AKT bind directly to PIP3 via their pleckstrin homology (PH) domains. AKT is then phosphorylated and activated by PDK1. The PI3K-AKT pathway activates and inhibits many target molecules. For example, AKT phosphorylates TSC2 (tuberous sclerosis complex 2) leading to its inactivation. This again leads to activation of the mTOR (mammalian target of rapamycin) complex [11, 60]. AKT also phosphorylates pro-apoptotic proteins such as BAD (Bcl-2 antagonist of cell death) and inhibits their function [61]. Thus AKT activation is often connected with cell survival.

To activate the PLC γ /PKC pathway, PLC γ has to bind directly, via its SH2 domain, to the phosphorylated Y766 in the FGFR C-terminal part. Once bound, PLC γ is activated by a phosphorylation event mediated by the FGFR tyrosine kinase [54]. Activated PLC γ catalyzes the hydrolysis of PIP2 to produce inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 production leads to an increase in intracellular Ca²⁺ resulting in activation of Ca²⁺/calmodulin-dependent kinase II (CaMKII). DAG recruits and activates PKC. Adaptor protein GRB14 also binds to phosphorylated Y766 and inhibits activation of PLC γ [11].

Transcription factors in the STAT family such as STAT1, STAT3, and STAT5 can also be phosphorylated by active FGFRs [11]. STAT3 binds directly to the phosphorylated Y677 of FGFR1 and is thought to be activated upon overexpression of FGFR1 or FGFR2 and in the presence of JAK2 (Janus kinase 2) and tyrosine-protein kinase Src [62].

In addition to the main signaling pathways, other signaling events downstream of FGFRs have been described. Interestingly, SHB (SH2 domain-containing adaptor protein B) has been shown to bind phosphorylated Y766 in FGFR1 and might contribute to activation of the RAS/MAPK pathway via FRS2 α phosphorylation [63]. It is not well understood how binding of SHB and PLC γ to the same phosphorylated tyrosine (Y766) in FGFR1 is regulated. The adaptor proteins, CRK and CRKL (CRK-like proto-oncogene), bind directly to phosphorylated Y463 in FGFR1 [64, 65]. FGFR activation and subsequent phosphorylation of FRS2 might lead to a release of CKS1 (cyclin-dependent kinase regulatory subunit 1) from FRS2. The release of CKS1 leads to ubiquitination and degradation of the cell cycle regulatory protein p27kip1

(cyclin-dependent kinase inhibitor 1B). p27kip1 is a negative regulator of the G1/S transition during cell cycle and therefore possibly mediates mitogenic signals from FGFR1 [66]. Activation of FGFRs can also lead to phosphorylation of focal adhesion kinase (FAK) [67].

Despite the fact that FGFRs usually activate only four main signaling pathways in addition to some signaling molecules, their activation influences a plethora of cellular functions in the human embryonic, fetal, and adult life.

Noncanonical FGFR signaling

Several atypical receptors and cell-surface proteins can interact with FGFRs/FGFs and might play a role in FGFR signaling. It has been shown that the transmembrane cysteine-rich FGF receptor (CFR) can bind FGF1, FGF2, FGF3, and FGF18 [68, 69, 70]. The role of CFR in FGFR signaling is not clear but it has been shown that binding of FGF18 to CFR enhanced FGF18-dependent cell proliferation. Integrins, receptors for extracellular matrix molecules, can also form signaling complexes with FGFR1 [71]. A complex of $\alpha\beta3$ integrin and FGFR1 seems to increase FGF2-induced ERK1/2 activation [72]. Moreover, receptors like the tyrosine kinase receptor, ephrin type-A receptor 4 (EphA4), and the G-protein coupled receptor, adenosine A2A, can also interact with FGFRs and influence activation and downstream signaling [73, 74].

It has also been demonstrated that FGFRs can be activated by noncanonical ligands such as cell adhesion molecules (CAMs) including L1, N-cadherin, integrins and neural cell adhesion molecules (NCAM) [75, 76]. N-cadherin is a transmembrane adhesion protein that has been shown to play a role in FGF-dependent and independent activation of FGFRs [77]. In cancer cells, N-cadherin has been shown to decrease ligand-induced internalization and lysosomal degradation of FGFR1, resulting in prolonged signaling [78]. Stimulation of FGFRs by NCAM has been shown to alter FGFR1 dynamics at the cell surface, leading to a changed cellular response compared to FGF2 stimulation [79, 80]. FGF2 induces dimerization and lysosomal degradation of FGFR1, while NCAM induces receptor recycling [79, 80]. NCAM-induced recycling of FGFR1 to the cell surface requires RAB11 and Src kinase and leads to sustained signaling [79]. NCAM can also form a complex with FGFR4 and N-cadherin [81].

Negative regulation of FGFRs

Many mechanisms exist to down-regulate FGFR signaling [87]. Tight regulation of signaling is necessary to maintain homeostasis in the body. The regulation of FGFR activity is

not fully understood. It has been shown that certain molecules such as Sprouty [82, 83] and related protein SPRED (Sprouty related with EVH1 [Ena/VASP homology 1]), SEF (similar expression to Fgf), ubiquitin ligase CBL [11], phosphatases SHP2 [56], PTPRG (protein tyrosine phosphatase receptor type G) [84], and MKP3 (mitogen-activated protein kinase phosphatase 3) [85] act at different steps in the signaling pathways and may down-regulate FGFR signaling. However, the main mechanism that regulates the duration and strength of receptor signaling is endocytosis and subsequently its lysosomal degradation [87].

Future perspective

The FGF/FGFR signaling system is complex and has to work precisely in order to prevent diseases such as dwarfism and cancer. Therefore, a better understanding of the signaling, and the regulation of the FGF/FGFR signaling, is needed. Questions such as how the FGF/FGFR family can generate a plethora of biological effects in different receiver cells, using the same pathways, should be explored. Moreover, research on possible ways to manipulate the FGF/FGFR signaling system (such as inhibitors, etc.) should have the highest priority. Inappropriate signaling must be controlled to stop diseases [86].

Abbreviations

aFGF - acidic fibroblast growth factor, **BAD** - Bcl-2 antagonist of cell death, **bFGF** - basic fibroblast growth factor, **CAM** - cell adhesion molecule, **CFR** - cysteine-rich FGF receptor, **Cks1** - cyclin-dependent kinase regulatory subunit 1, **CRKL** - CRK-like proto-oncogene, **DAG** - diacylglycerol, **EphA4** - ephrin type-A receptor 4, **ER** - endoplasmic reticulum, **ERK** - extracellular signal-regulated kinase, **ETS** - E26 transformation-specific, **FAK** - focal adhesion kinase, **FGF** - fibroblast growth factor, **FGFR** - fibroblast growth factor receptor, **FRS2** - FGFR substrate 2, **GAB1** - GRB2-associated-binding protein1, **GRB2** - growth factor receptor-bound 2, **Hp** - heparin, **HS** - heparan sulfate, **HSPG** - heparan sulfate proteoglycan, **Ig** - immunoglobulin, **IP₃** - inositol triphosphate, **JAK** - Janus kinase, **JNK** - c-Jun N-terminal kinase, **KLPH** - Klotho-LPH related protein, **MAPK** - mitogen-activated protein kinase, **MEK** - mitogen-activated protein kinase kinase, **MKP3** - MAPK phosphatase 3, **mTOR** - mammalian target of rapamycin, **NCAM** - neural cell adhesion molecules, **p27kip1** - cyclin-dependent kinase inhibitor 1B, **PDK1** - phosphoinositide-dependent kinase 1, **PI3K** - phosphatidylinositol-4,5-bisphosphate 3-kinase, **PIP₂** - Phosphatidylinositol-3,4-bisphosphate, **PIP₃** - phosphatidylinositol-3,4,5-trisphosphate, **PKB** - protein

kinase B, **PKC** - protein kinase C, **PLC γ** - phospholipase C γ , **PLSD-SD** - platyspondylic lethal skeletal dysplasia, San Diego type, **PTB** - phosphotyrosine binding, **PTPN** - protein tyrosine phosphatase nonreceptor type, **PTPR** - protein tyrosine phosphatase receptor type, **RSK** - ribosomal s6 kinase, **SEF** - similar expression to *Fgf*, **SH** - Src homology, **SHB** - SH2 domain-containing adaptor protein B, **SHP** - Src homology region 2 domain-containing phosphatase, **SOS** - son of sevenless homology, **SPRED** - sprouty related with EVH1 (Ena/VASP homology 1), **SPRY** - sprout, **STAT** - signal transducer and activator of transcription, **TSC2** - tuberous sclerosis complex 2.

Authors' Contributions

P.S.: writing the manuscript, literature review, designing the figures, final proofreading, and approval of the version for publication; **E.M.H.:** writing the manuscript, designing the figures, final proofreading, and approval of the version for publication; **A.W.:** research concept and design, literature review, final proofreading, and approval of the version for publication.

Conflict of Interest

The authors have no potential conflicts of interest to declare.

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