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Role of fibroblast growth factors in elicitation of cell responses

C. Laestander and W. Engström

Department of Biomedical Sciences and Veterinary Public Health, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, Uppsala, Sweden

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Abstract

Fibroblast growth factors (FGFs) are signalling peptides that control important cell processes such as proliferation, differentiation, migration, adhesion and survival. Through binding to different types of receptor on the cell surface, these peptides can have different effects on a target cell, the effect achieved depending on many features. Thus, each of the known FGFs elicits specific biological responses. FGF receptors (FGFR 1–5) initiate diverse intracellular pathways, which in turn lead to a variety of results. FGFs also bind the range of FGFRs with a series of affinities and each type of cells expresses FGFRs in different qualitative and quantitative patterns, which also affect responses. To summarize, cell response to binding of an FGF ligand depends on type of FGF, FGF receptor and target cell, all interacting in concert. This review aims to examine properties of the FGF family and its members receptors. It also aims to summarize features of intracellular signalling and highlight differential effects of the various FGFs in different circumstances.

Introduction

Fibroblast growth factors (FGFs) are secreted polypeptide ligands that can act in either paracrine or endocrine fashions to stimulate or uphold particular cell functions required for metabolism, tissue homeostasis and development. The mammalian FGF family consists of 23 related polypeptides. Eighteen of these have been grouped into five paracrine-acting groups and one endocrine-acting group, on the basis of amino acid sequence and structural analysis (reviewed in 1). These molecules have been studied for many years, but their functions are yet to be fully understood. The first FGFs were isolated from bovine brain tissue in the late 1970s (2,3) and since then scientists have been working to understand their effects, as each member elicits different biological responses in different cells (reviewed in 4–6). It has been demonstrated that FGFs are involved in processes including cell proliferation, migration, differentiation, adhesion and survival. Targets of the FGFs are mainly two classes of receptor, the tyrosine kinase receptor family (7) and co-receptors heparin sulphate proteoglycans (5). FGF–tyrosine kinase receptor interaction is not a straightforward process; different FGFs have different affinities for different receptors. Moreover, activation of multiple receptors affects subsequent downstream activity. Full understanding of fibroblast growth factor–receptor interactions however, will help comprehension of how any specific biological response is achieved. The variety of FGF affinities for the receptors, lead in turn to different effects on the cell (8).

Fibroblast growth factors and their receptors have important functions in development, metabolism, angiogenesis and tumourigenesis, and some also play an important role in embryogenesis, organ development and wound healing (1); thus it is essential to understand their wide-ranging effects. In particular, the role of FGFs in eliciting PKC gamma, Ras-MAP kinase (Ras-MAPK) and Src-mediated pathways (cf below) is crucial in tumourigenesis, and further understanding of this may be a further step in combatting cancer. Here, the members of the fibroblast growth factor family will be described with their receptors, and how interactions between them can lead to a plethora of cellular responses.

The FGFs

Fibroblast growth factors are secreted polypeptide ligands that bind to a variety of receptors located on the surface of target cells, of many tissues. Structure of

Correspondence: W. Engström, Department of Biomedical Sciences and Veterinary Public Health, Faculty of Veterinary Medicine, Swedish University of Agricultural Sciences, PO Box 7028, 75007 Uppsala, Sweden. Tel.: +46-18-671000; Fax: +46-18-673532; E-mail: wilhelm.engstrom@slu.se

FGFs has been revealed by high-resolution X-ray diffraction as well as by nuclear magnetic resonance imaging (9) and shows that the archetypal structure consists of 12 strands, linked together forming a 3-fold symmetrical structure of beta sheets (10). All members of the FGF family have the same core of around 140 amino acids. Twenty-two types of FGF have been recognized in humans, while FGF-24 has been identified only in zebrafish embryos (11). FGF-15 occurs in mice and is closely related to human FGF-19 (12) (Fig. 1).

First FGFs to be discovered were FGF 1 and FGF 2, originally called acidic and basic FGFs (aFGF and bFGF) (2,3,13). These were found to have significant effects on cell migration, proliferation, differentiation and angiogenesis. Also, FGFs are sometimes referred to as heparin-binding growth factors, as they have high affinity for heparin and heparan sulphate. Binding of FGFs to different glucosaminoglycans (such as heparan sulphate) makes FGFs resistant to degradation, hence they can remain as a reservoir in the extracellular matrix.

Fibroblast growth factors are commonly divided into subgroups (14) although members of each family have similar qualities. First, FGFs11–FGF15 are not always included in the FGF family; this is because unlike other FGFs, they do not have the ability to bind to and activate FGF receptors. Instead, they are called homologous factors, acknowledging that their genomic structure highly resembles FGFs. FGF1 and FGF2 (aFGF and bFGF) are members of the FGF1 subfamily. They were the first to be discovered, yet their physiological roles are still unclear. It is likely that they affect vascular tone or reduce blood pressure (15). However, it is known that FGF2 has angiogenic properties, promotes proliferation and migration, and inhibits apoptosis of endothelial cells (16). The FGF4 subfamily includes FGF4, FGF5 and FGF6. FGF4 is particularly important during organ development. It affects processes such as trophoblast proliferation as well as limb and heart valve development, while FGF5 is an important factor in hair growth cycle regulation (17). FGF3, FGF7, FGF10 and FGF22 are members of the FGF7 subfamily (14). FGF3 is crucial in development of inner ear structure, while FGF7 is central to kidney development; it is sometimes referred to as keratinocyte growth factor (KGF) (14). FGF22, FGF7 and FGF10 are presynaptic organizers involved in vesicle clustering and neurite branching (18). A further subfamily includes FGF8, FGF17 and FGF18. FGF8 is a significant player in limb, ear, eye and brain development and together with FGF17, FGF8 also produces effects on forebrain development (19); FGF18 is required for correct development of bone. The FGF9 subfamily consists of FGF9, FGF16 and FGF20. FGF9 upregulates proliferation of mesenchymal tissues, and initiates secretion of ligands for FGF3, FGF7, FGF10 and FGF22 subgroups. Accordingly, FGF9 knockout leads to reduced production of certain ligands and reduced mesenchymal–epithelial signalling (20).

FGF19, FGF21 and FGF23 belong to a subfamily normally referred to as endocrine FGF ligands (21). One property that distinguishes these from other FGFs is that they need the presence of two types of klotho-protein to form the FGF–receptor complex in tissue; this is a consequence of their low affinity to heparin sulphate. The two types of klotho protein (αKlotho and βKlotho) (22–28) are selectively used as co-receptors by FGF19 subfamily members (21). FGF19 stimulates bile acid synthesis and initiates oxidation of fatty acids. FGF21 causes a fasting response by stimulating glucose uptake in adipocytes, thus reducing levels of glucose in the bloodstream. Injections of FGF21 to diabetic and obese mice leads to reduced blood concentration of insulin,

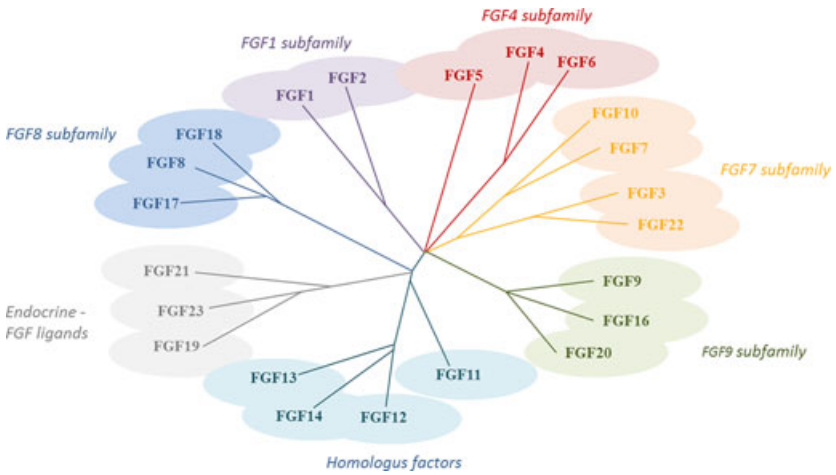

Figure 1. Fibroblast growth factor family tree. Modified after (14).

glucagon, glucose and triglycerides. Continuous injection of FGF21 to obese mice reduces their body weight in the order of 20%. Finally, FGF23 is an important vitamin D regulator (21).

FGF receptors

The FGFs bind to two different classes of receptor and it is important to appreciate that many different ligands can activate the same receptor. FGFs bind simultaneously to low-affinity, heparin sulphate proteoglycans (29) and high-affinity FGF receptors. High-affinity receptors consist of one extracellular component containing between one and three Ig-SF domains, one transmembrane domain and one intracellular tyrosine kinase domain. High-affinity receptors have one unique structure that distinguishes them from others namely an 'acid box' consisting of eight acidic residues located between the first and second Ig-SF domains. First Ig-SF domain and the acid box probably contribute to autoinhibition, while domains 2 and 3 are FGF ligand-binding sites (6).

Five signalling FGF receptors (expressed as multiple splice variants) have been identified to date (30,31), but it is believed that there are others hitherto undiscovered. The common name for these receptors is FGFR 1–5 and they are coded for by separate genes; differential splicing gives rise to multiple alternative forms of the receptors. For example, splicing of the gene that codes for Ig-like domain 3 causes variants with different specificities for the binding site. Different isoforms of the receptors are expressed in different organs, for example, FGFR3IIIb is mainly located in epithelial tissues and IIIc forms are preferentially expressed in tissues of mesenchymal origin. Both isoforms have separate ligands, which only bind to the specific receptor (30). This means that mesenchymal cells produce ligands that only activate IIIc receptor, achieving a paracrine signal (30).

Fibroblast growth factors receptors are unevenly distributed between body tissues and patterns in which they occur are specific to each tissue. Studies have shown that FGFR-1 is expressed in skin, calvarial bones and growth plates and at high levels in the foetal brain (32,33). FGFR-2 is also found in the brain, growth plates and calvarial bone, but also in liver, lungs, intestine and kidneys (34,35). FGFR-3 also is expressed in brain, growth plates and calvarial bone as well as in lung, kidney and intestines (36,37). FGFR-4 can be found in lungs, kidney, liver, pancreas, intestine, foetal adrenals, spleen and striated muscle (38).

Low-affinity FGF receptors are present on surfaces of most cells; they can also be called heparan sulphate proteoglycans (HSPGs). Heparan sulphate is a linear glycan, containing disaccharide unit repeats of glucuronic acid and *N*-substituted glucosamine (39,40). *N*-sulphated molecules 5–10 disaccharide units in length with various modifications alternate along the glycan chain, with *N*-acetylated stretches that are mainly unaltered (39,40). These changes in *N*-sulphated regions include C5 epimerization of glucuronic acid 3-0- as well as 6-0-sulphation of *N*-sulphated glucosamine (39,40); FGFs mainly bind to these modified sulphated domains (40). Interestingly, alteration of heparan sulphate by sulphation and epimerization is controlled in a tissue-specific fashion, contributing to a better finetuning of FGF activity.

Thus, HSPGs have two different very important functions. First, is that binding FGF to an HSPG protects the growth factor from degradation, thus it can act as an extracellular buffer. Secondly, HSPGs are involved in complex formation between FGFs and their FGFR. Binding FGFs to their respective receptors induces dimerization and formation of a ternary complex containing FGF, FGFR and heparan sulphate. However, the primary importance of HSPGs appear to be to maintain functional role of paracrine FGF activity, as they immobilize FGFs in the extracellular matrix neighbouring their site of secretion; this limits their activity on cells in the immediate proximity. Conversely, as endocrine FGFs have a poor affinity to HSPGs, they can easily enter the bloodstream and act at a distance from their site of synthesis. In contrast to paracrine FGFs that depend heavily on HSPGs, endocrine FGFs instead require Klotho co-receptors to become biologically active (22–28).

FGF signalling

Signalling *via* activation of an FGFR requires receptor dimerization, a prerequisite for moving intracellular kinases closer to each other, initiating onset of different intracellular signalling pathways that lead to adjustment of gene expression (41). Formation of receptor dimers activates their intracellular tyrosine kinases, allowing them to transphosphorylate tyrosine residues on each dimer of the receptor (42). These residues can act as binding sites for signalling molecules containing Src homology-2 or phosphotyrosine-binding domains. Signalling molecules are often bound to different docking proteins and activated receptor kinases phosphorylate and activate their intracellular substrates. Most prominent of these are FGFR substrate 2a (FRS2a) (43) and phospholipase Cy1 (PLCy1) (44,45). Upon activation, FRS2a initiates downstream signalling *via* one of two pathways – Ras-MAPK or PI3K-AKT (43–45) (Fig. 2).

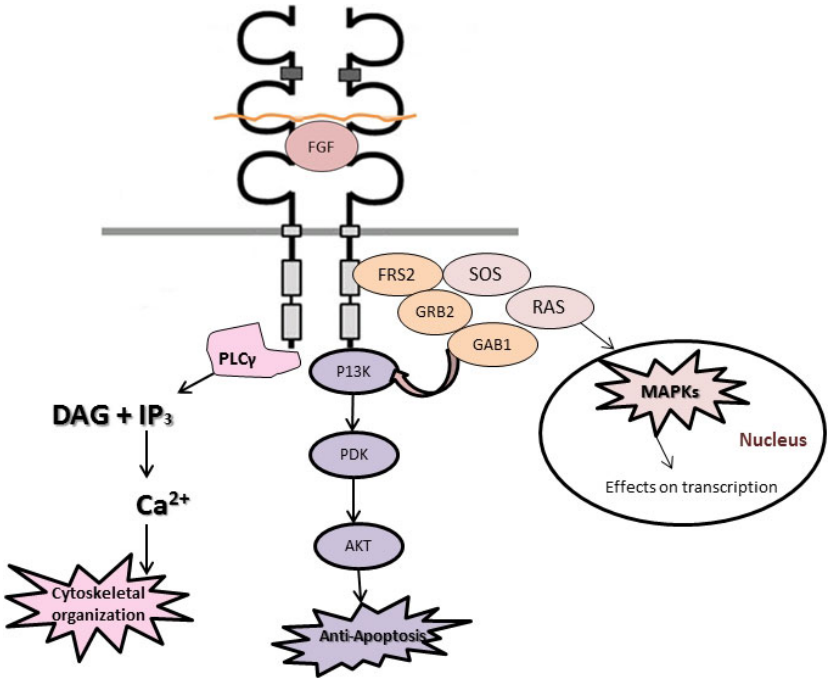

Figure 2. Overview of fibroblast growth factor (FGF) receptor signalling.

Ras-MAPK is one of the best studied signalling pathways. It involves docking protein FRS2α, which becomes activated by tyrosine residues on the activated FGF receptor. FRS2α is the core of a complex formed by adaptor Grb2, tyrosine phosphatase Shp2 and docking protein GAB1 (46). To Grb2 binds guanine nucleotide exchange factor SOS, which in turn activates the Ras-MAP kinase. The MAP kinases are regulatory proteins that affect different kinases and transcription factors and thereby regulate target genes. Effects gained by stimulating Ras-MAP kinase are mainly mitogenic (6).

GAB1 also leads to activation of a further important pathway, by activation of PI-3 kinase. P-I-3 activates PDK, which in turn activates ATK/PKB (43). Effects of ATK are anti-apoptotic. Activated FGF receptors can also lead to hydrolysis of PtdIns and activation of PDK and ATK. FGFs can act on intracellular calcium levels through recruitment of Src homology-2 domain of PLCγ to the receptor. Activation of PLCγ allows it to hydrolyse PIP2, which leads to formation of diaglycerol and IP3. Effect of diaglycerol and Ins P3 is release of calcium and activation of calcium-dependent protein kinases, which affect cytoskeletal organization (41).

In addition, Src plays an unexpected role in receptor signalling. FGFR activation stimulates clathrin-mediated endocytosis. FGF exposure increases number of CCPs, including those undergoing endocytosis, and this is mediated by Src and its phosphorylation target Eps8. Eps8 interacts with clathrin-mediated endocytosis machinery and depletion of Eps8 inhibits FGFR signalling and immediate Erk signalling. Once internalized, FGFR passes through peripheral early endosomes on its way to recycling, *via* a Src- and Eps8-dependent mechanism (45).

Diversity in cellular responses

Effects achieved by these signalling cascades are not shared through all cell types. There appears to be an apparently redundant number of receptors and ligands. Moreover, cross-binding activity between ligands and receptors further complicates the picture and makes it difficult to use gene manipulation as a way forward. The MAPK pathway is always seen as a response to FGF ligand binding, while others, such as AKT activation, differ between cell types (47). Moreover, FGF signalling is affected by tissue-specific HSPGs that can either amplify or block FGFR activation. Varying effects also seem to depend on cell state of differentiation, receptor phenotype and presence of other growth factors or cytokines (47).

A further explanation for the variety between cell responses is that intracellular signalling pathways are influenced by a number of regulators. Examples of these are the Sproty proteins, MAPK phosphatase 3 and SEF, which are inhibitory molecules that either bind to different molecules (Sproty protein) and inhibit them, or act as regulatory feedback [SEF has similar expression to FGF (48)]. There are also excitatory molecules, which

upregulate signalling pathways such as FLRT, a family of transmembrane proteins (14).

Finally, responses to FGFs are in some instances, concentration dependent. One study on lens epithelial cells has indicated that low concentrations (150 pg/ml) of FGF-1 and FGF-2 initiated proliferation; as higher concentrations (3 ng/ml) of FGF were added, the cells started to migrate. To achieve cell differentiation, an even higher concentration (40 ng/ml) of FGF was required (49). It also seems that time interval over which cells are exposed to FGFs matters. Proliferation and migration were achieved within 24 h, while their differentiation was seen after 4 days. It was also shown that proliferation and migration could occur simultaneously and higher concentration lead to more pronounced response.

Embryogenesis

Fibroblast growth factors and their receptors have long been implicated in control of embryogenesis (50). In the mouse, FGF-4 is the first FGF to be expressed, from the 4-cell stage and onwards (51); FGF-4 knockout mice have been shown to suffer peri-implantation lethality at stages E4–5. Development progresses normally up to the blastocyst stage, but they die immediately after implantation due to ICM (inner cell mass) defect formation (52). Moreover, along with FGF-4 expression, the fifth cell division appears to be crucial for blastocyst formation (53). It is believed that FGF-4 effects are mediated *via* FGFR2 as this receptor is the first to be expressed (54). Indeed, FGFR2 knockout mice suffer from early embryonic lethality comparable to FGF-4 KO mice. In both cases, cause of death seems to be deficient formation and maintenance of the ICM (54).

Three receptors, FGFR1, 3 and 4, are all expressed in early development (54). Deletion of FGFR1 is lethal at stages E7.5–E9.5, as it prevents normal gastrulation and inhibits mesoderm/endoderm differentiation (55). FGFR 3 knockout mice survive, but display skeletal malformations, whereas FGFR4 knockout mice appear to have normal development (56).

Use of murine embryonic stem cells has shed further light on the role of FGFs in development. Homozygous FGF4 −/− ES cells proliferate normally when grown in culture. They maintain pluripotency, but their differentiated progeny have a much reduced lifespan. By blocking FGFR1 and FGFR3 in ES cells, it has been shown that these cells continue to proliferate and maintain their state of pluripotency, suggesting that this particular signalling mechanism does not initiate differentiation itself, but rather exerts a permissive effect (57). FGFR2, on the other hand, stimulates proliferation of ES cells from the 5th cell division to ICM formation, whereas FGFR1 and 3 studied in epiblast EC cells affected germ layer specification (58). Thus, it appears probable that FGF signalling in embryos has clear stage-specific effects.

Teratocarcinoma

Different biological responses to FGF exposure can, in some instances, be concentration-dependent, as for example, in the human teratocarcinoma cell line Tera 2 (59,60). These cells have been extensively used as a target line for FGFs as they express four FGF receptors (FGFR1-4); the cells have been shown to respond differently to changes in concentration of FGFs. Cell population multiplication was stimulated by addition of 1–10 ng bFGF/ml, but bFGF-effect was abrogated by addition of protamine sulphate. When high concentrations of bFGF were added, preferential effects on cell locomotion were observed. One hundred nanograms bFGF/ml stimulated cell movement, but only exerted a marginal effect on cell proliferationion (61). Moreover, when effects of four other members of the fibroblast growth factor family, FGF-10, FGF-16, FGF-17 and FGF-18, were examined, it was found that all four enhanced survival levels of Tera-2 cells by counteracting apoptosis at concentrations between 1–10 ng/ml. When higher concentrations of any of the four FGFs were added, preferential effects on cell motility were observed (4). Greater difference were revealed when effects of FGF-8, FGF-9 and KGF on Tera 2 cells were examined. It was found that each of these factors promoted Tera 2 cell proliferation, albeit with different efficacy. Whereas dramatic effects on cell numbers were observed after addition of 1–10 μg FGF-9/ml, a lower effect could be achieved by FGF-8 or KGF. In contrast, KGF expressed the most potent effect on cell locomotion at higher concentration (100 μg/ml). Even though high concentrations of FGF-8 and FGF-9 stimulated cell movement, this effect was substantially lower than that of KGF (62). Likewise, FGF-19, as well as FGF-20, promoted Tera 2 cell proliferation. Whereas FGF-20 promoted cell population expansion at low doses, FGF-19 was required at high doses to achieve a comparable effect. Moreover, FGF-19 did not significantly stimulate cell locomotion, while FGF-20 promoted motility at high doses (63). In one recent study, it was demonstrated that FGF23 acted in a similar way to FGF19, whereas FGF24 was virtually without effect on proliferation or expansion of Tera 2 cell numbers (64).

Oligodendrocytes

A number of studies has shown that activation of MAPK leads to proliferation of oligodendrocyte

precursors and endothelial cells. Inhibition of PLCγ, on the other hand, did not affect oligodendrocytes (5). Whereas ligand activation of the MAPK pathway has been shown to stimulate proliferation in other cell types, investigations of chondrocytes has shown that MAPK activation, unlike PLCγ and PI-3, leads to interruption of the cell cycle. Effects of FGF2 on development of oligodendrocytes have been studied and it has been found that FGF2 induces different, stage-specific responses in the cells (65,66). One experiment on oligodendrocytes has shown that FGF-2 induced proliferation in the cells while FGFs-8, -9 and -17 had no effect, no matter how high the concentration was or how long the duration. This difference in response is due to FGF-2 rapidly activating the MAPK pathway, while FGF-8, -9 and -17 had much weaker and slower effects on it. Consequences of FGF-2 on differentiation have also been closely studied (66) the conclusions being that FGF-2 inhibits differentiation of oligodendrocyte progenitors. However, further studies showed that FGF-9, unlike FGF-2, did not block oligodendrocyte differentiation.

Effects of FGFs on mature oligodendrocytes have also been examined: FGF-2 induces multiple responses in mature cell line such as elongation, inhibition of myelin protein synthesis and re-entering the cell cycle. These were studied after FGF-8, -9, -17 and -18 addition, when it was shown that only FGF-2 had any cell cycle effect. FGFs-9 and -18 provided the same results as FGF-2 on oligodendrocyte differentiation, but treatment with FGF-9 and -18 did not result in loss of myelin-like membranes, as observed in FGF-2 treated cells. FGF-8 and FGF-17 did not increase cell size, which suggests that these cells' response distinguished between different FGFs (66).

Expression patterns of the receptors may also play a certain role in response of FGF signalling. It has been confirmed that cells express different FGFRs during different developmental phases. FGFR expression pattern changes dramatically as cells differentiate. An example of this is FGFR expression in oligodendrocytes during differentiation where FGFR1 is expressed throughout development; while FGFR2 is more prominent during terminal differentiation, FGFR3 is downregulated at the end of oligodendrocyte differentiation (65,66).

It is also possible that different FGF-FGFR interactions lead to different responses. This theory has been tested by adding FGFR inhibitors to oligodendrocytes during different phases of development. Results indicated that during the progenitor phase, activation of FGFR-1 only (by FGF-2) (67,68) induced proliferation, while inhibition of proliferation required FGF-8, FGF-17 or FGF-18, bound to FGFR-3. When the same experiment was performed on differentiated oligodendrocytes

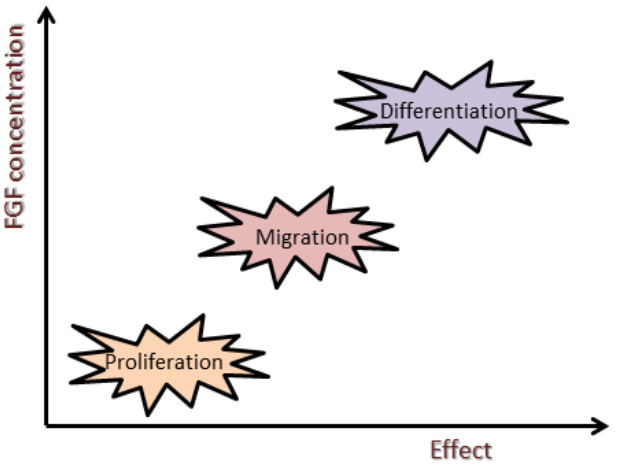

Figure 3. Relationship between concentration and biological effects.

it revealed that activation of FGFR-1 was required for cells to re-enter the cell cycle, not FGFR-3. Cell elongation required activation of FGFR-2 by FGF-2, FGF-9 and FGF-18 (67,68) (Fig. 3).

Concluding remarks

Fibroblast growth factors play a pivotal role in regulation of key developmental processes. There is mounting evidence for importance of correct spatial and temporal regulation of expression of FGFs and their receptors. This review has highlighted differential effects of the 23 hitherto discovered mammalian members of the FGF family. These ligands interact with a family of tyrosine kinase receptors that can elicit a variety of biological responses. We conclude that different FGFs do not necessarily have the same effect on one type of cell, as different FGFs exert different responses. Moreover, a variety of intracellular pathways is activated to differing extents depending on which ligand initiates activation.

One particular type of FGF can also give rise to varied responses at different stages of development. The picture is further complicated by different cellular expression patterns of tyrosine kinase receptors during different phases of development. Finally, response to FGF activation may depend on availability of substrates and other intracellular regulators. A key question is then how these different responses are generated and more specifically how very similar elicitations can lead to changes in secondary patterns of gene expression, which direct intracellular signal transduction to generate any particular cell response.

One obvious result is that even though FGF and FGFR families are large, there does not appear to be any functional redundancy in the system. This issue has

been addressed in studies where one FGF has been deleted in mouse knockout experiments and other FGFs have been increased to compensate for the lacking factor. A good example is knockout mice lacking FGF10 that are characterized by mammary gland hypoplasia, salivary gland aplasia and pulmonary agenesis (69–71). In contrast, knockout of the closely related FGF7 fails in kidney development – a phenotype quite distinct from FGF10 knockout mice (72). Even though FGF7 and FGF10 are branching morphogens (73), structurally very similar, it seems clear that they cannot substitute for each other. Moreover, FGF7 and FGF22 are both involved in development of presynaptic terminals of hippocampal pyramidal neurons. However, these two ligands affect formation of different synapses, and it has been conclusively demonstrated that they cannot replace each other (74).

Vast amounts of genetic data on mammalian development have pointed at the importance of a finely orchestrated role for the FGF family in normal development. *FGF*s are part of an extended gene family including *TGFbeta/BMP*s, *Hedgehog*, *Notch* and *Wnt* (41). Albeit structurally different, they all combine their efforts to steer undifferentiated cells toward lineage determination, proliferation, locomotion and differentiation. This concept also suggests that there is a role for crosstalk between activation of FGF-receptors and other signalling pathways. There is mounting evidence that FGF-activation may activate or repress other signalling pathways such as TGFbeta/BMP, IGF, IHH/PTHIH and Notch (5). However, the best-characterized developmental crosstalk is that between FGF and Wnt. In such distinct areas as trachea development in Drosophila, mesoderm induction in Xenopus, and CNS, kidney and tooth development in lower mammals (75) crosstalk between FGFs and Wnt can lead to convergence or divergence of signalling routes activated by each pathway. Moreover, it has been suggested that activation of one signal can confer competence of another (76).

However, still puzzling questions remain to be explored. Interplay between endocrine and paracrine FGFs is an area that has recently attracted some attention. Some clues have emerged from studies of adipose tissue where postprandial induction of FGF1 and FGF21 link back to peroxisome proliferator-activated receptor gamma (PPAR gamma) (77,78). FGF1 is an adipose tissue morphogen acting *via* PPAR gamma, a role that is believed to safeguard nutrient supply (79,80). This process also requires FGF21 for a successful outcome (81). FGF21, however, stimulates PPAR gamma in such a way that this could be seen as a positive feedback loop (82). Hence, nuclear receptors could be seen as one way of integrating endocrine FGF and paracrine FGF action.

Much attention has been given to the role of FGFs in tumourigenesis and development (83). Recently, the role of FGFs in ageing has attracted some promising attention. A series of studies using FGF23 knockout mice has pointed at accelerated ageing and reduced endogenous expression of other FGFs. Moreover, ability to respond to FGFs appears to be obscured in such mice. This has been the basis for testing FGFs on a therapeutic scale for such age-related diseases as osteoarthritis, cardiovascular disease and type II diabetes. (14).

In conclusion, the FGF signalling systems must be tightly regulated and integrated to uphold metabolism, ensure tissue repair and avoid dysregulation of development (84). Even minor mutations or changes in gene activation can eventually cause tissue damage or malignant disease (5). However, on the bright side, the FGF–FGFR system still remains a promising area for future drug development.

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