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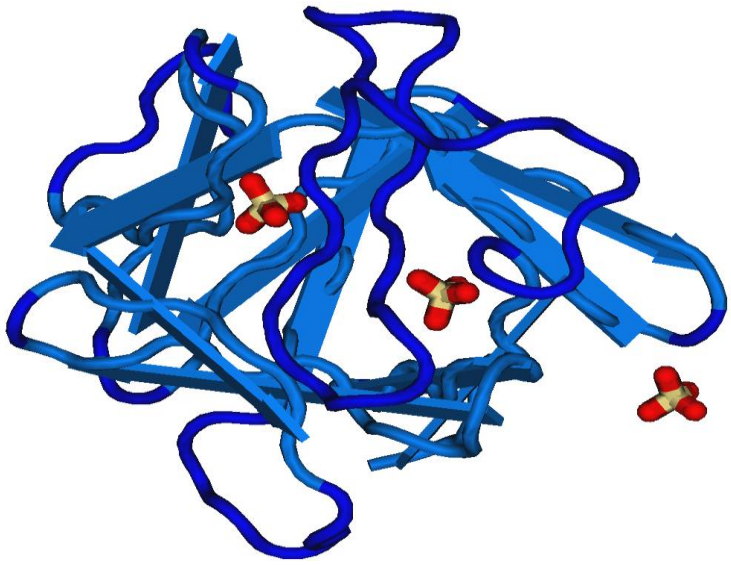
Caroline Laestander



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Fibroblastlika tillväxtfaktorer, deras receptorer och biologiska effekter

The role of FGFs in the elicitation of different cellular responses

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SAMMANFATTNING

Fibroblastlika tillväxtfaktorer är proteinhormoner som styr viktiga cellulära processer som till exempel proliferation, differentiering, migration, adhesion och överlevnad. Genom att binda till olika typer av membranbundna receptorer kan dessa peptider utöva olika effekter på målcellen. Vilka effekter de ger upphov till har olika förklaringsgrunder. Sålunda ger var och en av de 24 hittills kända FGF-varianterna delvis olika biologiska svar. Dessutom initierar olika receptorer (ffa FGF-R 1-4) olika intracellulära signalvägar som i sin tur ger upphov till olika biologiska sluteffekter. Receptorerna binder olika FGF–ligander med olika affinitet. Slutligen har olika målceller olika receptoruppsättningar vilket i sin tur påverkar det slutliga svaret. Således samspelar själva tillväxtfaktorerna med olika receptorer, med olika affinitetsegenskaper, som uttrycks i olika relativa tal på olika cellslag. Vilket biologiskt svar som utlöses beror på vilken kombination av fibroblastlik tillväxtfaktor, receptor och celltyp som samverkar.

ABSTRACT

Fibroblast growth factors are signaling peptides that controls important cellular processes such as proliferation, differentiation, migration, adhesion and survival. Through binding to different types of receptors on the cell surface, these peptides can have different effect on the target cell. Which effect that is achieved depends on many factors. Thus, each of the 24 known FGFs different biological responses. The FGF receptors (FGFR 1-4) initiates different intracellular pathways which in turn leads to different biological responses. The FGFs also binds different FGFs with different affinities. Finally, different types of cells express the FGFRs in different patterns and in altered extent which also affects the response. To summarize it, the response from a FGF depends on the combination of FGF, receptor and the type of cell that collaborate.

INTRODUCTION

The fibroblast growth factors (FGFs) are members of a large family consisting of 24 polypeptides. (Engström & Granerus, 2006). These molecules have been studied for many years but their functions are not yet fully understood. The first fibroblast growth factors were isolated from bovine brain tissue in 1977 (Gospodarowicz et al., 1978), and since then scientists have worked hard to understand the effects of these peptides as each member elicits different biological responses in different cells (Granerus & Engström, 2000). It has been shown that the fibroblast growth factors are involved in processes including cell proliferation, cell migration, differentiation, adhesion and survival. The targets of the FGFs are mainly two classes of receptors; the tyrosine kinase receptor family and the co-receptors heparin sulfate proteoglycans. The growth factor – receptor interaction is not a straight forward process. Different FGFs have different affinities for different receptors. Moreover, activation of multiple receptors affects which action that will follow. In other words, a full understanding of growth factor – receptor interaction will help understand how a specific biological response is achieved. The different FGFs have different affinities for these receptors which will lead to different effects on the cell.

The reason for trying to fully understand these growth factors, their receptors and the effects they lead to, is that scientists have reasons to believe that they play an important role in tumor development and angiogenesis, and that further understanding of the growth factors exact role may lead to a further step in combatting cancer.

The purpose of this review is to describe the different members of the fibroblast growth factor family, their receptors and how the interactions between the growth factor and the receptors can lead to various cellular responses.

MATERIAL AND METHODS

To find the literature needed to write this review, databases such as Web Of Knowledge and PubMed were used. While searching for relevant articles I used keywords such as “Fibroblast growth factors”, “effects on proliferation and migration”, “fibroblast growth factors and their receptors”, “FGF-1” and so on. Some of the articles were hard to find, while many of them is available online. A couple of articles were kindly provided by the supervisor.

LITTERATURE REVIEW

The fibroblast growth factors

Fibroblast growth factors are secreted polypeptide ligands that bind to different receptors that are located on the cell surface of target cells in a variety off tissues. The structure of the FGFs has been revealed through high-resolution X-ray diffraction as well as with, nuclear magnetic resonance, NMR (Arunkumar et al., 2002) and shows that the FGF archetype consists of 12 strands, which are linked together forming a three-fold symmetrical structure of beta sheets (Zhu et al., 1991). All 23 members of the FGF family have the same core of around 140 amino acids. Twenty two of the FGFs have been recognized in humans while FGF-24 has only been identified in zebrafish embryos (Fischer et al., 2003). FGF-15 occurs in mice and it is closely related to human FGF-19 (Nishimura et al., 1999).

The first FGFs ever to be found was the FGF 1 and FGF 2, also called the acidic and basic fibroblast growth factors (Fitzgerald et al., 2001). They were found to have a significant effect on cell migration, proliferation, differention and angiogenesis. Another name often used for the fibroblast growth factors are heparin binding growth factors, since they all have high affinity for heparan sulfate. Binding of fibroblast growth factors to different glucosaminoglycans, such as heparan sulfate, makes FGFs resistant to degradation and so FGFs can exist in the extracellular matrix as a reservoir. Some of the fibroblast growth factors also play an important role in embryogenesis, organ development and wound healing, which makes them an interesting object for research.

The FGFs are often divided into subgroups (Beenken & Moosa, 2009). The members of each family have similar qualities. Firstly, the FGF11-FGF14 aren't always included in the FGF-family because unlike the other FGFs they do not have the ability to bind to and activate FGF-receptors. Instead, they are called homologous factors, because their genomic structure highly resembles the FGFs. FGF1 and FGF2, also called the acidic and basic FGFs are members of the FGF1 subfamily. They were the first FGFs to be discovered but despite that, their physiological roles are still unclear. It is likely that they affect the vascular tone and lowers bloodpressure (Cuevas et. al., 1991). However, it is known that FGF2 have angiogenic properties and promotes proliferation and migration and inhibits apoptosis of endothelial cells (Zhou at al., 1998). The FGF4 subfamily contains FGF4 and FGF5. FGF4 is particularly important during organ development. It affects processes like trophoblast proliferation as well as limb and heart valve development while FGF5 is an important factor in the hair growth cycle regulation (Pethö-Schramm et al., 1996). FGF3, FGF7 and FGF10 and FGF22 are members of the FGF7 subfamily. FGF3 plays and important part in development of the inner ear structure while FGF7 is an important factor in kidney development and it is sometimes refered to as keratinocyte growth factor (KGF). FGF22, FGF7 and FGF10 are presynaptic organisers involved in vesicle clustering and neurite branching (Umemori et al., 2004). Another subfamily contains FGF8, FGF, 17 and FGF18. FGF8 is an important factor in limb, ear, eye and brain development and together with FGF17, FGF8 also have an effect on the development of the forebrain (Bernhard et al., 2003). FGF18 is required for correct development of bone tissue. The FGF9 subfamily consists of FGF9, FGF16 and FGF20.

FGF9 up regulates proliferation of mesenchymal tissue which initiates secretion of ligands from the FGF3, FGF7, FGF10 and FGF22 subgroups. Accordingly, knockout of the FGF9 leads to reduced production of different ligands and reduced mesenchymal-epithelial signaling (Colvin et al., 2001).

FGF19, FGF21 and FGF23 belong to a subfamily called the endocrine FGF ligands (Wu et al., 2011). One of the properties that distinguish them from the other FGFs is the fact that they need the presence of two types of klotho-proteins to form the FGF – receptor complex in the tissue. This is a consequence of their low affinity toward heparin sulfate proteoglycan. The two types of klotho proteins (αKlotho and βKlotho) are selectively used as co-receptors by the FGF19 subfamily members (Wu, 2012). FGF19 stimulates bile acid synthesis and initiates oxidation of fatty acids. FGF21 gives a fasting response, by stimulating glucose uptake in adipocytes and so, reducing the levels of glucose in the bloodstream. Injections of FGF21 in diabetic and obese mice lead to reduced concentration of insulin, glucagon, glucose and triglycerides in the blood. Continues injections of FGF21 in obese mice reduced the body weight by 20%. Finally FGF23 is an important vitamin D regulator.

The FGF receptors

The FGFs binds to two different classes of receptors and it is important to state that many different ligands can activate the same receptor. FGFs bind simultaneously to low-affinity, heparin sulfate proteoglycans and high-affinity FGF-receptors. The high affinity receptors consist of one extracellular part that contains between one and three Ig-SF (domains, one transmembrane domain and one intracellular tyrosine kinase domain. The high-affinity receptors have one unique part that distinguishes it from other receptors. That is an “acid box” that consists of eight acidic residues located between the first and second Ig-SF domains. The first Ig-SF domain and the acid box probably contribute to autoinhibition while domains 2 and 3 are FGF ligand binding sites (Wesche et al,. 2011).

Four FGF-receptors have been identified to date, but it is believed that there are others hitherto undiscovered. The common name for these receptors is FGFR 1-4 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 the Ig like domain 3 causes variants with different specificities of the binding site. The different isoforms of the receptors are expressed in different organs, for instance, the FGFR3IIIb is mainly located in epithelial tissues and the IIIc forms are mainly expressed in tissues of mesenchymal origin. Both isoforms have separate ligands which only bind to the specific receptor. This means that mesenchymal cells produce ligands that only activate IIIc receptor in order to achieve a paracrine signal.

The FGF-receptors are unevenly distributed in several tissues in the body, and the patterns in which they occur is specific to each tissue. Studies have shown that FGFR-1 is expressed in the skin, calvarial bones, growth plates and in high amounts in the fetal brain. FGFR-2 also

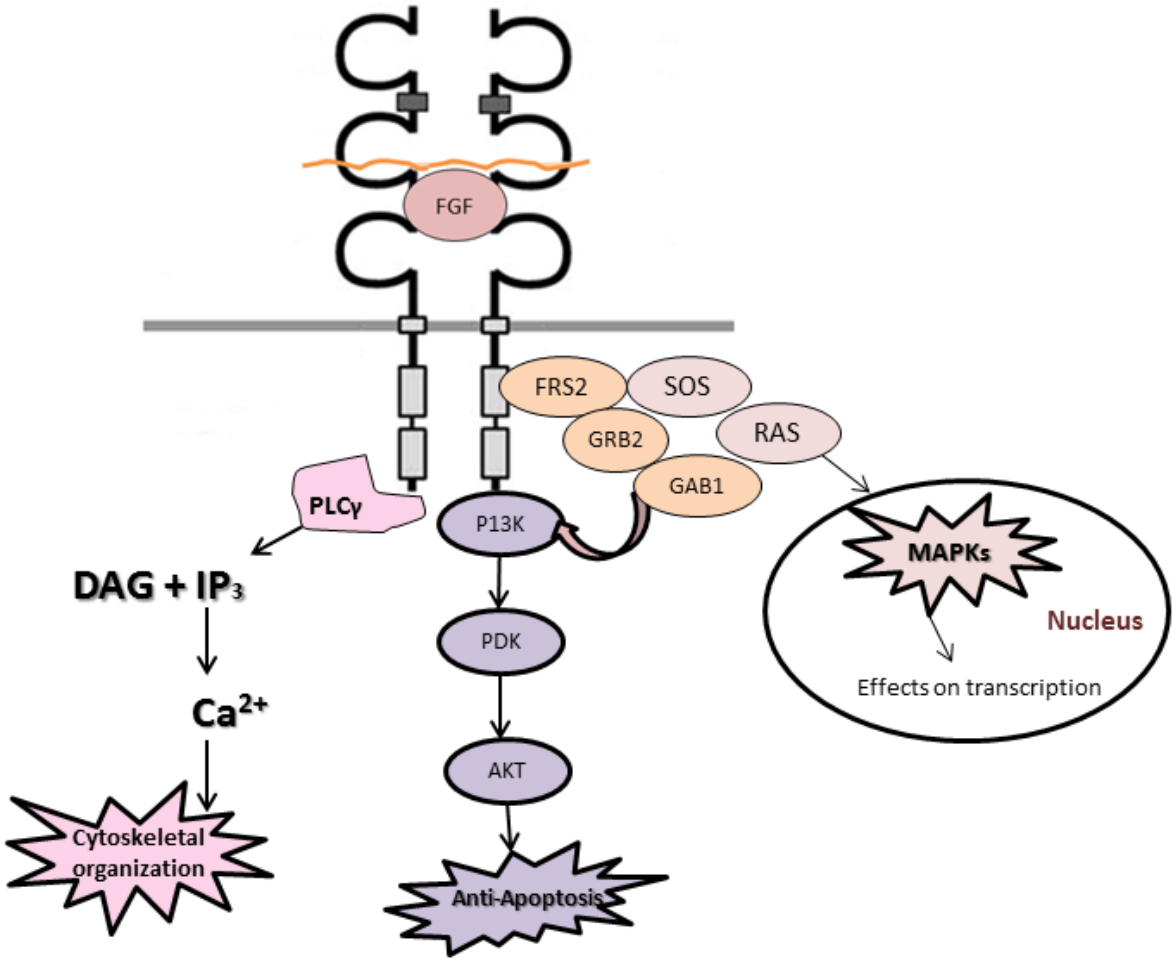
exists in the brain, growth plates and calvarial bone, but also in the liver, lungs, intestine and kidneys. The FGFR-3 is also expressed in the brain, growth plates and calvarial bone as well as in the lung, kidney and intestines. FGFR-4 can be found in lungs, kidney, liver, pancreas, intestine, fetal adrenals, spleen and striated muscle (Fitzgerald et. al. 2001).

The low affinity receptors are present on the surface of most cells. They are so called heparan sulfate proteoglycans, HSPGs for short. They have a much simpler structure then the high affinity receptors with their single proteoglycan core that can bind to 2 or 3 negatively charged heparan sulfate chains. The binding site for the FGF ligands is the polysaccharides (heparan sulfate chains). The HSPGs has two different very important functions. The first is that binding of the FGF to the HSPGs protect the growth factors from from degradation and so it can act as a extracellular buffert. HSPGs are also involved in the complex formation between the FGFs and the FGFR. Binding of the FGFs to their respective receptors induces dimerisation and formation of a ternary complex containing FGF, FGFR and heparan sulfate.

FGF signaling

A signal through activation of the FGFR requires a dimerisation, a prerequisite for moving the intracellular kinases closer to each other which initiates the onset of different intracellular signaling pathways that leads to adjustment of gene expression (Schlessinger, 2010). Formation of the receptor dimers activates their intracellular tyrosine kinases which allow them to transphosphorylate the tyrosine residues on both dimers of the receptor. These residues can act as a binding site for signaling molecules containing src homology-2 (SH2) or phosphotyrosine binding (PTB) domains. The signaling molecules are often bound to different docking proteins. There are a few known signal transduction pathways and some of them are better understood then others. The RAS-MAP kinase pathway is one of the most studied signaling pathways. It involves the docking protein FRS2α which becomes activated by the tyrosine residues on the activated FGF receptor. FRS2α is the core of a complex formed by the adaptor Grb2, tyrosine phosphatase Shp2 and the docking protein GAB1. To Grb2 binds the guanine nucleotide exchange factor SOS, which in turn activates the Ras-MAP kinas. The MAP kinases are regulatory proteins that affect different kinases and transcription factors and thereby regulating target genes. The effects gained by stimulating the Ras-Map kinase pathway are mainly mitogenic (Wesche et al., 2011).

GAB1 also leads to activation of another important pathway through activation of PI-3 kinase. P-I-3 activates PDK which in turn activates ATK/PKB. The effects of ATK are anti-apoptotic. The activated FGF receptor can also lead to hydrolysis of Pt Ins and activation of PDK and ATK. FGFs can also act on intracellular calcium levels through recruitment of the SH2 domain of PLCγ to the receptor. Activation of PLCγ allows it to hydrolyze Pt Ins P2 which leads to formation of diaglycerol (DAG) and Ins P3. The effect of DAG and Ins P3 is release of calcium and activation of calcium-dependent protein kinases which affects cytoskeletal organization (Schlessinger, 2000).



Diversity in cellular responses

The effects achieved by these signaling cascades are not always the same on all cells. The MAPK pathway is always seen as a response to FGF ligand binding while others, such as AKT activation, differs between cell types. Studies have shown that activation of MAPK leads to proliferation of oligodendrocyte precursors and endothelial cells. Inhibition of PLCγ on the other hand, did not affect the oligodendrocytes (Dailey et al., 2005). Ligand activation of the MAPK pathway has been shown to stimulate proliferation in fibroblasts. Studies performed on chondrocytes showed that MAPK activation, unlike PLCγ and PI-3, leads to interruption of the cell cycle.

Another explanation to the variety between cell responses is the fact that the intracellular signaling pathways are influenced by a number of regulators. A few example of these are the Sproty proteins, MAPK phosphatase 3 and SEF which are inhibitory molecules that either bind to different molecules (Sproty protein) and inhibits them or act as regulatory feedback (SEF – has similar expression to FGF). There are also excitatory molecules which up regulate the signaling pathways such as some FLRT, a family of transmembrane proteins (Beenken et al., 2009).

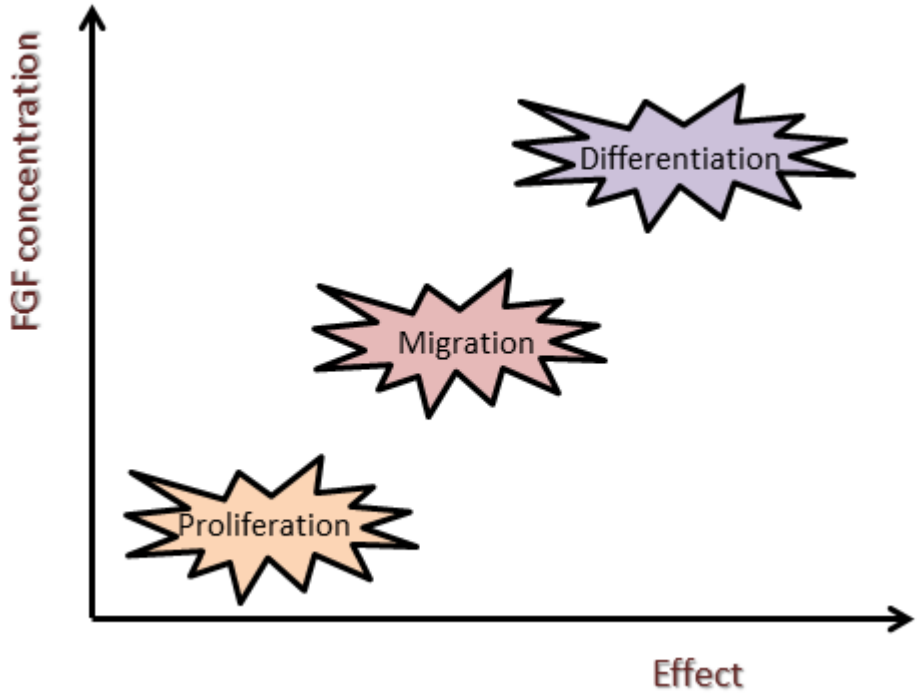
It is known that the FGFs have different effects on different tissues depending on the developmental stage of the organ and the concentration of growth factor present there. The effects of FGF2 on the development of oligodendrocytes have been studied and it has been found that FGF2 induces different, stage-specific responses in the cells (Fortin et al., 2005). An experiment on oligodendrocytes showed that FGF-2 induced proliferation in the cells while FGF-8,9 and 17 had no effect no matter how high the concentration was or how long the duration was. The reason to this difference in response is the fact that FGF-2 rapidly activates the MAPK pathway while FGF-8,9 and 17 had a much weaker and slower effect on the MAPK pathway. The effects of FGF-2 on differentiation have also been closely studied (Fortin et al., 2005) and the conclusion was that FGF-2 inhibits the differentiation of oligodendrocyte progenitors. However, further studies showed that FGF-9, unlike FGF-2, did not block the differentiation of the oligodendrocytes.

The effects of FGFs on mature oligodendrocytes were also examined: FGF-2 induces multiple responses in the mature cell line such as elongation, inhibition of myelin protein synthesis and reentering into the cellcycle. These effects where studied after of FGF-8,9, 17 and 18 addition and it was shown that only FGF-2 had any effect on the cell-cycle. FGF-9 and 18 gave the same results as FGF-2 on differentiation of the oligodendrocytes but treatment with FGF-9 and 18 did not result in loss of myelin-like membranes which was observed in FGF-2 treated cells. FGF-8 and FGF-17 did not increase cell size, which suggests that these cells can distinguish between different FGFs.

The expression patterns of the receptors may also play a certain part in the response of FGF signaling. It has been confirmed that cells express different FGFRs during different developmental phases. The patterns in which the FGFRs occurs changes as the cells differentiate. An example is the FGFR expression in oligodendrocytes during their differentiation where FGFR1 are expressed throughout the development while FGFR2 was more prominent during terminal differentiation and FGFR3 was downregulated at the end of oligodendrocyte differentiation.

It is also possible that different FGF-FGFR interactions lead to different responses. This theory was tested by adding FGFR inhibitors to oligodendrocytes during different phases of development. The result was that during progenitor phase, only activation of FGFR1 (by FGF-2) induced proliferation while inhibition of proliferation requires FGF-8, FGF-17 or FGF-18 bound to FGFR-3. The same experiment was performed on differentiated oligodendrocytes and it showed that activation of FGFR1 is required for cells to re-enter cell cycle and not FGFR3. The cell elongation requires activation of FGFR2 by FGF-2, FGF-9 and FGF-18.

It is also known that the responses from FGFs are concentration dependent, which adds yet another factor to determine the response. A study on lens epithelial cells showed that low concentrations (150pg/ml) of FGF-1 and FGF-2 initiated proliferation. As higher concentrations (3ng/ml) of FGF were added, the cells started to migrate. To achieve cell differentiation, an even higher concentration (40ng/ml) of FGFs was required (McAvoy & C Chamberlain, 1989). It also seems like the time interval in which the cells are exposed to the FGFs matters. Proliferation and migration of the cells were achieved within 24 h while differentiation of the cells was seen after 4 days. It was also shown that proliferation and migration can occur simultaneously and higher concentration leads to a more pronounced response.



DISCUSSION

Fibroblast growth factors play a pivotal role in the regulation of key developmental processes. There is mounting evidence for the importance of correct spatial and temporal regulation of the expression of FGF:s and their receptors. Deregulation of this signaling system can lead to a variety of developmental aberrations as e.g. skeletal disorders and cancer (Dailey et al., 2005).

This review has highlighted the differential effects of the 24 hitherto discovered 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, because the 24 FGFs exert different cellular responses. Moreover, different intracellular pathways are activated to a different extent depending on which ligand that initiates the activation.

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

Vast genetic data on mammalian development has pointed at the importance of a finely orchestrated role for the FGF family for normal development. The FGF;s are part of an extended gene family including TGFBeta/BMP;s, Hedgehog, Notch and Wnt (Goldfarb, 1996). Albeit structurally different, they all combine their efforts to steer undifferentiated cells towards lineage determination, proliferation, locomotion and differentiation.

This concept also suggests that there is a role for cross talk between the activation of FGF-receptors and other signaling pathways. There is mounting evidence that FGF-activation may activate or repress other signaling pathways as e.g. TGFBeta/BMP, IGF, IHH/PTHIH and Notch (Dailey et al 2005). 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 (Moon et al., 1995) cross talk between FGFs and Wnt can lead to convergence or divergence of the signal routes activated by each pathway. Moreover it has been suggested that activation of one signal can confer competence on the other.

This signaling system must be tightly regulated to avoid dysregulation of development. Even minor mutations or changes in gene activation can lead to tissue damage or even malignant disease.

REFERENCES

Arunkumar, A.I., Srisailam, T., Krishnaswamy, T., Kumar, S., Kathir, K.M., Chi, Y., Wang, H., Chang, G., Chiu, I & Yu, C. (2002) Structure and Stability if an Acidic Fibroblast Growth Factor from *Notophthalamus viridescens*. *The Journal of biological chemistry*. 277, 46424-46432.

Beenken, A. & Moosa, M. (2009). The FGF family: biology, pathophysiology and therapy. *Nature Reviews Drug Discovery.* 8, 235-253.

Bernhard, R. & von Bohlen, O.H. (2003) Fibroblast growth factors and their receptors in the central nervous system. *Cell tissue Research*. 313, 139-157.

Colvin, J.S., White, A.C., Pratt, S.J. & Ornitz, D.M. (2001). Lung Hypoplasia and neonatal death in FGF9-null mice identify gene as an essential regulator of lung mesenchyme. *Development.* 128, 2095-2106.

Cuevas, P., Carceller, F., Ortega, S., Nieto, I. & Giménez-Gallego, G. (1991). Hypotensive Activity of Fibroblast Growth Factor. *Science, New series.* 254, 1208-1210.

Dailey L, Ambrosetti, D., Mansukhani, A. & Basilico, C. (2005). Mechanisms underlying differential responses to FGF signaling. *Cytokine & Growth Factor Reviews.* 16, 233-247.

Engström, W & Granerus, M. (2006). Effects of Fibroblast Growth Factors 19 and 20 on cell multiplication and locomotion in a human embryonal carcinoma call line (Teraa.2) *In Vitro*. *Anticancer research*, 26, 3307-3310.

Fisher S., Draper B.W. & Neumann, C.J. (2003) The zebrafish fgf24 mutant identifies an additional level of Fgf signaling involved in vertebrate forelimb initiation. *Development.* 130, 3515-3524.

Fitzgerald, K.A., O'neill, L.A., Geraring A.J & Callard, R.E. (2001) *The Cytokine Factsbook and Webfacts.* 2nd ed. Burlington: Elsevier Siene.

Goldfarb, M. (1996). Functions of Fibroblast Growth Factors in Veterbrate Development. *Cytokine and Growth Factor Reviews.* 7, 311-325.

Gospodarowicz, D., Bialecki, T & Greenburg G. (1978). Purification of the Fibroblast Growth Factor activity from bovine brain. *The Journal of Biological* Chemistry, 253, 3736-3743.

Granerus, M & Engström, W. (2000). Dual effects of Four Members of the Fibroblast Growth Factor Member Family on Multiplication and Motility in Human Teratocarcinoma Cells In Vitro. *Anticancer Research*. 20, 3527-3532.

McAvoy, J.V., & Chamberlain C.G. (1989). Fibroblast growth factor (FGF) induces different responses in lens epithelial cells depending on its concentration. *Development.* 107, 221-228.

Moon, R.T., Brown, J.D. & Torres, M. (1997). WNTs modulate cell fate and behavior during vertebrate development. *Trends in Genetics.* 13, 157-162.

Nishimura, T., Utsunomiya, M., Hoshikawa, M., Ohuchi H & Itoh, N. (1999). Structure and expression of a novel human FGF, FGF-19, expressed in the fetal brain. *Biochemica et Biohysica Acta.* 1444, 148-151.

Pethö-Schramm, A., Müller, H.J. & Paus, R. (1996). FGF5 and the murine hair cycle. *Archives of Dermatological Research.* 288, 264-266.

Schlessinger J. (2000). Cell signaling by Receptor Tyrosine Kinases. *Cell*. 103, 211-225.

Umemori, H., Linhoff M.W., Ornitz, D.M. & Sanes J.R. (2004). FGF22 and Its Close Relatives Are Presynaptic Organizing Molecules in the Mammalian Brain. *Cell Press.* 118, 257-270.

Wesche, J., Haglund, K. & Haugsten, M. (2011). Fibroblast growth factors and their receptors in cancer. *Biochemical Journal.* 437, 199-213.

Wu, A. Coulter, S., Liddle, C., Wong, A., Eastham-Anderson, J., French, D.M., Peterson, A.S. & Sonoda, J. (2011). FGF19 Regulates Cell proliferation, Glucose and Bile Acid Metabolism via FGFR4-Dependent and Independent Pathways. *PLoS One.* [online] (2011-03-18) Tillgänglig: http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0017868. [2013-05-07]

Zhou, M., Sutliff, R.L., Paul, R.J., Lorenz, J.N., Hoying, J.B., Haudenschild, C.C., Yin, M., Coffin, D.J., Kong, L., Kranias, E.G., Lou, W., Boivin G.P., Duffy, J.J., Pawlowski, S.A. & Doetschman T. (1998). Fibroblast Growth Factor 2 control of vascular tone. *Nature Medicine.* 4, 201-207.

Zhu, X., Komiya, H., Chirino, A., Faham, S., Fox, G.M., Arakawa, T., Hsu, B.T & Rees D.C. (1991) Three-Dimensional Structures of Acidic and Basic Fibroblast Growth Factors. *Science, New Series.* 251, 90-93.