

The FGF Family

The first two fibroblast growth factors (FGFs), acidic FGF (FGF-1) and basic FGF (FGF-2) were originally identified as growth factors for fibroblasts. However, FGFs are now recognized as polypeptide growth factors with diverse biological activities and expression profiles. Today the human FGF family consists of 22 members (FGF-1 to 14 and FGF-16 to 23) and these family members are further divided into six subfamilies (Figure 1). The FGF-11 subfamily is generally not considered to be a member of the FGF family [for a recent review see A. Beenken & M. Mohammadi; *Nat. Rev. Drug Discov.* **8**, 235 (2009)]. All FGFs, except those of the FGF-1 and FGF-9 subfamily, have signal peptides. The FGF-9 subfamily is nonetheless secreted through the traditional endoplasmic reticulum (ER) – Golgi secretory pathway, whereas the FGF-1 subfamily is secreted independently.

The various FGFs have been reported to regulate complex biological processes such as embryon-

ic development, angiogenesis, wound healing, nerve regeneration, chronic inflammation and cancer. These processes require spatial and temporal integration of several cell responses, including cell survival, proliferation, migration and invasion, and cell differentiation. All these responses or functions are induced or modulated by the interaction of FGFs with tyrosine kinase FGF receptors (FGFRs). There exist four FGFRs (FGFR-1 to 4) which consist of three extracellular immunoglobulin domains (D1-D3), a single transmembrane domain and a cytoplasmic tyrosine kinase domain. Unlike other growth factors, FGFs act in concert with heparin or heparan sulfate proteoglycan (HSPG) to activate FGFRs. The binding of FGF and HSPG to the extracellular ligand domain of FGFR induces receptor dimerization, activation and autophosphorylation of multiple tyrosine residues in the cytoplasmic domain of the receptor

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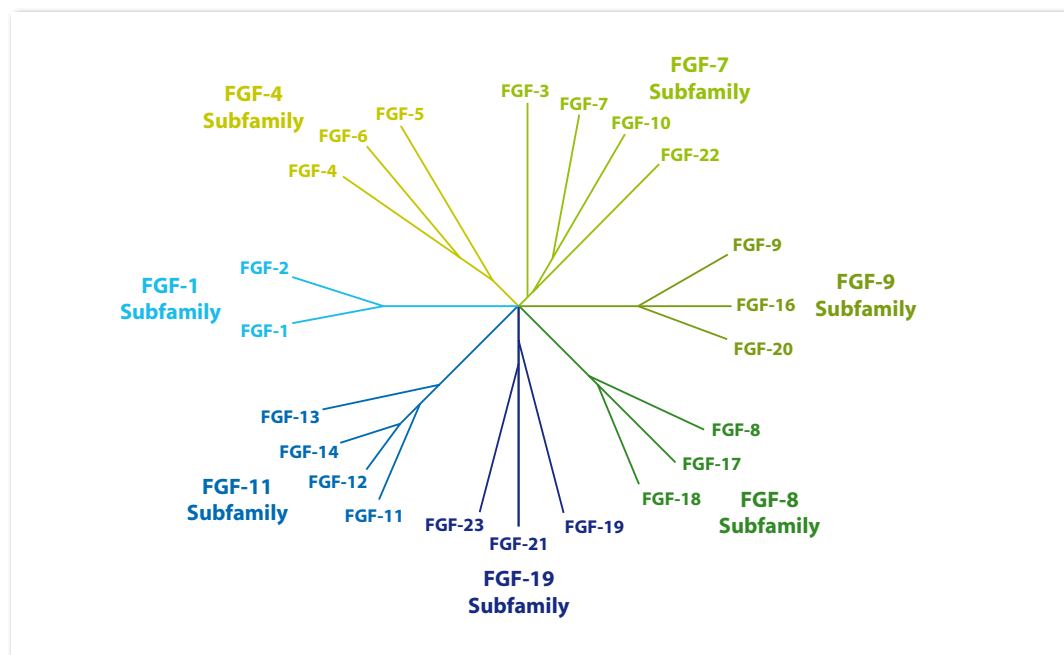
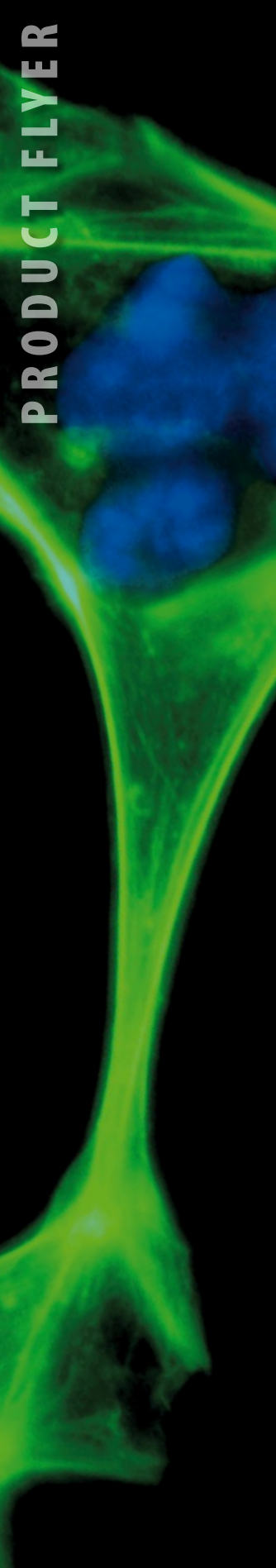


FIGURE 1: The human FGF Gene Family. Adapted from: *The Fgf families in humans, mice, and zebrafish: their evolutionary processes and roles in development, metabolism, and disease*; N. Itoh; *Biol. Pharm. Bull.* **30**, 1819 (2007)



molecule. A variety of signaling proteins are phosphorylated in response to FGF stimulation including Shc, phospholipase C γ , Gab1 and FRS2 α . In addition the interaction of FGFs with these receptors also mediates FGFR cell trafficking. In fact, most FGFs are imported or exported in and out of cells and are translocated to the cell nucleus complexed with their receptors. Internalization and nuclear translocation of receptors results in specific signaling pathways that appear to be different from those elicited at the cell surface. Thus, most FGFs act on cells through autocrine, paracrine and endocrine effects that are modulated by both receptor activation and trafficking. Future application of the FGFs in renal disease, glucose and phosphate homeostasis, stem cell research, angiogenesis, tissue repair and bioengineering are under investigation.

FGF-19 Subfamily

Fibroblast growth factors (FGFs) are humoral factors with diverse biological functions. While most FGFs work as local factors regulating cell growth and differentiation, the FGF-19 subfamily members FGF-19 (the human ortholog of mouse FGF-15), FGF-21 and FGF-23 work as systemic factors (Figure 2). β Klotho has been identified as co-factor/co-receptor required for FGF-19 and FGF-21 signaling whereas Klotho is essential for FGF-23 signaling. It has been proposed that the tissue-specific expression of FGF receptor (FGFR) subtypes together with the limited expression of the co-factors/co-receptors β Klotho and Klotho determine the tissue specific metabolic activities of the FGF-19 subfamily members. For a recent review see H. Kurosu & M. Kuro-o; *Mol. Cell Endocrinol.* **299**, 72 (2009).

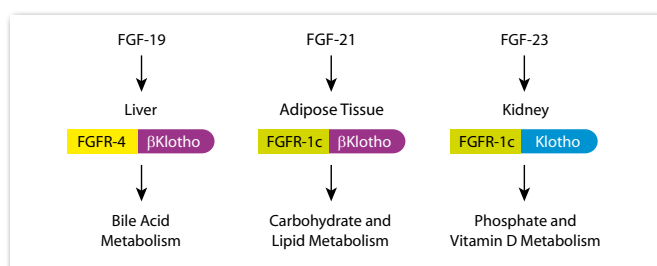


FIGURE 2: Mode of actions of FGF-19 subfamily members.

FGF-19 subfamily members require klotho or β klotho in addition to canonical FGFRs for their actions. Representative target tissues and FGFRs are indicated. Adapted from: *Actions and mode of actions of FGF19 subfamily members*; S. Fukumoto; *Endocr. J.* **55**, 23 (2008)

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Tissue-specific expression of betaKlotho and fibroblast growth factor (FGF) receptor isoforms determines metabolic activity of FGF19 and FGF21: H. Kurosu, et al.; *J. Biol. Chem.* **282**, 26687 (2007) ■ Liver-specific activities of FGF19 require Klotho beta: B.C. Lin, et al.; *J. Biol. Chem.* **282**, 27277 (2007) ■ betaKlotho is required for metabolic activity of fibroblast growth factors 21: Y. Ogawa, et al.; *PNAS* **104**, 7432 (2007) ■ Actions and mode of actions of FGF19 subfamily members: S. Fukumoto; *Endocr. J.* **55**, 23 (2008) (Review) ■ The Klotho gene family as a regulator of endocrine fibroblast growth factors: H. Kurosu & M. Kuro-o; *Mol. Cell Endocrinol.* **299**, 72 (2009) (Review)

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FGF-19

FGF-19 (human) (rec.) (His)

AG-40A-0111-C010

10 μ g

AG-40A-0111-C050

50 μ g

Produced in HEK 293 cells. The original signal peptide and the mature peptide of human FGF-19 (aa 1-216) are fused at the C-terminus to a His-tag. **PURITY:** \geq 90% (SDS-PAGE). **ENDOTOXIN CONTENT:** $<$ 0.1 EU/ μ g protein (LAL-test).

new anti-FGF-19 (human), mAb (FG98-6)

AG-20A-0065-C050

50 μ g

AG-20A-0065-C100

100 μ g

CLONE: FG98-6. **ISOTYPE:** Mouse IgG2. **IMMUNOGEN:** Recombinant human FGF-19. **SPECIFICITY:** Recognizes human FGF-19. **APPLICATION:** ELISA, WB.

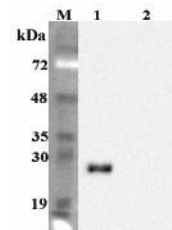


FIGURE: Western blot analysis of human FGF-19 using anti-FGF-19 (human), mAb (FG369-1) (Prod. No. AG-20A-0065) at dilution 1:2,000.

1. FGF-19 (human) (rec.) (His) (Prod. No. AG-40A-0111)
2. Recombinant mouse Vaspin-His (negative control)

new anti-FGF-19 (human), mAb (FG369-1)

AG-20A-0066-C050

50 μ g

AG-20A-0066-C100

100 μ g

CLONE: FG369-1. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human FGF-19. **SPECIFICITY:** Recognizes human FGF-19. **APPLICATION:** ELISA, WB.

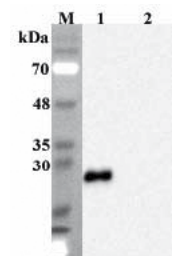


FIGURE: Western blot analysis of human FGF-19 using anti-FGF-19 (human), mAb (FG98-6) (Prod. No. AG-20A-0066) at 1:2,000 dilution.

1. FGF-19 (human) (rec.) (His) (Prod. No. AG-40A-0111)
2. Recombinant mouse Vaspin-His (negative control)

FGF-21

FGF-21 (human) (rec.)

AG-40A-0091-C010 10 µg
AG-40A-0091-C050 50 µg
Produced in HEK 293 cells. Mature human FGF-21 (aa 1-209) is fused at the C-terminus to a FLAG®-tag. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <0.1EU/µg protein (LAL-test).

FGF-21 (human) (rec.) (His)

AG-40A-0098-C010 10 µg
AG-40A-0098-C050 50 µg
Produced in HEK 293 cells. Mature human FGF-21 (aa 1-209) is fused at the C-terminus to a His-tag. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <0.1EU/µg protein (LAL-test).

FGF-21 (human):Fc (human) (rec.)

AG-40A-0095-C010 10 µg
AG-40A-0095-C050 50 µg
Produced in HEK 293 cells. Mature human FGF-21 (aa 1-209) is fused at the C-terminus to the Fc portion of human IgG. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <0.1EU/µg protein (LAL-test).

FGF-21 (mouse) (rec.)

AG-40A-0092-C010 10 µg
AG-40A-0092-C050 50 µg
Produced in HEK 293 cells. Mature mouse FGF-21 (aa 1-210) is fused at the C-terminus to a FLAG®-tag. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <0.1EU/µg protein (LAL-test).

FGF-21 (mouse) (rec.) (His)

AG-40A-0099-C010 10 µg
AG-40A-0099-C050 50 µg
Produced in HEK 293 cells. Mature mouse FGF-21 (aa 1-210) is fused at the C-terminus to a His-tag. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <0.1EU/µg protein (LAL-test).

FGF-21 (mouse):Fc (human) (rec.)

AG-40A-0097-C010 10 µg
AG-40A-0097-C050 50 µg
Produced in HEK 293 cells. Mature mouse FGF-21 (aa 1-210) is fused at the C-terminus to the Fc portion of human IgG. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <0.1EU/µg protein (LAL-test).

anti-FGF-21, mAb (FG224-7)

AG-20A-0051-C100 100 µg
CLONE: FG224-7. **ISOTYPE:** Rat IgG1. **IMMUNOGEN:** Recombinant mouse FGF-21. **SPECIFICITY:** Recognizes human and mouse FGF-21. **APPLICATION:** ELISA, WB.

new anti-FGF-21 (human), mAb (FG204-3)

AG-20A-0067-C050 50 µg
AG-20A-0067-C100 100 µg
CLONE: FG204-3. **ISOTYPE:** Mouse IgG2. **IMMUNOGEN:** Recombinant human FGF-21. **SPECIFICITY:** Recognizes human FGF-21. Does not cross-react with mouse FGF-21. **APPLICATION:** ELISA, WB.

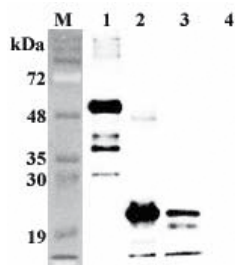


FIGURE: Western blot analysis of human FGF-21 using anti-FGF-21 (human), mAb (FG204-3) (Prod. No. AG-20A-0067) at 1:2,000 dilution.

1. FGF-21 (human):Fc (human) (rec.) (Prod. No. AG-40A-0095)
2. FGF-21 (human) (rec.) (Prod. No. AG-40A-0091)
3. FGF-21 (human) (rec.) (His) (Prod. No. AG-40A-0098)
4. Jagged-1 (human):Fc (human) (rec.) (Prod. No. AG-40A-0081) (negative control)

new anti-FGF-21 (human), mAb (FG348-1)

AG-20A-0068-C050 50 µg
AG-20A-0068-C100 100 µg
CLONE: FG348-1. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human FGF-21. **SPECIFICITY:** Recognizes human FGF-21. Does not cross-react with mouse FGF-21. **APPLICATION:** ELISA, WB.

anti-FGF-21 (human), PAb

AG-25A-0074-C100 100 µg
From rabbit. **IMMUNOGEN:** Recombinant human FGF-21. **SPECIFICITY:** Recognizes human and mouse FGF-21. **APPLICATION:** ELISA, WB.

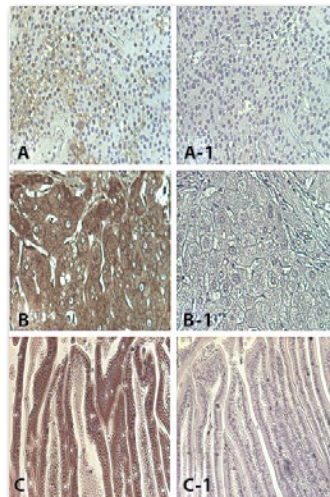


FIGURE: Immunoperoxidase staining of FGF-21 in formalin-fixed, paraffin-embedded human tissue using anti-FGF-21 (human), PAb (Prod. No. AG-25A-0074) at 1:5,000 dilution (A, B, C) or pre-immune rabbit serum at dilution 1:500 (A-1, B-1, C-1) as negative control.

A, A-1: Parathyroid (200x magnification)
B, B-1: Liver (200x magnification)
C, C-1: Small intestine (100x magnification)

anti-FGF-21 (mouse), PAb

AG-25A-0076-C100 100 µg
From rabbit. **IMMUNOGEN:** Recombinant mouse FGF-21. **SPECIFICITY:** Recognizes mouse FGF-21. Weakly cross-reacts with human FGF-21. **APPLICATION:** ELISA, WB.

LATEST INSIGHT

The FGF Family – FGFR Interaction – A Target For Developing New Therapeutics?

The involvement of FGF signaling in human disease is well documented. Therapeutic approaches using exogenous FGFs, antibodies or small molecules are still relatively new and many avenues of investigation remain open. Recombinant FGF-7 is already in use for the treatment of chemotherapy-induced oral mucositis. Continued efforts to understand the structural biology of FGF-FGFR interactions will play a key part in driving the discovery of new therapies. For the latest review on the current knowledge regarding FGF-FGFR signaling, the biology, pathology and recent developments regarding the pharmacological applications of each FGF ligand see The FGF family: biology, pathophysiology and therapy: A. Beenken & M. Mohammadi; Nat. Rev. Drug Discov. 8, 235 (2009).

FGF-23

FGF-23 (human) (rec.) (His)

AG-40A-0114-C010 10 ug
AG-40A-0114-C050 50 ug

Produced in HEK 293 cells. The original signal peptide and the mature peptide of human FGF-23 (aa 1-251) are fused at the C-terminus to a His-tag. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <0.1EU/μg protein (LAL-test).

FGF-23 (human):Fc (human) (rec.)

AG-40A-0109-C010 10 ug
AG-40A-0109-C050 50 ug

Produced in HEK 293 cells. The original signal peptide and the mature peptide of human FGF-23 (aa 1-251) are fused at the C-terminus to the Fc portion of human IgG. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <0.1EU/μg protein (LAL-test).

FGF-23 (R179Q Mutant) (human) (rec.) (His)

AG-40A-0126-C010 10 ug

Produced in HEK 293 cells. The original signal peptide and the mature peptide of human FGF-23 (aa 1-251) are fused at the C-terminus to a His-tag. The R179Q mutant is resistant to degradation by the endopeptidase PHEX. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <0.1EU/μg protein (LAL-test).

FGF-23 (mouse):Fc (human) (rec.)

AG-40A-0128-C010 10 ug
AG-40A-0128-C050 50 ug

Produced in HEK 293 cells. The original signal peptide and the mature peptide of mouse FGF-23 (aa 1-251) are fused at the C-terminus to the Fc portion of human IgG. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <0.1EU/μg protein (LAL-test).

new anti-FGF-23 (human), mAb (FG322-3)

AG-20A-0073-C050 50 ug
AG-20A-0073-C100 100 ug

CLONE: FG322-3. **ISOTYPE:** Mouse IgG1. **IMMUNOGEN:** Recombinant human FGF-23. **SPECIFICITY:** Recognizes human FGF-23. Does not cross-react with FGF-23 (mouse):Fc (human) (rec.). **APPLICATION:** ELISA, WB.

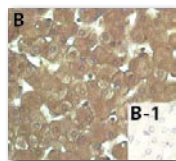


FIGURE: Immunohistochemical staining of FGF-23 with anti-FGF-23 (human), mAb (FG322-3) (Prod. No. AG-20A-0073) in human tissue (dilution 1:200).

1. Immunoperoxidase staining of formalin-fixed, paraffin-embedded human liver showing cytoplasmic staining (brown color, 200x).

FGFs & Stem Cells

Fibroblast growth factors (FGFs) play an important role in the regulation of proliferation and differentiation in stem cells. For research in stem cells and clinical application of these cells, it is important to establish *in vitro* culture conditions that maintain the self-renewal ability of stem cells along with their fully pluripotent or multipotent differentiation capacity as well as efficient proliferative ability. FGFs are amongst the most common growth factors used to expand stem cells, including human embryonic stem (hES) cells, trophoblast stem (TS) cells and neural stem (NS) cells. Moreover, it has been recently recognized that FGFs are useful for culturing cancer stem cells.

FGFs & Embryonic Stem Cells

Emerging evidence indicates that there are many differences between mouse embryonic stem (mES) cells and human embryonic stem (hES) cells. In particular, the required growth factors for maintaining self-renewal ability in culture are clearly different. Leukemia inhibitory factor (LIF) is essential for mES cells. In mES cells, FGF-4 acts as an autoinductive stimulus that propels mES cells toward lineage specification. The main role of LIF is to block the FGF-4 signaling to keep pluripotency of mES cells. However, LIF signaling does not support self-renewal ability of hES cells. In hES cells, under serum-free conditions, FGF-2 and activin/nodal factors maintain self-renewal ability. It was shown that FGF-4 perpetuates the pluripotency of hES cells as well. Interestingly, FGF-4 is secreted with a novel FGF-4 splice isoform (FGF-4si). FGF-4si is an antagonist of FGF-4, shutting down FGF-4 induced undifferentiated growth of hES cells.

FGFs & Trophoblast Stem Cells

Mouse trophoblast stem (mTS) cells are tissue-specific stem cells of the trophoblast lineage, that give rise to embryonic por-

tion of the placenta. FGF-4 in combination with conditioned medium are the key for the self-renewal ability of mTS cells *in vitro*. In the absence of either FGF-4, or conditioned medium, mTS cells lose multipotency and differentiate into giant cells, which resemble trophoblast giant cells. *In vivo*, FGF-4, produced from the inner cell mass (ICM) of the blastocyst and the epiblast of embryos, signals to neighboring mTS cells in a paracrine manner. Therefore, it is proposed that FGF-4 functions as a secretory factor in the mTS cell niche.

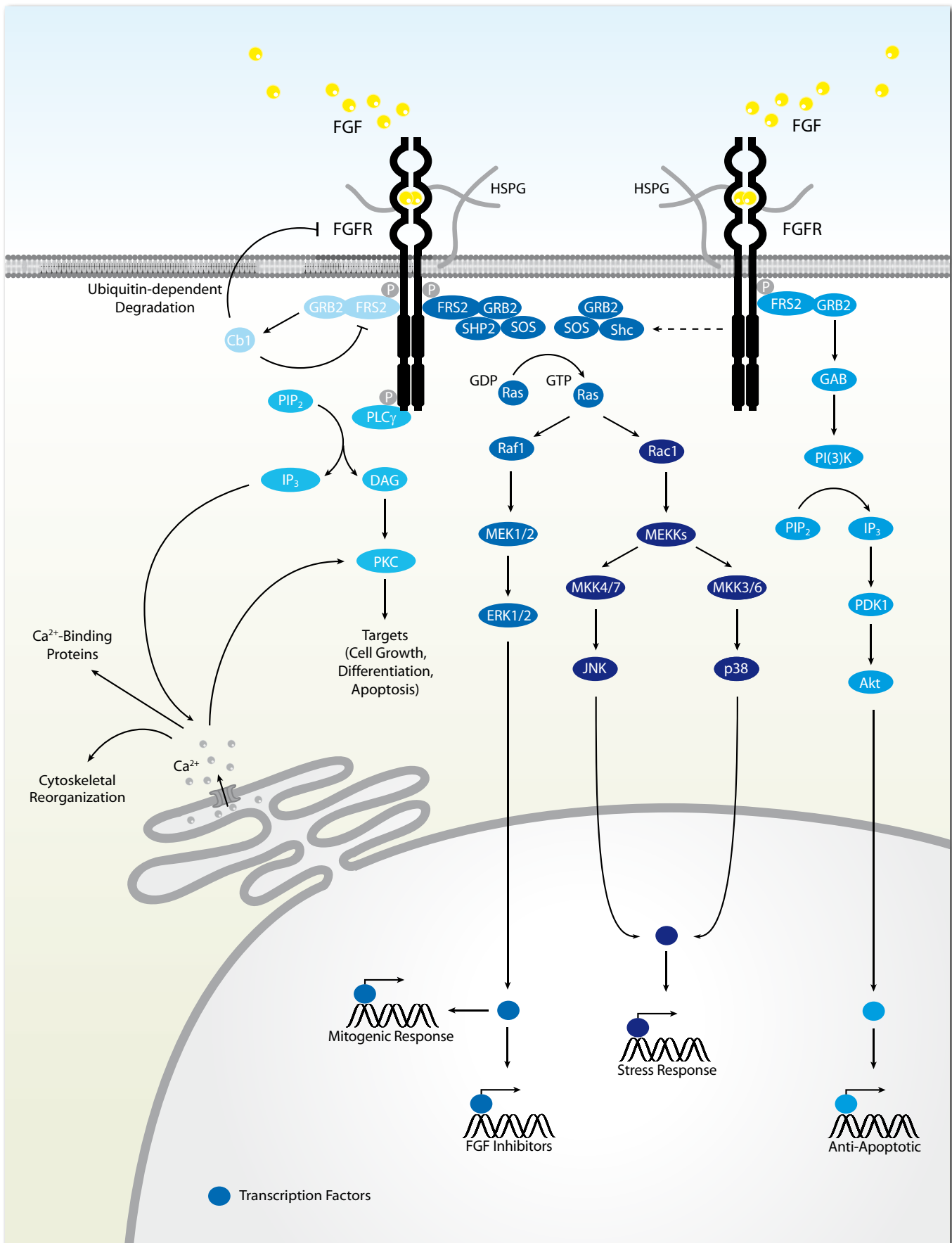
FGFs & Neural Stem Cells

Neural stem (NS) cells possess the characteristics of multipotent stem cells: first, the self-renewal ability and second, the ability to differentiate into neurons, astrocytes and oligodendrocytes. FGF-2 and/or epidermal growth factors (EGFs) are required for the *in vitro* culturing of NS cells. By withdrawal of FGF-2 *in vitro*, the differentiation of NS cells can be driven. *In vivo*, FGF-2 also plays an important role. In the event of brain damage, endogenous production of FGF-2 is necessary and sufficient to stimulate proliferation and differentiation of neural progenitor cells to repair brain lesions.

SELECTED LITERATURE REFERENCES

Fibroblast Growth Factor 4 and Its Novel Splice Isoform Have Opposing Effects on the Maintenance of Human Embryonic Stem Cell Self-Renewal: Y. Mayshar, et al.; *Stem Cells* **26**, 767 (2008) ■ Stem Cells and Early Lineage Development: J. Rossant; *Cell* **132**, 527 (2008) (Review) ■ Control of Stemness by Fibroblast Growth Factor Signaling in Stem Cells and Cancer Stem Cells: N. Gotoh; *Curr. Stem Cell Res. Ther.* **4**, 9 (2009) (Review)

FGF Signaling Pathway



Klotho – Beyond Spanning The Thread Of Life

The *klotho* gene was identified as a gene mutated in the klotho mouse [1]. The *klotho* gene encodes a single transmembrane protein and is highest expressed in distal convoluted tubules in the kidney and choroid plexus in the brain. Klotho plays a pivotal role in regulating aging and the development of age-related diseases. First, a loss of *klotho* results in multiple aging-like phenotypes [1]. Second, overexpression of the *klotho* gene extends lifespan [2]. Binding of the extracellular domain of Klotho directly to fibroblast growth factor receptors (FGFRs) increases their affinity for FGF-23. Therefore, Klotho functions as a co-factor/co-receptor of FGFRs and is essential for FGF-23 to activate FGF signaling [3].

It was demonstrated that the signal transduction pathways initiated by FGF-23-Klotho signaling prevents tissue atrophy by stimulation proliferation and preventing apoptosis caused by excessive systemic vitamin D [4]. Although Klotho-mediated FGF-23 signaling is well documented, it is not yet clear whether FGF-23 may also have Klotho-independent effects. In addition the extracellular domain of Klotho can be shed and secreted and may

- act as a circulating hormone,
- regulate insulin/insulin-like growth factor 1 (IGF1) signaling,
- suppress oxidative stress,
- act as a beta-glucuronidase and activate ion channels (such as TRPV5),
- protect against endothelial dysfunction, and
- regulate the production of nitric oxide (NO).

Furthermore, Klotho suppresses the insulin/IGF-1 signal pathway and influences p53/p21, cAMP, PKC and Wnt signaling pathways. Thus Klotho seems to be a multi-functional protein that regulates the phosphate/vitamin D metabolism through the bone derived hormone FGF-23 and plays a role in aging, cancer and stem cell biology. For a recent review see Y. Wang & Z. Sun: Ageing Res. Rev. **8**, 43 (2009).

LIT: [1] Mutation of the mouse klotho gene leads to a syndrome resembling ageing: M. Kuro-o, et al.; Nature **6**, 45 (1997) ■ [2] Suppression of aging in mice by the hormone Klotho: H. Kurosu, et al.; Science **309**, 1829 (2005) ■ [3] Klotho converts canonical FGF receptor into a specific receptor for FGF23: I. Urakawa, et al.; Nature **444**, 770 (2006) ■ [4] FGF-23-Klotho signaling stimulates proliferation and prevents vitamin D-induced apoptosis: D. Medici, et al.; J. Cell Biol. **182**, 459 (2008)

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Products

Klotho (extracellular domain) (human):Fc (human) (rec.)

AG-40A-0124-C010 10 µg
Produced in HEK 293 cells. The signal peptide and the extracellular domain of human klotho (aa 1-981) are fused at the C-terminus to the Fc portion of human IgG. **PURITY:** ≥90% (SDS-PAGE).

βKlotho (extracellular domain) (human):Fc (human) (rec.)

AG-40A-0125-C010 10 µg
Produced in HEK 293 cells. The extracellular domain of human βklotho (aa 1-996) is fused at the C-terminus to the Fc portion of human IgG. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <0.1EU/µg protein (LAL-test).

The FGF-11 Subfamily

FGF-11 to 14 are members of the FGF-11 subfamily and are also known as fibroblast growth factor-homologous factors (FHF). Generally, FHF are not considered as members of the FGF family. FHF have high sequence and structural homology with FGFs and bind HSPG with high affinity. However, they do not activate FGFRs, likely due to the structural incompatibility of the FGFR-interacting region. FHF act as intracellular signaling molecules via interaction with islet brain-2 scaffold protein and voltage-gated sodium channels.

SELECTED REVIEW ARTICLES

Molecular Pathology of the Fibroblast Growth Factor Family; P. Krejci, et al.; Hum. Mutat. **30**, 1245 (2009) ■ The FGF family: biology, pathophysiology and therapy; A. Beenen & M. Mohammadi; Nat. Rev. Drug Discov. **8**, 235 (2009)

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