Fibroblast Growth Factor Family

FGF Growth Factors, Receptors and Main Functions

Irvine Scientific R&D

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Introducing the FGFs

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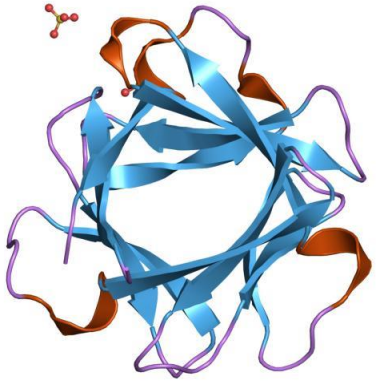
- The Fibroblast Growth Factor (FGF) family:
  - One of the largest peptide growth factors families
  - At least 28 distinct members identified in a variety of organisms
  - Pivotal roles in many cellular processes includ
    - Cell proliferation
    - Differentiation
    - Migration
    - Cell survival
  - In vertebrates, 22 multifunctional and structu related FGF members were found (FGF1 to 2
  - Ubiquitous involvement in embryo development and adult physiological processes



Introduction to Family Language

- Paracrine or canonical FGFs
  - Mediate biological responses by binding to and activating a cell surface tyrosine kinase receptors. They act as a local paracrine signaling molecules
- Endocrine or hormone-like FGFs
  - Mediate biological responses by binding a receptor, but function over long distance as endocrine hormones
- Intracrine or intracellular FGFs
  - Regulate the function of voltage gated sodium channels in a receptor independent manner

FGF Family Evolutionary History

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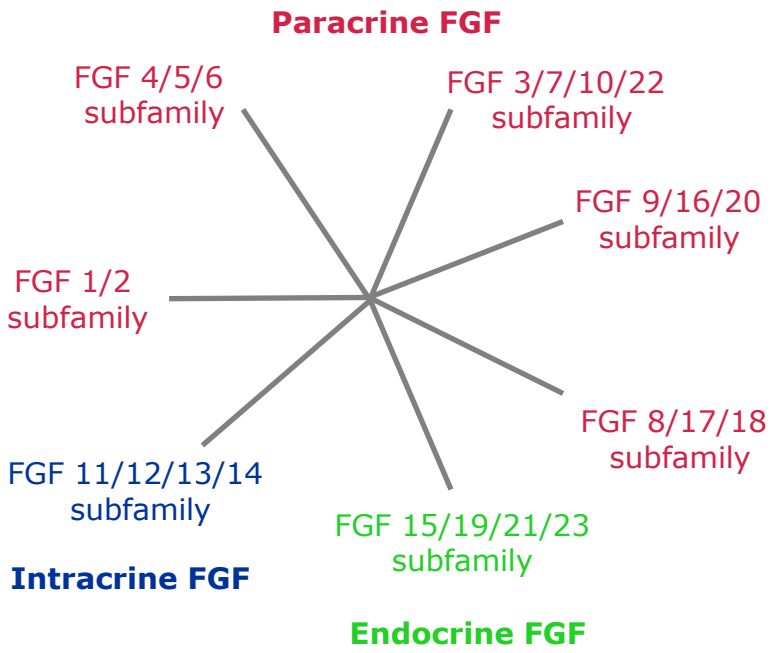
- FGF acidic (FGF1) and FGF basic (FGF2)
  - Purified from brain and pituitary as mitogenic factors for fibroblast grown *in vitro* in early 1970s
  - Since then FGF and FGFRs genes have been identified in multicellular organisms from worms to humans
- No FGF-like sequence have been found in unicellular organisms
- Evolutionary Comparison
  - Fewer FGF genes in *D. melanogaster* (1) and *C. elegans (2)* vs. large number in human and mouse (22) indicate gene duplication during evolution

Meet the FGF Family Members

- 7 FGF subfamilies, based on:
  - Phylogenetic analysis
  - Sequence similarities
  - Functional properties
- Endocrine FGFs
  - FGF15 has not been identified in humans
  - FGF19 has not been identified in mice nor rats



Adapted from [1] Itoh N & Ornitz DMJ. Biochem (2011)

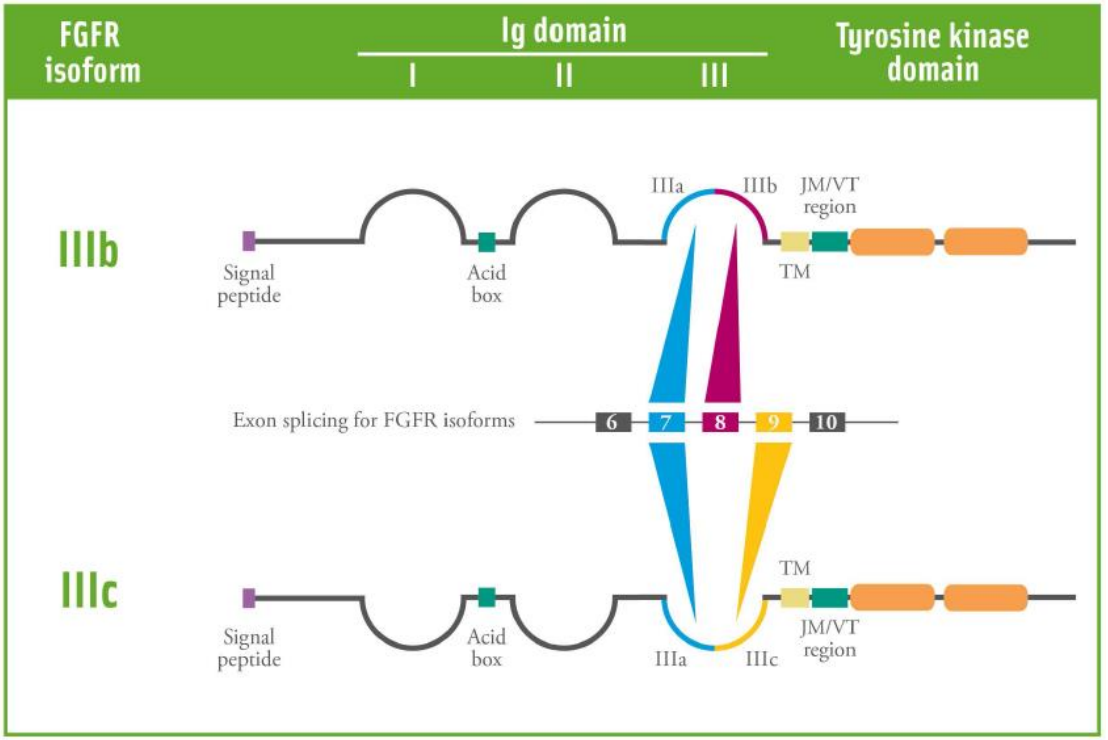
FGF Partners (Receptors)

- Fibroblast growth factor functions are induced or modulated by the interaction of FGFs with tyrosine kinase receptors (fibroblast growth factor receptors - FGFRs)
  - 4 different tyrosine kinase receptors named FGFRs1-4
- FGFRs consist of:
  - 3 extracellular immunoglobulin domains (D1-D3)
  - A single transmembrane domain
  - Cytoplasmic tyrosine kinase domain.
- Ligand specificity of FGFRs determined by acid box

[5] Beenken A & Mohammadi M. Nat Rev Drug Discov (2009)

The FGFRs

- Ligand binding specificity is mainly determined by Ig domain III
- Two forms of each FGFR are generated by alternative splicing of exons on the FGFR transcripts leading to two isoforms of the same receptor: IIIb and IIIc
- There are no isoforms of FGFR4



Adapted from Cotton M, O'Bryan MK, & Hinton BT. Endocrine Reviews. 2008

Alternative Splicing: process where a primary transcript is spliced into patterns for multiple proteins

FGF/FGFR Interactions

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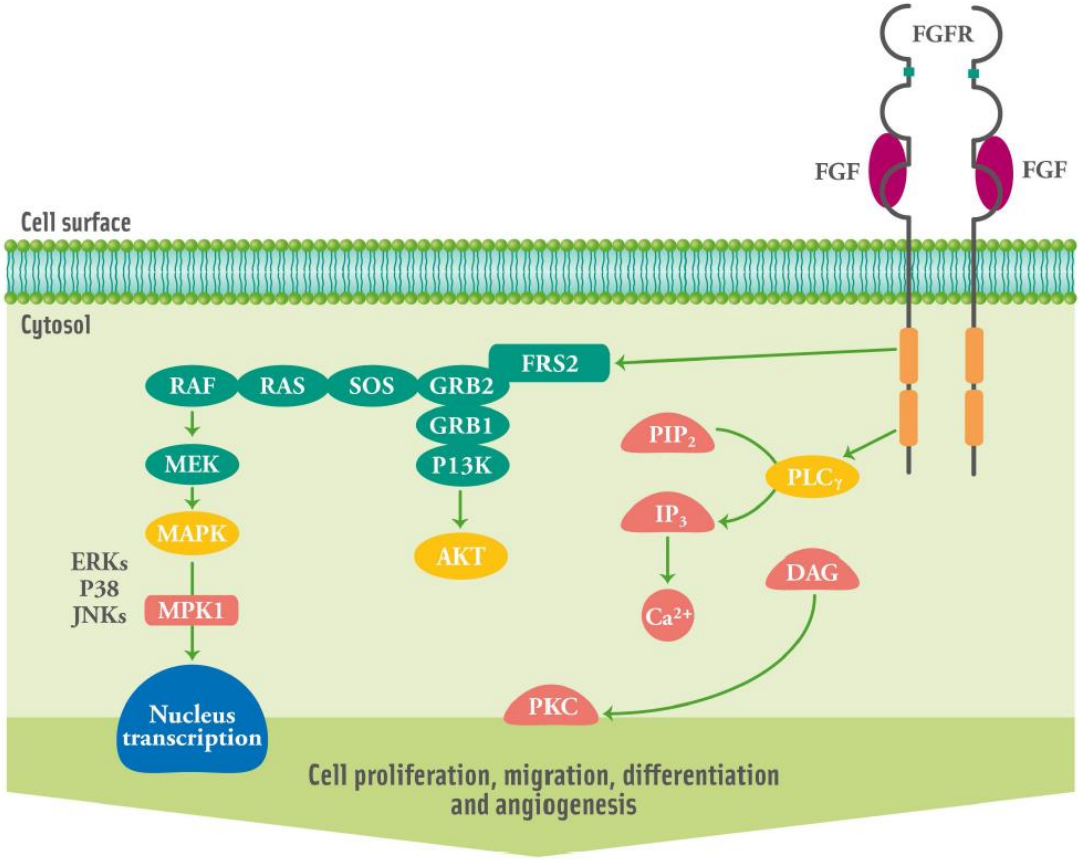
FGF (ligand)	Interaction with receptors			
	FGFR1	FGFR2	FGFR3	FGFR4
FGF1 (aFGF)	FGFR1: IIIb and IIIc	FGFR2: IIIb and IIIc	FGFR3: IIIb and IIIc	FGFR4
FGF2 (bFGF)	FGFR1: IIIb	FGFR2: IIIc	FGFR3: IIIc	FGFR4
FGF3	FGFR1: IIIb	FGFR2: IIIb		
FGF4	FGFR1: IIIc	FGFR2: IIIc	FGFR3: IIIc	FGFR4
FGF5	FGFR1: IIIc	FGFR2: IIIc	FGFR4	
FGF6	FGFR1: IIIc	FGFR2: IIIc		
FGF7		FGFR2: IIIb		FGFR4
FGF8	FGFR1[a]	FGFR2: IIIc	FGFR3: IIIc	FGFR4
FGF9		FGFR2: IIIc	FGFR3: IIIb and IIIc	FGFR4
FGF10	FGFR1: IIIb	FGFR2: IIIb		
FGF11-14	?			
FGF15	?			
FGF16, 18	?			
FGF17	FGFR1: IIIc	FGFR2: IIIc		FGFR4
FGF19, 21, 23	FGFR1: IIIc		FGFR3: IIIc	FGFR4
FGF20, 22, 24-25	?			

Based on Cotton M, O'Bryan MK, & Hinton BT. Endocrine Reviews. 2008

- Alternative splicing regulated in tissue specific manner
- Greatly affects ligand-receptor binding specificity:
  - FGFR2 IIIb isoform exclusively expressed in epithelial cells
  - FGFR2 IIIc isoform exclusively expressed in mesenchymal cells

FGFR Activation

- FGFR Activation: FGFs work with Heparin (H) and Heparan Sulfate Proteoglycan (HSPG) to activate FGFRs
- FGF + HSPG binding leads receptor homodimerization, activation and auto-phosphorylation of multiple tyrosine residues in the cytoplasmic domain of the receptor
- FGFRs can heterodimerize, thus adding another level of complexity of FGF responses modulation



Adapted from Yun et al. J Tissue Eng. 2010

Dimerization - chemical reaction that joins two subunits to form a dimer

Overview of Signaling Pathways

Dimerization of FGFRs results in trans-phosphorylation of specific intracellular tyrosine kinase residues, leading to the activation of different pathways:

- PLCγ → Morphology and migration
- PI3K/PKB → Survival
- Ras/ERK pathway → Proliferation and cell fate determination

[3] Dorey K & Amaya E. Development (2010)

The Enablers: Heparin Sulfate & Heparan Sulfate Proteoglycans

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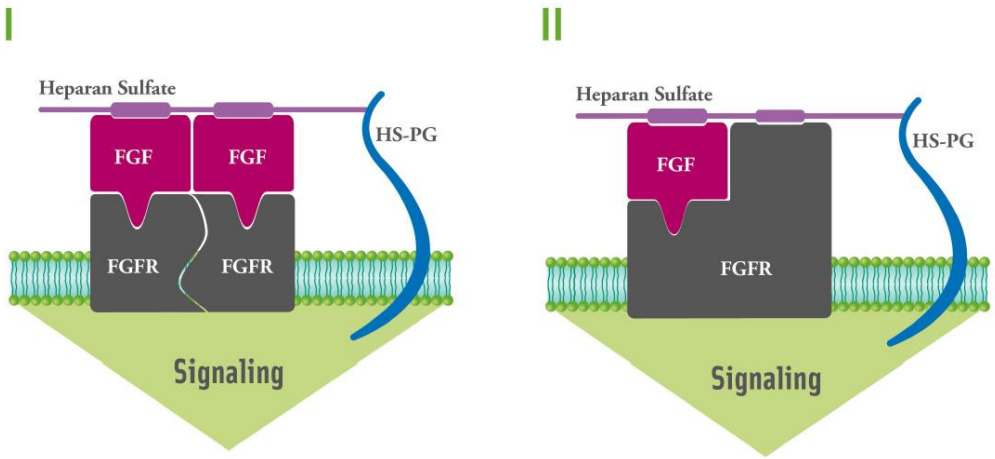
- Fibroblast growth factors act in concert with heparin sulfate (HS) or heparan sulfate proteoglycan (HSPG) and possibly other cell surface proteoglycans (syndecan family) to activate FGFRs
- In the absence of heparin sulfate, FGFs can activate their receptors but only at high concentrations in most cells
- FGFs do not induce cell growth in HS-deficient cells



Adapted from Lindahl, U., Lidholt, K., Spillmann, D., and Kjellen, L.: More to "heparin" than anticoagulation., Thrombosis Res., 75, 1-32, 1994

Noncanonical FGFR Signaling

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To add more complexity:

- FGF-FGFRs system can interact with a variety of proteins and glycolipids unrelated to the canonical FGF:FGFR-HSPG ligand receptor system:
  - Extracellular matrix protein fibronectin
  - Thrombospondin (TSP)
  - Long pentraxin 3 (PTX3)
- Non-FGF ligands can activate FGFR signaling in the absence of FGFs:
  - Neural cell adhesion molecule (NCAM)
  - N-cadherins

Role of FGF Signaling

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- FGF signaling plays an essential role in virtually every cell fate decision, patterning event and coordinated cell movement in the early embryo, but mechanisms not clearly understood
- In the adult, FGFs are homeostatic factors functioning in tissue repair, wound healing, control of the nervous system and tumor angiogenesis.
- Many paracrine FGFs are deregulated in cancers, as overexpression stimulates proliferation and angiogenesis, which contribute to cancer growth.

Physiological Role

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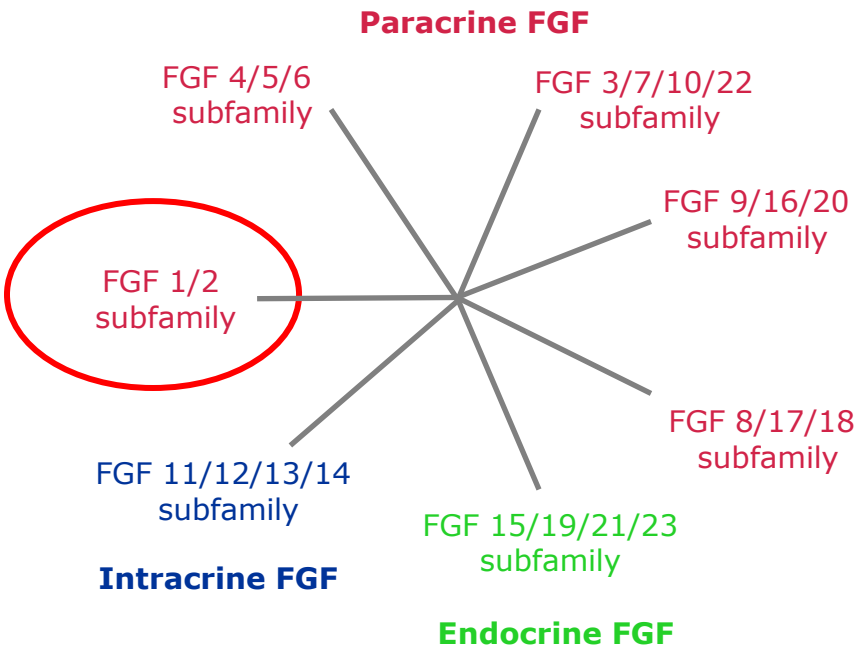
Fibroblast Growth Factor (FGF)	Phenotype of knockout mouse	Physiological Role
FGF1	Normal	Not established
FGF2	Loss of vascular tone Slight loss of cortex neurons	Not established
FGF3	Inner ear agenesis in humans	Inner ear development
FGF4	Embryonic lethal	Cardiac valve leaflet formation Limb development
FGF5	Abnormally long hair	Hair growth cycle regulation
FGF6	Defective muscle regeneration	Myogenesis
FGF7	Matted hair Reduced nephron branching in kidney	Branching morphogenesis
FGF8	Embryonic lethal	Brain, eye, ear and limb development
FGF9	Postnatal death Gender reversal Lung hypoplasia	Gonadal development Organogenesis
FGF10	Failed limb and lung development	Branching morphogenesis
FGF16	Embryonic lethal	Heart development
FGF17	Abnormal brain development	Cerebral and cerebella development
FGF18	Delayed long-bone ossification	Bone development
FGF19	Increased bile acid pool	Bile acid homeostasis Lipolysis Gall bladder filling
FGF20	No knockout model	Neurotrophic factor
FGF21	No knockout model	Fasting response Glucose homeostasis Lipolysis and lipogenesis
FGF22	No knockout model	Presynaptic neural organizer
FGF23	Hyperphosphataemia Hypoglycaemia Immature sexual organs	Phosphate homeostasis Vitamin D homeostasis

Based on [5] Beenken A & Mohammadi M. Nat Rev Drug Discov (2009)

Paracrine FGF Ligands: FGF1/2 subfamily

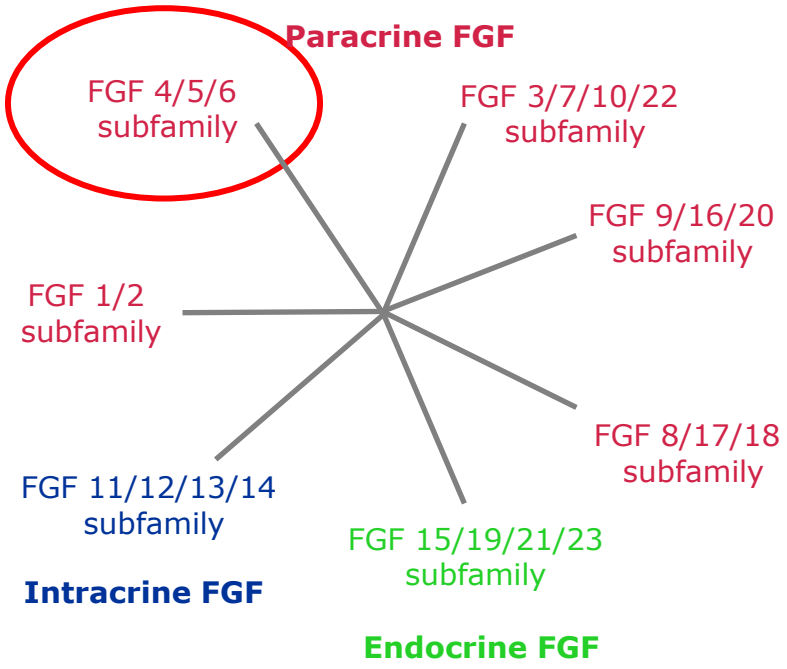
- Role of FGF1 and FGF2:
  - Physiological roles of are still unclear
  - Likely play a role in the maintenance of vascular tone
- FGF2 properties
  - Well established angiogenic properties in vivo
  - High anti-apoptotic activity



FGF2 Synonyms: FGF basic, bFGF, Prostatropin, HBFG-2

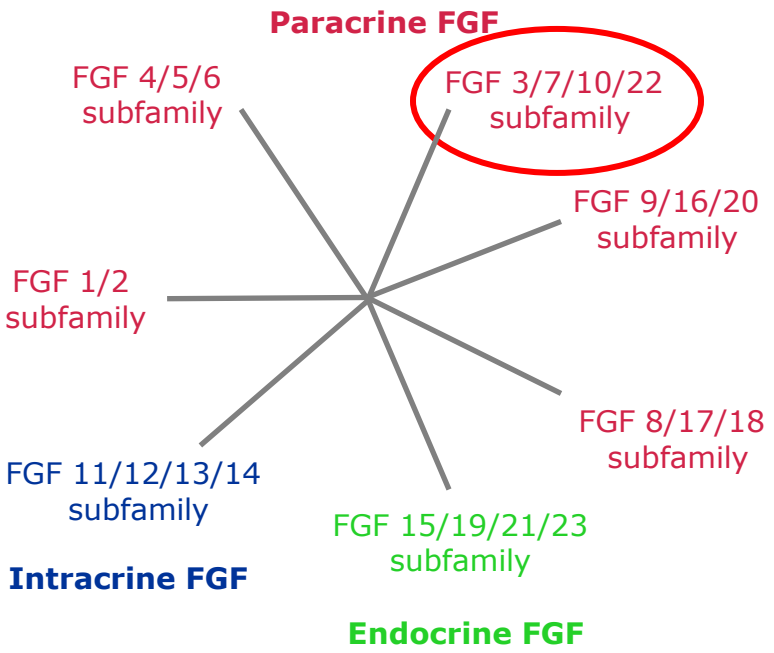
Paracrine FGF Ligands: FGF4 subfamily

- FGF4 has wide ranging functions in development:
  - Cardiac valve leaflet development
  - Limb development
- FGF5 negatively regulates hair follicle growth cycle.
- FGF6 plays a part in myogenesis



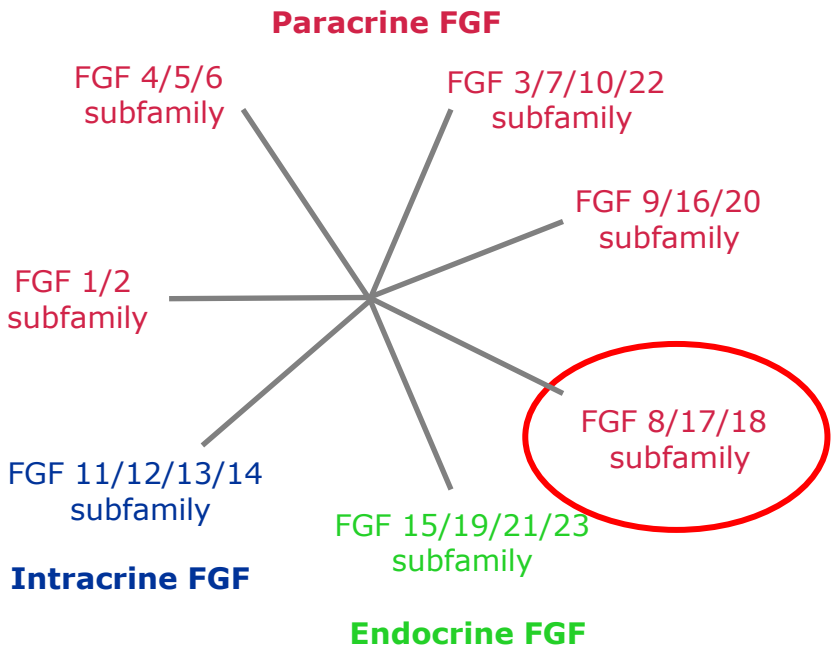
Paracrine FGF Ligands: FGF7 subfamily

- FGF7 (Keratinocyte growth factors) specifically expressed in the mesenchyme
- FGF7 family signals from mesenchyme to epithelium



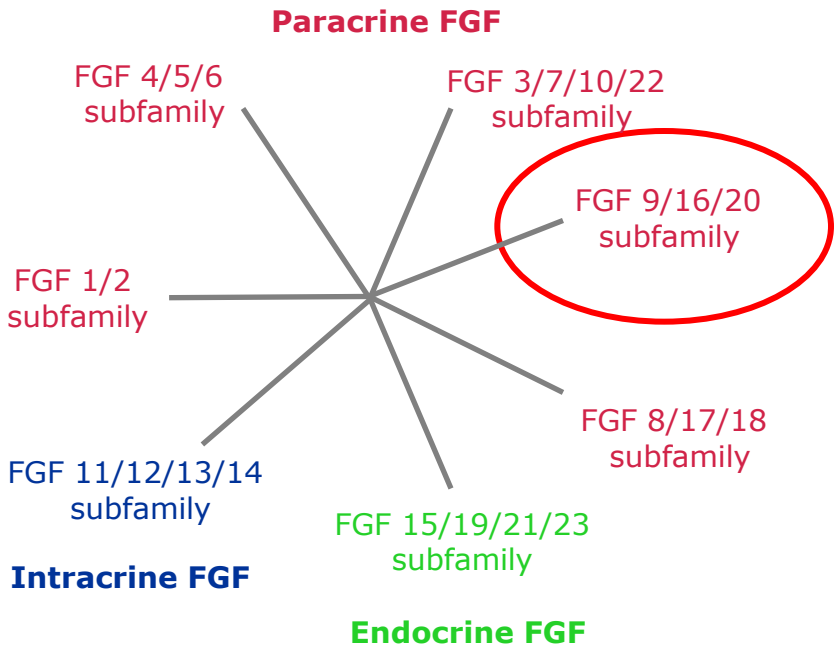
Paracrine FGF Ligands: FGF8 subfamily

- FGF8 involved in the development of:
  - Brain
  - Limb
  - Ear
  - Eye
- FGF17 and FGF8 are crucial for forebrain patterning
- FGF18 plays a role in osteogenesis



Paracrine FGF Ligands: FGF9 subfamily

- FGF9 family signals from epithelium to mesenchyme
- FGF9 stimulates mesenchymal proliferation which in turn produces ligands in FGF7 subfamily



The Adventurers: FGF-19 subfamily
a.k.a. Endocrine FGFs

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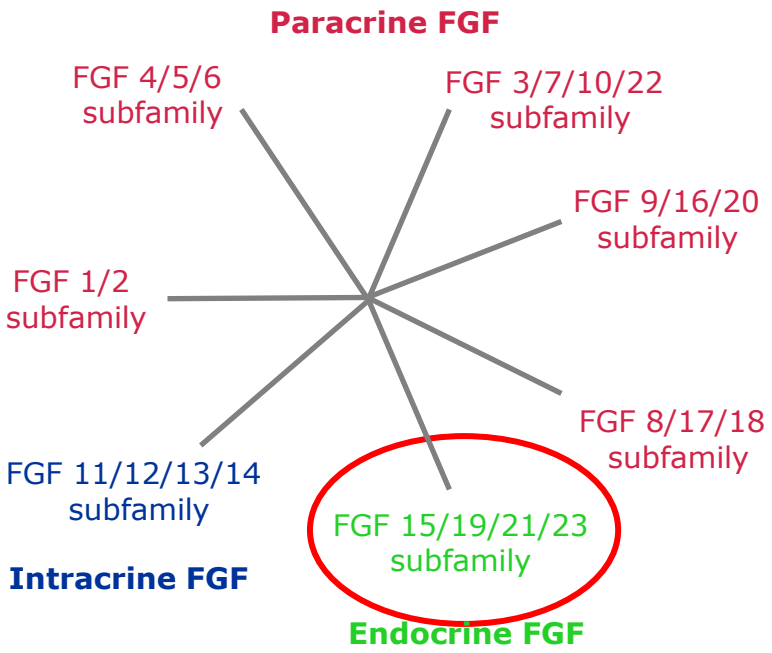
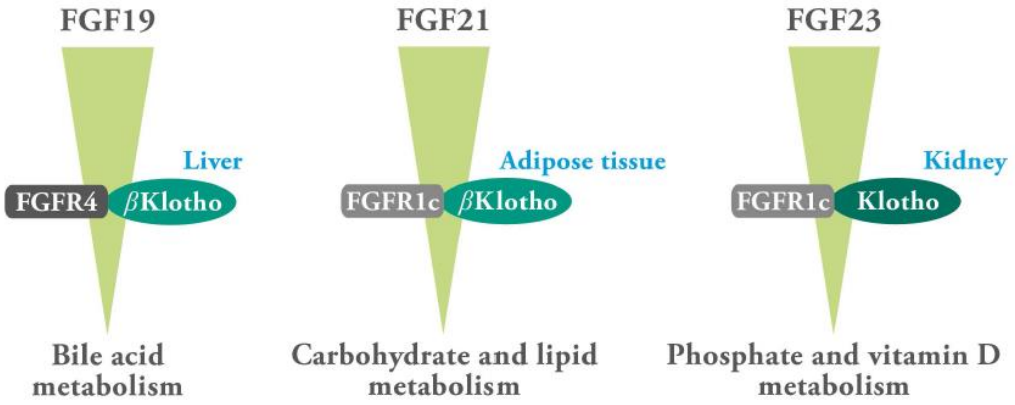
- Hormone-like growth factors (act far from expression site)
- Require additional cofactor beside HS to stabilize interaction with their FGFRs
- Protein Klotho identified as a necessary cofactor for FGF19, 21, and 23



FGF-19 Subfamily: Actions

- FGF15/19
  - Expressed in mice in regionally restricted patterns
  - Helps regulate cell division and patterning in brain
  - Role in energy metabolism
- FGF 21
  - Abundant expression in liver, pancreas, white adipose tissue and muscle
  - Role in energy metabolism
- FGF23
  - Mainly expressed in bone, role in regulation of vitamin D and phosphate metabolism in kidney.



Adapted from Fukumoto S. Actions and Mode of Actions of FGF19 Subfamily Members. Endocrine Journal 2008, 55(1), 23-31

The Misfits: FGF-11 subfamily
a.k.a. Intracrine FGFs

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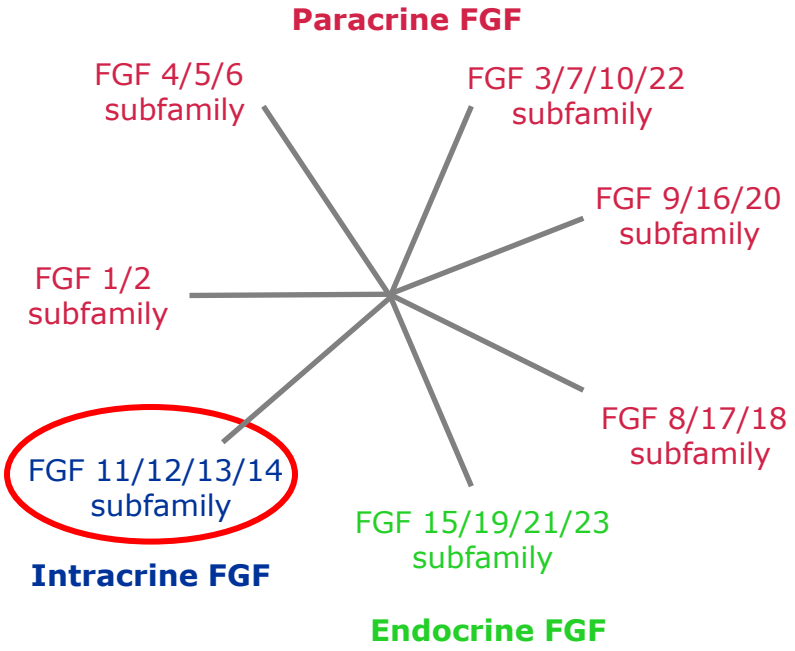
- Generally not considered a member of FGF family
- Also called FGF-homologous factors (FHFs)
- High sequence and structural homology with FGFs
- Bind HSPG with high affinity
- Do not activate FGFRs (structural incompatibility?)
- Act as intracellular signaling with voltage gated Na channels and islet brai-2 scaffold protein



Potential Clinical Applications

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Ligand or Receptor	Current/Potential Therapeutic Application
FGF1	Recombinant FGF1 used with nerve grafts Treatment of peripheral ischemia with FGF1 plasmid
FGF2	Use of thalidomide in prostate and renal cancer Implantation of FGF2-coated heparin beads post-MI Recombinant FGF2 modulates mood in mice
FGF4	Potential gene therapy for stable angina in women
FGF5	Potential of FGF5 inhibitors to aid hair growth
FGF7	Treatment of mucositis (known as the drug palifermin) Recombinant FGF7 improves wound healing
FGF18	Recombinant FGF18 has an anabolic effect on cartilage
FGF19	Potential of recombinant FGF19 in diabetes
FGF20	Potential in Parkinson's disease
FGF21	Potential of recombinant FGF21 in diabetes
FGF23	Use of anti-FGF23 antibodies in hypophosphatemia
FGFR1	PLC inhibitors in the treatment of EMS and as an adjunct to TKIs
FGFR2	Small molecule inhibitors and anti-FGFR2 antibodies in endometrial cancer
FGFR3	Small molecule inhibitors and anti-FGFR3 antibodies in multiple myeloma
FGFR4	Prognostic marker in prostate cancer and squamous cell carcinoma

Based on [5] Beenken A & Mohammadi M. Nat Rev Drug Discov (2009)

EMS: 8p11 myeloproliferative syndrome
MI: myocardial infarction
PLCγ: phospholipase C gamma
TKI: Tyrosine kinase inhibitor

Current Clinical Applications

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- Recombinant FGF7 is used to treat chemo-radiation induced mucositis
- Recombinant FGF1, FGF2 and FGF4 gene therapy for cardiovascular pathologies are being explored.
- FGF18 is in early stages of development for osteoarthritis treatment

About Irvine Scientific

- In the past 44 years, Irvine Scientific has become a worldwide leader in the design, manufacture and distribution of medical devices and cell culture media products for use in Cell Therapy & Regenerative Medicine, Industrial Cell Culture, Cytogenetics, and Assisted Reproductive Technology (ART).

- To supplement researchers' cell culture needs, Irvine Scientific offers a variety of high quality growth factors, including Recombinant Human FGF basic, Recombinant Human Activin A, Recombinant Human GM-CSF, and more

References

1) Itoh N & Ornitz DM. Fibroblast growth factors: from molecular evolution to roles in development, metabolism and disease. J. Biochem (2011) 149: 121-130 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3106964/
2) Itoh N. Hormone-like (endocrine) Fgfs: their evolutionary history and roles in development, metabolism, and disease. Cell Tissue Res. (2010) 342: 1-11 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2948652/
3) Dorey K & Amaya E. FGF signaling: Diverse roles during early vertebrate embryogenesis. Development (2010) 137: 3731-3742 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3747497/
4) Beenken A & Mohammadi M. The FGF family: Biology, pathophysiology and therapy. Nature Reviews (2009) 8: 235-253 http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3684054/
5) Polanska UM et al. Extracellular interactome of the FGF receptor-ligand system: complexities and the relative simplicity of the worm (2009) Developmental Dynamics 238: 277-293 http://onlinelibrary.wiley.com/doi/10.1002/dvdy.21757/pdf