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Paracrine Factors, Their Receptors, and Human Bone Growth

The fibroblast growth factor family constitutes one of the most important groups of paracrine factors that act during development. They are responsible for determining certain cells to become mesoderm, for the production of blood vessels, for limb outgrowth, and for the growth and differentiation of numerous cell types. As we study developmental biology, we will see fibroblast growth factors popping up all over the embryo.

In mammals, there are nine FGF genes and four FGF receptors (FGFRs). The actual number of FGFs and FGFRs is actually larger, since there are multiple splicing forms for the FGF receptors and alternate translation initiation sites for the FGFs.

Activation of the receptors

Fibroblast growth factor binding to their receptors causes the receptors to dimerize, and this results in the activation of their protein tyrosine kinases. These kinases phosphorylate each other and initiate downstream signaling. There are three components of this signal. The main signal involves the activation of the ras G protein and the MAP kinase cascade. In addition, the activated receptor stimulates phospholipase C to split PIP_2 into IP_3 and DAG. A third signal involves the phosphorylation of the Stat1 transcription factor and its subsequent translocation into the nucleus.

The binding of FGF to their receptors is accomplished with the aid of proteoglycans, namely heparin or heparan sulfate. Heparin proteoglycans act by binding several FGF molecules together in a web. It is this "web" of FGF that is presented to the receptors (Spivak-Kroizman et al., 1994). In this way, the receptors are crosslinked and brought together.

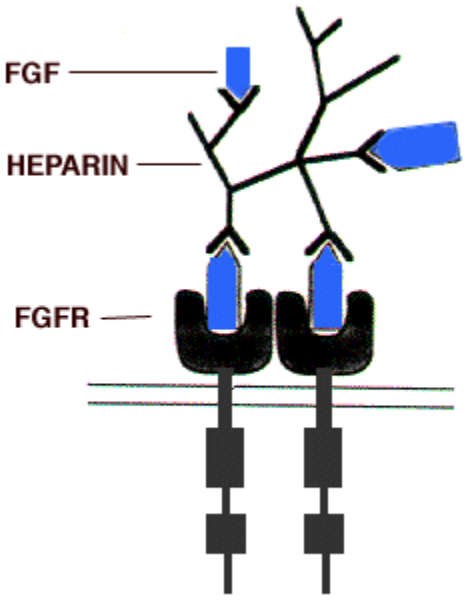

Figure 1 A model for heparin-induced dimerization and activation of the FGF receptors. (After Spivak-Kroizman et al., 1994).

Human developmental effects of FGF receptor mutations

Fibroblast growth factors play important roles in human limb and craniofacial development. This can be seen in dominant human conditions that are characterized by the premature differentiation of cartilage and bone. Mutations in FGFR1 can cause the Pfeiffer syndrome, a malformation syndrome characterized by limb defects and by the premature fusion of the cranial sutures (craniosynostosis) that results in abnormal skull and facial shape.

Mutations in FGFR2 can give a variety of syndromes (Figure 2). In one region of the protein, several mutations give rise to the Pfeiffer syndrome, just like the mutations of FGFR1. Overlapping this region is an area where mutations can give a different syndrome, the Crouzon Syndrome. Patients with Crouzon syndrome also have craniosynostosis, but have normal limbs. Also in this region are mutations that can give rise to the Jackson-Weiss syndrome, a syndrome of craniofacial malformation and foot abnormalities (enlarged great toes and the coalescence of the tarsals and metatarsals) and Apert Syndrome, a condition involving craniosynostosis and severe syndactyly (fusion of digits). The fact that identical FGFR2 mutations have been found in patients diagnosed with these three different syndromes suggests that these syndromes may actually constitute one spectrum of craniosynostosis and limb malformation anomalies, whose severity is modified by other genes (Park et al., 1995; Wilkie et al., 1995).

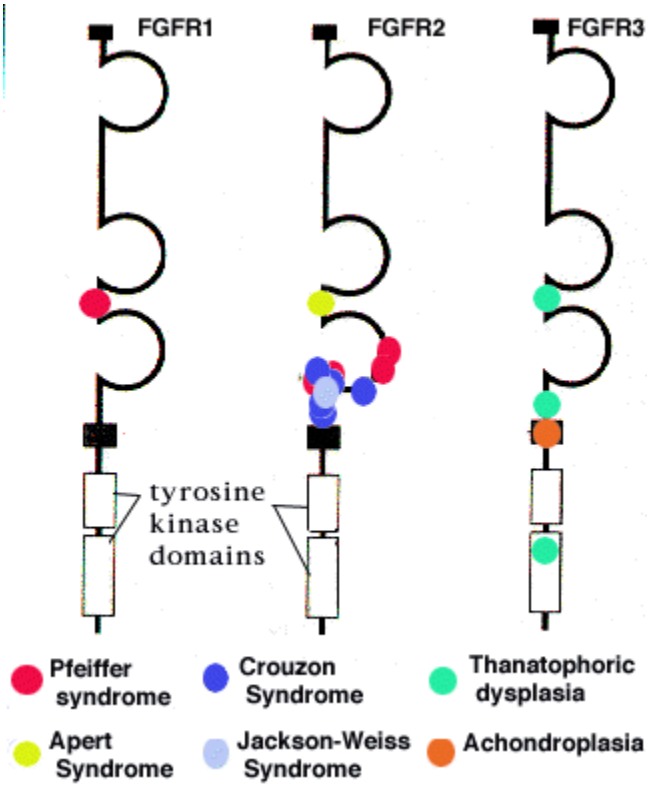

Figure 2 The different types of mutations in the human fibroblast growth factor receptors and their respective phenotypes. The black rectangle represents the transmembrane domain. The loops represent the immunoglobulin-like domains (and are stabilized by disulfide bridges) (After Yamaguchi and Rossant, 1995).

Mutations of FGFR3 give achondroplasia. Roughly 95% of the achondroplastic dwarfs have the same mutation of FGFR3, a base pair substitution that converts glycine to arginine at position 380 in the transmembrane region of the protein. In addition, mutations in the extracellular portion of the FGFR3 protein or in the tyrosine kinase intracellular domain have resulted in thanatophoric dysplasia, a lethal form of dwarfism that resembles homozygous achondroplasia (Bellus et al., 1995; Tavormina et al., 1995).

The mutations of fibroblast growth factor receptors that give rise to dwarfism and craniosynostosis are not loss-of-function mutations. Rather, they are gain-of-function mutations that enable the FGF receptor to become active without binding its FGF ligand (Webster and Donaghue, 1996; Deng et al., 1996). It appears that the normal role of the FGFs on cartilage cells is to limit cartilage cell growth. A constitutively active mutation would severely retard bone development in those areas (the skull and limb) where it normally functions. Conversely, the knockout of the FGFR3 gene from mice causes an expansion of endochondral bone growth. Most of these human FGFR mutations in single dosage do not appear to completely inhibit growth, as the homozygotes are more severely effected. The mutations of thanatophoric dwarfism appear to be most severe and resemble that of the achondroplasia homozygotes. The mutation in FGFR3 that characterizes thanatophoric dysplasia type II gives the FGFR3 constitutive tyrosine kinase activity. This particular activity has been seen to phosphorylate the Stat1 protein and to induce its translocation into the nucleus. Moreover, this kinase activity also promoted the expression of proteins that block the cell cycle (Su et al., 1997). Such constitutive Stat1 activation and cell cycle withdrawal were seen in cartilage cells from fetuses with thanatophoric dwarfism but not with cartilage cells from normal fetuses.

Literature Cited

Bellus, G. A. and several others. 1995. A recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. *Nature Genet.* 357-359.

Deng, C., Wynshaw-Boris, A., Zhou, F., Kuo, A., and Leder, P. 1996. Fibroblast growth factor receptor-3 is a negative regulator of bone growth. *Cell* 84: 911-921.

Park, W.-J., Bellus, G., Jabs, E. W. 1995. Mutations in fibroblast growth factor receptors: Phenotypic consequences during eukaryotic development. *Amer. J. Hum. Genet.* 57: 748-754.

Spivak-Kroizman, T., and several others. Heparin-induced oligomerization of FGF molecules is responsible for FGF receptor dimerization, activation, and cell proliferation. *Cell* 78: 1015-1024.

Su, W.-C. S., and eight others. 1997. Activation of Stat1 by mutant fibroblast growth-factor receptor in thanatophoric dysplasia type II dwarfism. *Nature* 386: 288-292.

Tavormina, P. L. and several others. 1995. Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor receptor 3. *Nature Genet.* 9: 321-328.

Webster, M. K. and Donoghue, D. J. 1996. Constitutive activation of fibroblast growth factor receptor 3 by the transmembrane domain point mutation found in achondroplasia. *EMBO J.* 15: 520-527.

Wilkie, A. O. M., Morriss-Kay, G. M., Jones, E. Y., and Heath, J. K. 1995. Functions of fibroblast growth factors and their receptors. *Curr. Biol.* 5: 500-507.

Yamaguchi, T. P. and Rossant, J. 1995. Fibroblast growth factors in mammalian development. *Curr. Opin. Genet. Devel.* 5: 485-491.