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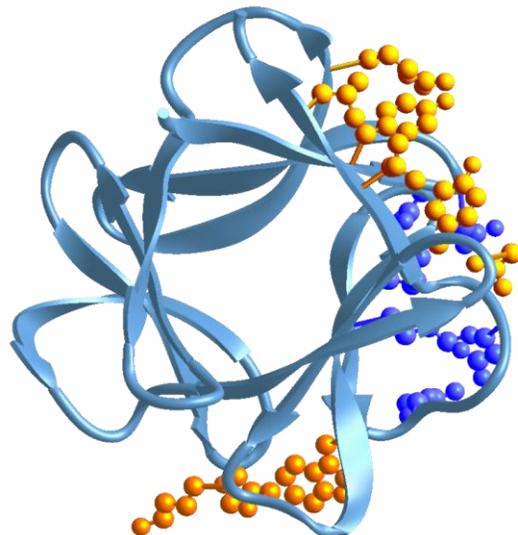
Human Fibroblast Growth Factor 1 (FGF-1): A New Treatment to Heal Diabetic Foot Ulcers

Summary: FGF-1 has shown remarkable efficacy in closing chronic diabetic ulcers, both in animal models and FDA-authorized clinical trials. In a Phase IIb trial, FGF-1 closed 100% of all ulcers treated over a 5 month period whereas roughly only 50% of the ulcers closed in the placebo arm. This paper will discuss the biological properties of FGF-1 which allow this molecule to be such a potent wound healing agent. The paper will also review preclinical and clinical data that is publicly available and supports an important role for FGF-1 in diabetic ulcer wound healing.

FGF-1 Structure and Receptor Binding: Human FGF-1 is a 141 amino acid monomeric protein devoid of any requisite post-translational modifications such as glycosylation. It was first isolated in its pure form in the early 1980s in the laboratory of Dr. Ralph Bradshaw at the

Figure 1: *Ribbon structure of the 141 amino acid FGF-1 showing the receptor binding residues (yellow) and the sites which tightly bind heparin (blue).*

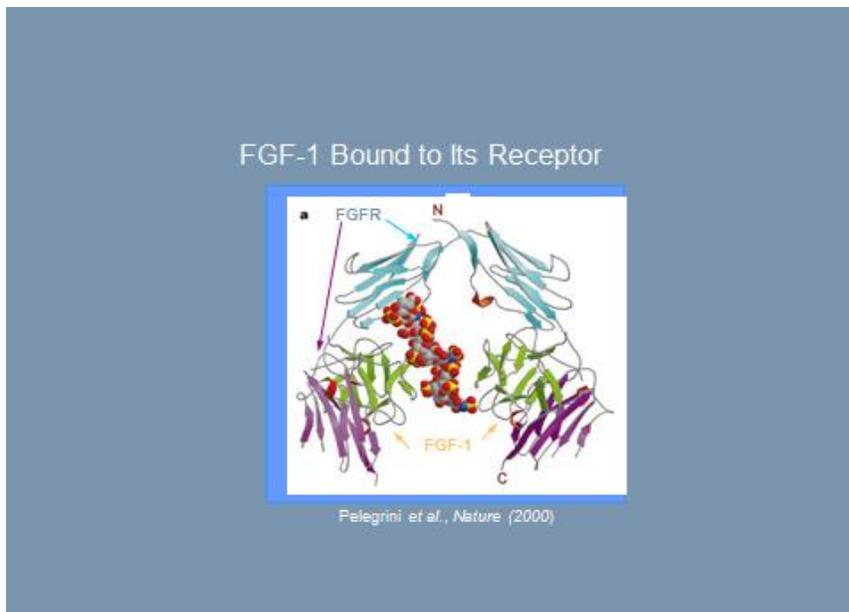
Blaber, Disalvo & Thomas Biochemistry (1996)



Washington University School of Medicine in St. Louis. The amino acid sequence of the protein was subsequently determined at Merck by a team led by Dr. Ken Thomas¹¹. Dr. Thomas then went on to determine the three dimensional structure of FGF-1 (see Figure 1 above). Human FGF-1 can be made as a recombinant protein in *E. coli* and its ability to bind strongly to heparin allows for a relatively easy purification by heparin affinity chromatography. This heparin binding ability is one reason FGF-1 is such a potent wound healing agent as it can stay resident in the wound bed for days bound to heparin moieties found in abundance on basement membranes of damaged tissues. The simplicity of the FGF-1 structure also makes it a very stable molecule and in the presence of heparin, FGF-1 is stable for 18 months at 4° C, a desirable quality for a pharmaceutical.

A second group of scientists were then able to co-crystallize FGF-1 together with its receptor to show that the receptor complex consisted of 2 FGF-1 molecules bound to each receptor molecule as shown in Figure 2 below.

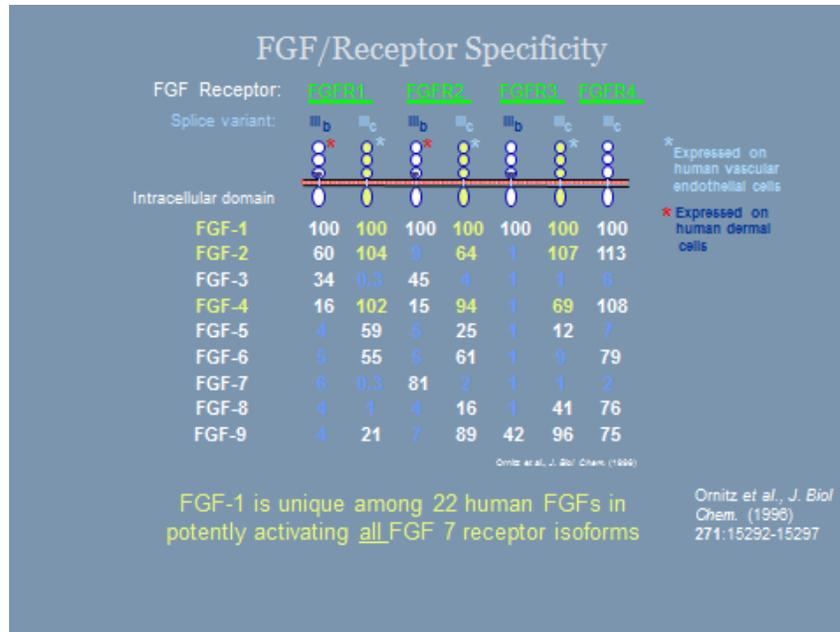
Figure 2: *The interaction of FGF-1 with its receptor where two molecules of FGF-1 fill one receptor site.*



FGF-1 is a member of a family that includes 22 FGF proteins. FGF-2 or basic FGF has also been extensively characterized and was in development for the treatment of stroke. FGF-7 or keratinocyte growth factor is an FDA approved drug and is used to regenerate the epithelium inside of the mouths of cancer patients undergoing chemotherapy. The 22 members of the FGF family interact with seven

distinct FGF cell surface receptors. FGF-1 is the only member of the family of 22 FGFs that binds to all 7 receptor isoforms with high affinity (as depicted in Figure 3 below). Also, FGF-1 is the only growth factor known to be potently mitogenic for dermal fibroblasts, vascular endothelial cells, and epidermal keratinocytes, the three major cell types present in skin. These structural properties and biologic activities make it an extremely attractive therapeutic agent to promote dermal healing.

Figure 3: *The interaction of FGFs with the seven known receptors for this family of growth factors. Only the first nine members of the family are depicted. FGF-1 is unique in showing a strong interaction with all seven receptor subtypes.*



FGF-1 biological actions: FGF-1 is present in a wide range of tissue types and is implicated in a broad array of biological functions including embryonic development, cell proliferation and differentiation, and tissue repair including dermal wound healing. As mentioned above, FGF-1 is the only growth factor known to be mitogenic and chemotactic for the three major cell types present in skin: dermal fibroblasts, vascular endothelial cells, and epidermal keratinocytes as demonstrated by *in vitro* studies⁸⁻¹². FGF-1 is also mitogenic for pericytes, capillary smooth muscle cell-like cells that decorate the microvasculature and are a necessary component for the formation of new capillaries¹³. In addition, FGF-1 has been shown to induce angiogenesis in specially designed assays for blood vessel growth employing embryonic chick chorioallantoic membranes and rabbit corneas^{10, 14}.

Successful wound healing acceleration in healthy animal models. Early animal studies at Merck demonstrated FGF-1's ability to accelerate wound healing in both healthy mice and rats, with low dose therapy resulting in a two-fold increase in the rate of full-thickness wound closure⁸. Further, these studies demonstrated *in vivo* stimulation of angiogenesis, granulation tissue formation, and the growth of new epithelium as measured by quantitative histomorphometric analyses.



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