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Functional Adaptations in Fibroblast Growth Factor (FGFs) Family

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Abstract: *Fibroblast Growth Factor Homologous Factors (FHFs) are member of Fibroblast Growth Factors (FGFs) family and shares high degree of structural and sequence similarity but are functionally distinct. Only a few residues seem to be responsible for functional divergence. The high conservation of Asn 95 in most FGFs can be explained by the ability of the Asn side chain to interact with residues conserved Arg 250 of Fibroblast Growth Factor Receptors (FGFRs), thereby stabilizing FGF-FGFR complex. Presence of Val95 in the case of FHFs indicates it to be a key residue for functional adaptation. When considering the "function/stability trade-off" hypothesis, the β-trefoil architecture appears capable of diverse functional adaptation.*

Keywords: *Fibroblast Growth Factor; Fibroblast Growth Factor Homologous Factor; β-trefoil; Functional Adaptation*

I INTRODUCTION

Fibroblast growth factors (FGFs) are family of 23 polypeptide growth factors that shares a common core (Ornitz and Itoh 2001). FGFs stimulate a verity of cellular functions like cell proliferation, division, embryonic differentiation and morphogenesis by binding to cell surface FGF receptor (FGFRs). In the adult, FGFs play important roles in regulating homeostasis, wound healing, and tissue repair (Finch, Rubin et al. 2004) by activating four distinct FGFRs which consist of three extra cellular immunoglobulin like domains (D1, DII and DIII), single transmembrane domain and a single intracellular tyrosine kinase domain. Alternative RNA splicing that utilizes one of two unique exons results in two different versions of Ig-like domain III (referred to as domains IIIb and IIIc) in FGFRs 1–3. This alternative splicing is an essential determinant of ligand binding specificity of FGFRs (Johnson, et al. 1991; Chellaiah, et al. 1994; Ornitz, et al. 1996; Yeh, et al. 2003).

FGFs differ significantly in both size (17-25kDa) and sequence (13-71% sequence similarity), but all contains a homologous core region of 120-130 residues. FGFs can be grouped into seven subfamilies based on their sequence similarities and functional properties (Itoh and Ornitz 2004; Zhang, et al. 2006). Human acidic fibroblast growth factor (FGF1) is considered as a universal FGF and can activate all FGFRs. Comparison of the crystal structures of FGF1-FGFR1c, FGF1-FGFR2c, and FGF1-FGFR3c complexes has provided key insights into the unique FGFR binding promiscuity of FGF1 (Olsen, Ibrahimi et al. 2004). The FGF core homology region assumes a β-trefoil fold consisting of 12β strands arranged in three sets of four-stranded β-sheet. (Eriksson, Cousens et al. 1991; Zhu, Komiya et al. 1991; Bernett, Somasundaram et al. 2004). As a member of the β-trefoil superfold (Murzin, Lesk et al. 1992), FGF-1 exhibits a characteristic pseudo-threefold symmetry when viewed down the β-barrel axis. The repeating structural unit of this threefold symmetry comprises of a pair of anti-parallel β-sheets, referred to as a "β-trefoil fold." The tertiary structure can be described as a six-stranded β-barrel closed off at one end by a β-hairpin triplet. The β-trefoil structure is hypothesized to have evolved by successive gene duplication and fusion events, and the β-trefoil has been identified as a monomeric structural element in epidermal growth factor, as a dimeric element in the structure of the protease inhibitor ecotine and as a trimeric arrangement in the β-trefoil superfold (Mukhopadhyay 2000; Ponting and Russell 2000).

II FUNCTIONAL ADAPTATION

Fibroblast growth factor homologous factors 1, 2, 3, 4 (also designated as FGFs-12, 13, 11 and 14, respectively) are member of Fibroblast Growth Factor family and were discovered within vertebrate DNA sequence databases by virtue of their similarity to FGFs **(**Smallwood et al., 1996; Hartung et al., 1997). Insipte of high sequence similarity, FHFs shares features that set them apart from

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other FGFs. Unlike most of the FGFs (except FGF-1), FHF proteins are synthesized without secretion signal sequence and can not be secreted outside the cell (Smallwood et al., 1996; Wang et al., 2000). Fibroblast growth factor homologous factors (FHFs) do not binds to FGFRs and functionally distinct from FGFs and have no detectable affinity for FGFRs. (Zhang et al., 2006). Crystal structure of FGF-12 has been recently reported (PDB accession number 1Q1U). An overlay of the structure of FGF-12 (FHF-2) with FGF-1 shows a high degree of the structural similarity. Both have β-trefoil structure with longer heparin binding loop in case FHF-12 (Fig 2). Similarly, an analysis of the sequence of all FGFs shows a high sequence homology **(**Smallwood et al., 1996; Hartung et al., 1997). This raises some interesting questions. Why these two highly homologous proteins have different function? Are Arg52 and Val95 residues in FHFs are responsible for different functions?

FGF shows three fold symmetry of tertiary structure (Brych et al., 2001; Brych et al., 2005; Dubey et al., 2005). A sequence analysis derived from a comparison of 23 members of the FGF family indicates that a Val residue at position 54 is present with a frequency of occurrence of 87.0 %. The only other residue found at this position (13.0% occurrence) is another β-branched residue Ile. Other symmetry related position 95, where Val occurs with 17.3 % frequency while Asx (Asn/Asp) is highly conserved. Careful sequence analysis shows that the Val residue is present only in fibroblast growth factor homologous factors (FGF-11, FGF-12, FGF-13 and FGF-14).

Analysis of symmetric FGFR-FGF complex (PDB accession 1E0O) indicates Asn95 is important for FGFR binding(Plotnikov, Schlessinger et al. 1999). Asn 95 makes an important hydrogen bonding interaction with Arg 250 in the D2 and D3 linker of FGFR. Interestingly, Arg 250 is conserved in all FGFRs indicating that Arg 250 in FGFRs and Asn 95 are co-evolved for functionality (Fig 1). Fibroblast growth factor homologous factors (FHFs) do not binds to FGFRs and functionally distinct from FGFs and have no detectable affinity for FGFRs. The function of FHFs are not clearly understood but several reports about its affinity for intracellular domains of voltage-gated sodium channels (VGSCs), neuronal MAP kinase scaffold protein and islet-brain-2 (IB2) have been published (Olsen, Garbi et al. 2003; Goldfarb 2005). Structures of FHFs in complex with known protein targets (IB2 and VGSCs) have not been reported as of yet. However, FHF mutagenesis in conjunction with binding studies has provided some important insights. Arginine-52 in the β4–β5 loop and valine-95 in the β9 strand are FHF surface residues conserved in all FHFs and not present in FGFs. Mutation of either residue to its most common FGF counterpart (R52G or V95N) prevented FHF binding to either IB2 or to VGSCs (Olsen, Garbi et al. 2003). These studies indicates that the Val95 and Asn 95 residues is functional requirement of FHFs and FGFs respectively and shows a protein is capable of diverse functional adaptation. Asn 95Val mutation in FGF-1 shows a substantial increase in stability (Dubey et al., unpublished data). We propose that the hypothesized capacity for extreme thermostability of the β-trefoil architecture underscores the selection of the β-trefoil architecture as one of the fundamental protein superfolds. Given the evidence for a stability\function trade-off, diverse functional radiation requires a protein architecture with the capacity to offset a wide range of destabilizing mutations. Conversely, a protein architecture with limited capacity for thermal stability is unlikely to be capable of diverse functional adaptation.

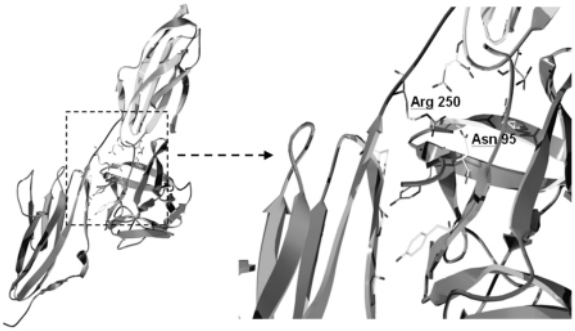

Figure 1: Diagram Showing Interactions of Asn 95 and with FGFR-1 (PDB Accession:1EVT). Fibroblast Growth Factor Homologous Factors (FGF-11, FGF-12, FGF-13, FGF-14) Which do not Bind to Receptor, have Val Residue at Homologous Position of 95.

Figure 2 : Diagram Showing an Overlay of Structure of FGF-12 (PDB Accession: 1Q1U) and FGF-1 (PDB Accession: 1JQZ).

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