Student: [redacted]
Mentor: [redacted]
Institution: University of Arkansas- Fayetteville
Classification: Senior
Grade Point Average: 3.7
Area of Study: Nursing
Title of Project: Characterization of Stable Fibroblast Growth Factor 2 using Consensus Protein Design

Project Summary

Belonging to a family of twenty-two, fibroblast growth factor 2 (FGF2) is a protein that specializes in the roles of wound healing, regeneration, angiogenesis, and cell proliferation. These critical functions provide promise for future medical application and tissue engineering, especially in populations that are immunocompromised and struggle overall with wound healing (i.e. diabetics). While such potential exists for FGF2, the wild type exhibits stability complications. Using evolutionary informatics, a new computerized sequence has been generated to contain the most frequently observed amino acids from a variety of mammalian relatives (mice, humans, etc): this is known as consensus design, which is proposed to be a more stable model for the application of FGF2. The wild type (wtFGF2) primarily carries out its function by binding and dimerizing with its receptor (FGFR), however, the generated Consensus FGF2 (cFGF2) lacks the amino acids involved in FGF2-receptor binding. In this context, a Quadruple mutant of consensus FGF2 (R39Q/V41E/F71V/K76F) has been generated by replacing some of the cFGF2 amino acids with the receptor-binding residues. In my project, I will use biophysical techniques to first obtain pure proteins, test its stability under various thermal conditions, and then characterize the function of the wild-type, consensus, and mutant forms of FGF2. After determining the stability and the proliferative activity of the cFGF2 and mutant, the future perspective will be to develop a topical medication to improve wound healing among high-risk individuals.

Project Proposal

Specific Aims:

1. Design, overexpression, and purification of "decoy" consensus FGF2
2. Characterization of the structure and stability of consensus FGF2 and Quadruple mutant (R39Q/V41E/F71V/K76F)
3. Comparison of the cell proliferation activity of wild-type FGF2, consensus FGF2 and Quadruple mutant (R39Q/V41E/F71V/K76F)

Introduction

Injury to the skin stimulates the initiation of the immune response's protective measures against the invasion of foreign bodies. Events such as inflammation, new tissue formation, and tissue remodeling are a result of the body's shift in defense; the human body immediately releases growth factors, cytokines, and low-molecular weight compounds to initiate the healing process[1]. Human acidic fibroblast growth factor 2 (FGF2) is a protein that belongs to a multifunctional and highly conserved subfamily of growth factors found within adult and embryonic cell types[1]. It plays a significant role in wound healing, regeneration, angiogenesis, and cell proliferation as it is one of the first agents to appear at the site of injury to influence the healing process[2].

While such critical roles exist, there are populations of people who are at risk for poor wound healing due to limited access of FGF2 stores in the body (immunocompromised individuals, diabetics, etc). For those with significant healing disorders, to treat a wound effectively, the goal would be to create an exogenous source of FGF2 to apply directly on the wound. While biologically powerful in its wild-type form, FGF2 has proven to present stability

complications both *in vivo* and *in vitro*[3]. This dilemma has been recognized by the Kumar Lab and provides the reasoning behind my research in the lab.

A popular strategy to combat instability is to use the evolutionary information found within homologous protein sequences of mammalian relatives (humans, mice, etc). This is performed by pulling the most frequently observed amino acids from each position in the alignment to create a new, diverse sequence known as the "consensus" design[4]. Using this model, consensus FGF2 (cFGF2) has been generated and found to interact with high-affinity transmembrane receptors to influence cell proliferation; this has been assessed for clinical use in the regeneration of damaged tissue[5]. FGF2 naturally carries out its function by binding and dimerizing with its receptor (FGFR), however, the generated cFGF2 lacks the amino acids involved in FGF2-receptor binding. In this context, a Quadruple mutant (R39Q/V41E/F71V/K76F) of the consensus was generated to include the receptor-binding residues necessary for structural stability.

My project aims to use biophysical techniques to first obtain pure proteins, test its stability under various thermal conditions, and then characterize the function of the wild-type, consensus, and mutant forms of FGF2. After determining the stability and the proliferative activity of the Consensus FGF2 and mutant, the future perspective will be to develop a topical medication to improve wound healing among high-risk individuals.

Preliminary Data:

Sequence of the Quadruple mutant (R39Q/V41E/F71V/K76F)

[redacted]

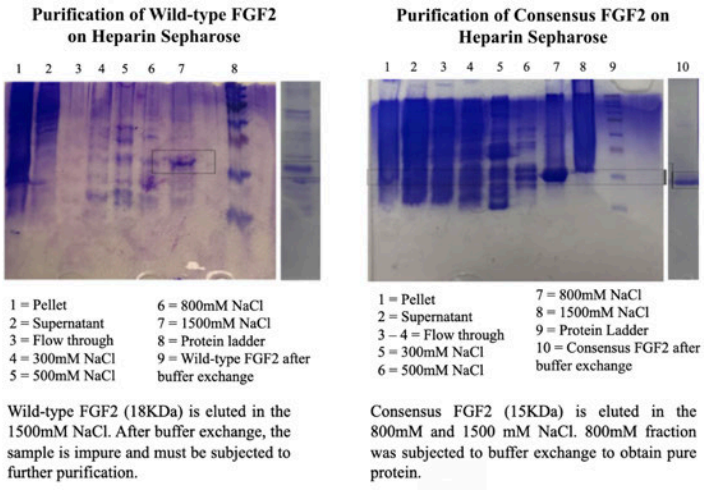
Consensus ("Decoy") FGF2 Sequence

[redacted]

Wild-Type FGF2 Sequence

[redacted]

Preliminary Data Related to the Consensus and Wild Type Forms



Source from [redacted] 2023 National Conference for Undergraduate Research presentation;

Conclusory data for the purification of wild type and consensus FGF2.

Experimental Methods:

I will use the following techniques to determine the structure and, therefore, the structural forces that contribute to enhanced stability and function of the R39Q/V41E/F71V/K76F mutant FGF2 protein. Control studies of wild-type and consensus FGF2 have already been performed as a comparison in this lab.

Over-expression and Purification of FGF2 Mutant*:* Using well established methods within the Kumar lab, I will transform Escherichia coli (BL21 star) cells with the plasmid containing the recombinant Quadruple mutant FGF2 via sonication. I will use Affinity chromatography as the method used to purify the desired protein; this will be achieved by using a heparin-Sepharose column with an increasing NaCl gradient in 10mM sodium phosphate buffer pH 7.2. I will verify the results of purification using SDS-PAGE analysis.

Limited Trypsin Digestion*:* I will use this method to measure the proteolytic resistance and structural flexibility of the Quadruple mutant. Given the protein to trypsin ratio should be 1:3, I will perform this experiment with 0.5 μM of protein sample in 1M tris-HCl with pH-8.0 at 37 °C. I will assess the results via SDS-PAGE. Then, I can plot a graph of the intensity of the undigested to the digested sample using UN-SCAN-IT gel software.

CD and Fluorescence Spectroscopy: Using the change in fluorescence emission profile of the FGF2 mutant, I will make an assessment of the secondary and tertiary structure of Wild-type FGF2 and Consensus FGF2.

NMR Spectroscopy*:* Due to its superior dispersion, I will perform the 15N-heteronuclear single quantum correlation (HSQC) experiment to track chemical shifts in wtFGF2, Consensus FGF2 and mutant. I will acquire two-dimensional proton NMR experiments on Bruker 700 MHz NMR

spectrometer equipped with a cryoprobe. I will monitor 1H-15N chemical shift perturbation by acquiring 1H15N HSQC spectra of wtFGF2 and the mutant.

Timeline

August-October: Large scale expression and purification of Quadruple mutant, consensus FGF2 and wtFGF2 using a Heparin Sepharose column.

November: Protein backbone flexibility testing using limited trypsin digestion.

December: Bioactivity of Quadruple mutant, consensus FGF2 and wtFGF2 .

January-February: Structural characterization of Quadruple mutant, consensus FGF2 and wtFGF2 using circular dichroism and fluorescence spectroscopy.

March-April: High-resolution NMR spectroscopy on the Quadruple mutant and the ligand-binding domain.

May: Verification of results to ensure accuracy and reproducibility; final analysis and drafting of project findings report.

References

1. Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. *Physiol Rev.* 2003;83(3):835-870. doi:10.1152/physrev.2003.83.3.835
2. Holoubek J, Lipový B, Knoz M, et al. The future for the application of fibroblast growth factor 2 in modern wound healing. *Burns*. 2023;49(2):484-486. doi:10.1016/j.burns.2022.10.007
3. Ribatti D, Vacca A, Rusnati M, Presta M. The discovery of basic fibroblast growth factor/fibroblast growth factor-2 and its role in haematological malignancies. Cytokine Growth Factor Rev. 2007;18(3-4):327-334. doi:10.1016/j.cytogfr.2007.04.011
4. Porebski BT, Buckle AM. Consensus protein design. *Protein Eng Des Sel.* 2016;29(7):245-251. doi:10.1093/protein/gzw015
5. Lee JH, Lee JE, Kang KJ, Jang YJ. Functional efficacy of human recombinant FGF-2s tagged with (His)6 and (His-Asn)6 at the N- and C-termini in human gingival fibroblast and periodontal ligament-derived cells. *Protein Expr Purif.* 2017;135:37-44. doi:10.1016/j.pep.2017.05.001