Investigations into the Significance of Epidermal Fatty Acid Binding Protein (FABP5) in Breast Cancer Survival and Design of Novel FABP5 Inhibitors

A.) Rationale

In the United States, on average, a woman is diagnosed with breast cancer every two minutes. Treatment strategies for combating tumors typically focus on the use of small chemical compounds (drugs) to block the function of one or more related proteins, which are large molecules that carry out a variety of chemical reactions vital to the normal functions of all cells. Recent evidence has highlighted the role of a specific protein (called FABP5) in the progression of breast cancer, suggesting that any action that interferes with FABP5 activity may slow or halt the growth of breast tumors. The current study will investigate the effectiveness of two drugs known to bind FABP5 on the growth of human breast cancer cells. An IC50 will be determined for both compounds over a 48 and 72-hour time frame. Similarly, computational tools will be utilized to design and screen for a new class of potential drug molecules. The most promising candidate will be used for further computational and biological studies.

B.) Research Question(s), Hypothesis, Expected Outcomes

- Are FABP5 inhibitors SB-FI-26 and SB-FI-103 capable of inhibiting MCF-7 cell viability when incubated at different concentrations for 48 and 72 hours?
- How do we create an analog that has a lower free binding energy to FABP5 than current FABP5 inhibitors?

→ Null Hypothesis (H_0) - When used in the MTT assay with MCF-7 cells, SB-FI-31 and SB-FI-128 won't be able to inhibit cell viability

→ Alternative Hypothesis(H_a) - When used in the MTT assay with MCF-7 cells, SB-FI-31 and SB-FI-138 will succeed in cleaving enough "flap" structures to create an efficient repair rate.

Expected Outcome: Through observations of SB-FI-31 and SB-FI128's function in the MTT cell viability assay, a significant decrease in cell viability will be detected, leading to the assumption that FABP5 inhibitors are capable of playing a role combating breast cancer.

C.) Procedure

Cell Lines, Culture, and Treatment

MCF-7, a human breast cancer cell line, will be obtained from the American Type Culture Collection (ATCC HTB-22). Cells will be kept in Roswell Park Memorial Institute (RPMI) 1640 medium with 10% Fetal Bovine Serum (FBS) and 1% Penicillin G Sodium Salt and incubated in 5% Carbon Dioxide (CO2) at 37 °C. Medium will be changed every 3 days.

Cell Viability Assay (MTT Assay)

MCF-7 cell cultures will be inspected and confluent plates will be chosen for MTT assay. Cells will be detached using Trypsin-EDTA and plated onto a cell counting plate. Cells will be counted to a final concentration of 7,000 cells per 100 µL of RPMI solution. The cell solution will be pipetted into the wells and the 96 well plate will be then incubated for 24 hours.

After a 24-hour incubation period, serial dilutions of SB-FI-26 and SB-FI-103 will be prepared with 0.50% DMSO in RPMI medium containing 1% FBS. Drug solutions will be then pipetted into the 96 well plate in increasing concentrations, from 0 µM (control) to 100 µM .

After incubation, MTT powder will be dissolved in RPMI solution to a final concentration of 0.5 mg/mL. The existing medium in the 96 well plate will be then aspirated out and 100 µL of MTT solution will be added to each well. After a 4 hour incubation, the MTT solution will be aspirated out and the remaining crystals will be solubilized using DMSO. The plate will be then read using a VICTOR Multilabel Plate Reader at an absorbance of 570 nm.

Computational Design of SB-FI-31 Analogs

Chemdraw 18.0 - a PerkinElmer software product - will be used to computationally design novel FABP inhibiting compounds, in which the lead compound SB-FI-31 will be used as a scaffold in the design process. 2-D analogs will be primarily made by adding constituents onto different portions of SB-FI-31. ChemDraw will also be utilized to obtain the cLogP score of the created compounds.

Energy Minimization of Designed Compounds

To visualize the created analogs, a three-dimensional molecule will be generated with Avagadro 1.90.0, a molecular editor and visualizer program. Using Avogadro, the SB-FI-31 derivatives will be refined through the minimization of intermolecular energies (using the MMFF94S force field), optimization of bond angles, and the adjustment of hydrogens to mimic the human physiological pH of 7.4.

Molecular Docking of Created Compounds

The program AutoDock-1.5.6, will be used to molecularly "dock" the created compounds with the target protein (FABP5), in order to investigate favorable ligand/protein interactions. Autodock will be able to generate a variety of binding poses for the compounds, which will be then docked within FABP5. Autodock then "scored" each compound to show the estimated free binding energy.

Visualization of Docked Compounds with Fatty Acid Binding Protein 5

The highest scoring compounds will be visualized with FABP5 in the program UCFS Chimera. Through Chimera, the bonds between the compounds and FABP5 will be visualized. The analogs will be then checked to see if they made canonical interactions with Arginine 129 and Tyrosine 131 in FABP5.

Prediction of Pharmacokinetic Properties

The highest scoring compounds that made canonical interactions will be then screened *in silico* through a pharmacokinetic properties predictor - pkCSM. Through pkCSM, the ADMES toxicity, hepatotoxicity, and the Oral Rat Acute Toxicity (LD50) of the selected compounds will be predicted. Additionally, compounds will be tested to see if they will be HERGI or HERGII inhibitors. Testing these specific properties of the created compounds ensures that these derivatives are safe to use in future clinical trials.

D.) Bibliography

1.) *Hotamisligil GS Nature. 2006 Dec 14; 444(7121):860-7.*

2) G. S. Hotamisligil and D. A. Bernlohr, "Metabolic functions of FABPs—mechanisms and therapeutic implications," *Nat. Rev. Endocrinol.*, vol. 11, p. 592, Aug. 2015.

3) Insulin signalling and the regulation of glucose and lipid metabolism. *Saltiel AR, KahnCNature. 2001 Dec 13; 414(6865):799-806.*

4) Ueda, Natsuo, et al. "Metabolic Enzymes for Endocannabinoids and Endocannabinoid- Like Mediators." *The Endocannabinoidome*, 2015, pp. 111–135., doi:10.1016/b978-0-12-420126-2.00008-0.

5) Reggio, Patricia H. "Endocannabinoid binding to the cannabinoid receptors: what is known and what remains unknown." *Current medicinal chemistry* vol. 17,14 (2010): 1468-86. doi:10.2174/092986710790980005

1. Human participant research: NA

2. Vertebrate animal research: NA

3. Potentially hazardous biological agents research:

MCF-7 cell cultures will be utilized in this study and are provided by the mentor. Safety goggles and gloves will be worn when handling these cells. After use, cells will be disposed of in red biological wastebaskets. They will be kept sterile during experimentation in a -80°C freezer. MCF-7 cells will be exposed to SB-FI-31 and SB-FI-128 to observe the effects of FABP5 inhibitors in breast cancer cells.

4. Hazardous chemicals, activities, and devices:

- RPMI Medium 1640 Phenol Red/No Phenol Red
    - Growth medium for MCF-7 cells

- Will cause skin and eye irritation
- Protective gloves and goggles will be worn at all times

- PBS Buffer, pH 7.4
  - Buffer solution
  - Will cause skin irritation
  - Protective clothing and gloves will be worn

- Trypsin-EDTA (0.25%)
  - Used for cell dissociation
  - May cause eye, skin, and respiratory tract irritation
  - Appropriate protective eyeglasses and gloves will be worn to prevent exposure

- MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
  - Stain to measure cell metabolism
  - Causes severe skin burns and eye damage
  - TEMED will be only be used under a fume hood and with gloves

– No additional risks exist –