Drug Discovery for Triple Negative Breast Cancer

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Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, and there are few treatments currently available. Patients with TNBC have a poor prognosis, and the chance of recurrence is high because this type of cancer is difficult to treat due to its chemoresistance. Signal transducer and activator of transcription 3 (STAT3) is a protein that regulates the expression of genes and has been found to be inappropriately active in TNBC cells (Qin et al., 2019). STAT3 is associated with metastasis (spread of cancer cells to other parts of the body) and it promotes resistance to chemotherapy, meaning that the cancer cells no longer respond to treatment.

Recent studies have shown that inhibition of the STAT3 signaling pathway enhances the efficacy of chemotherapy against TNBC (Qin et al., 2019). Therefore, STAT3 is a protein of interest for TNBC. During my 2021 Summer Undergraduate Research Fellowship (SURF), I investigated the direct inhibition of STAT3 by using bioinformatics, the process of collecting and analyzing biological data—most commonly genetic codes—through computer programming. I identified potential drugs that could disrupt STAT3’s signaling pathway, and performed different assays to test each drug’s effect on TNBC cell death.

Drugs that target transcription factors like STAT3 can be identified using a bioinformatics method that uses a “STAT3 signature” to screen for drugs. To analyze the gene signature that represents a specific STAT3 inhibition characteristic, I used the free online bioinformatics tool called CLUE that has been made available by the Broad Institute (the Connectivity Map) (Subramanian et al., 2017). CLUE produced a list of hundreds of drugs with a similar signature that are predicted to increase or decrease the expression of STAT3 target genes. However, I chose five out of the top fifty based on what they are already used for, whether they have been studied in breast cancer, and the biological pathway they are known to interrupt.

I focused on the TNBC cell line MDA-MB-231 and frequently tested viability using CellTiter-Glo (CTG). CellTiter-Glo measures the amount of ATP (a form of cellular energy), and therefore how many cells are present. CTG causes the cells to produce a luminescent signal based on ATP production, which can be analyzed by an instrument called a luminometer. The TNBC cells were treated with each drug for seventy-two hours before testing viability. I tested multiple concentrations of each drug and completed viability assays to determine if the drugs were effective. I observed a decrease in the number of cells present as the concentration of the drug increased. I also conducted a combination treatment viability assay to determine if some of the drugs worked well together. The results were promising, and the focus of my research in fall 2021 is the drug combination.

Next, to analyze the effect of the drugs specifically on STAT3, I isolated RNA and performed a quantitative polymerase chain reaction (qPCR). qPCR measures the amount of STAT3 target gene mRNA.
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present, and therefore reveals whether the drugs were able to inhibit STAT3 activity. The results of the qPCR have not been fully analyzed at this time; however, if there is a reduction of STAT3 genes present, the drug(s) were successful in inhibiting STAT3.

I also performed a migration assay on the drugs that had proven effective in my viability assay. This assay revealed whether the drugs would also prohibit the movement of cancer cells into empty space if given the opportunity. The initial experiment provided positive results, and replications of this study have been another priority of my research in fall 2021. Looking ahead, I plan to transition into using 3-D cell culture, so the cells grow into spheroids rather than a flat layer on a culture plate. 3-D cell culture is important in drug discovery research, because the spheroids more closely mimic a tumor in a patient, and therefore reveal how the drug may affect a tumor in vivo.

TNBC can be resistant to chemotherapy and patients often don’t survive due to the lack of alternative therapeutic options. My project has been part of a larger goal in the field of breast cancer research to develop new treatments that aim to decrease the rate of recurrence and change the prognosis of patients diagnosed with TNBC. STAT3 inhibitors have the potential to make a positive impact on the lives of the thousands of patients suffering from TNBC around the world.

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References


AUTHOR AND MENTOR BIOS

Emily Pratt, from Beverly, Massachusetts, will graduate in May 2023. A notable STEM individual at the University of New Hampshire, she is majoring in biochemistry, molecular, and cellular biology. Emily is part of the University Honors Program and plans to join Phi Sigma Biological Sciences Honor Society this semester. She performed research on breast and ovarian cancer this past summer through the Summer Undergraduate Research Fellowship (SURF) program recommended by her advisor. Emily discovered that the interdisciplinary value of her field is crucial to being successful in research projects. She enjoys doing independent lab work and analyzing data in order to make decisions about next steps in the process. Ever ambitious, she will continue this line of cancer research as part of her honors thesis next year and also wishes to participate in drug discovery research. Following graduation, Emily is determined to earn a master’s degree in biochemistry and after that, a PhD in pharmacology. She defines her recent research experience as what reinforced her original decision to study in this field. Her passion for writing in general and for Inquiry both prove to her how writing will play a great role in her research career.

Sarah Walker is an assistant professor in the Department of Molecular, Cellular, and Biomedical Sciences at the University of New Hampshire, College of Life Sciences and Agriculture. Dr. Walker’s research focuses on understanding the roles of STATs and related transcription factors in breast and ovarian cancer and using that knowledge to identify drugs to target the pathways associated with these transcription factors.