Stony Brook University
The Graduate School

Doctoral Defense Announcement

Abstract

Combinatorial CRISPR screening for synergistic

therapeutic targets in breast cancer

By

Shaobo Qin

The overarching goal of this project was to identify novel combinatorial therapeutic targets for devising effective treatments for triple-negative breast cancer (TNBC). This investigation aimed to overcome the difficulties encountered in previous attempts to identify cancer synthetic lethal targets. We developed a methodology for the positive selection of CRISPR-Cas9 library sgRNAs that negatively affected the ability to survive in the absence of mitogenic growth factors. To overcome these limitations inherent in negative CRISPR dropout screens, we conducted a kinome wide positive CRISPR/ Cas9 screen by enriching for dying cells by their selective binding to Annexin V.

The primary purpose of this single gene CRISPR/ Cas9 screen was to identify candidates for the combinatorial screening. The top ten most critical genes for survival in reduced growth factor conditions included several glycolytic kinases, including hexokinase and phosphofructokinase, along with key mitogenic signal transduction kinases IGF1R, ERK1, JAK2, and PIK3CD. Notably, none of these genes were critical in standard culture conditions. Dependencies identified in this step were used to construct a paired combinatorial CRISPR library.

We conducted combinatorial CRISPR library screening in both standard cell culture conditions as well as serum-deprived conditions. Based on our analysis, we propose the combinations of PIK3CA/IGFR1 and PIK3CA/JAK2 as combinatorial therapeutic targets in triple-negative breast cancer. Both single gene targets and gene pair targets identified from screens were validated *in vitro.*

Date: July 29, 2024 **Program**: Genetics
Time: 1:00 pm **Dissertation Advisor**: Scott Powers
Place: Room 145, Basic Science Tower Level 9