Preclinical Strategies Evaluating the Treatment of Triple Negative Breast Cancer

Elena Skarupa, Shanese "Lani" Jasper, and Robert "Rusty" Arnold

Breast cancer is the second leading cause of cancer death among women. Early detection and diagnosis, along with targeted therapies, have drastically improved survival rates. However, this improvement does not extend to patients with metastatic, triple-negative breast cancer (TNBC). TNBC is an aggressive cancer that is estrogen receptor-negative, progesterone receptor-negative, and HER-2 negative. Therefore, TNBC cannot be targeted via hormone therapies or novel molecularly targeted therapies, such as anti-HER2 therapies. Toxic chemotherapeutics, such as Doxorubicin, remain the primary treatment option. However, Doxorubicin exhibits cumulative dose-limiting cardiotoxicity that limits its clinical utility. In order to create and optimize therapeutic options for the treatment of TNBC, a better understanding of these tumors and their drug interactions is necessary. Often, two-dimensional (2D) *in vitro* models are employed to assess these interactions, but these models do not effectively recapitulate the complexity and multicellular nature of in vivo tumors. Three-dimensional (3D) *in vitro* models present more physiologically relevant models for assessing the interplay between drug and tumor by attempting to mimic the complexity of the tumor microenvironment.

For this study, the potency of Doxorubicin was determined using conventional cell toxicity assays. Both the SRB assay, which measures the total protein content of cells, and MTT assay, which evaluates the mitochondrial enzymatic activity of cells, were performed. The assays were conducted using classic 96-well flat-bottom plates with drug exposures ranging from 100 to 0.0001 uM Doxorubicin in the human TNBC cell-line, MDA-MB-231-LUC-GFP. They were evaluated following 24, 48, and 72 hours of drug exposure. The potency (IC50 value) was calculated after 72 hours of drug exposure. Cytotoxicity correlated positively with Doxorubicin concentration and length of Doxorubicin exposure.

The development and evaluation of the 3D model have been conducted in parallel with the 2D experiments, which serve as a reference platform for evaluating the 3D model using tumor spheroids. Future studies will focus on determining potency of Doxorubicin after the 72-hour time point using a single cell (tumor only) and multicellular (tumor and cells associated with tumor stroma, such as macrophages or fibroblasts) 3D models, as well. Future studies will also focus on evaluating differences in gene and protein expression between the 2D and 3D cell models with particular interest in HIF-1, P21, and other markers associated with tumor growth and metastases. HIF-1 is the Hypoxia-Inducible Factor, which is a transcription factor that responds to decreased available oxygen in the cellular environment. P21 is a cyclin-dependent kinase inhibitor, which inhibits the normal cell cycle. The goal of this work is to determine if 3D models can be used to give a more accurate depiction of the *in vivo* tumor response in an effort to develop better treatment options for TNBC.

Statement of Research Advisor

Over the last year Elena has worked to establish and test a 2D- and 3D-tumor spheroid platform that can be used to examine the effect of individual cell types on tumor growth and responsiveness to chemotherapy. This platform will permit examination of different drug treatment schedules and performance of various nanomedicines with the goal of improving the treatment of aggressive metastatic cancers.

– Robert "Rusty" Arnold, Harrison School of Pharmacy