

FINAL Progress Report - METavivor Grant 6-11-14

PI - Candace L. Gladson and Co-PI - Ruth Keri

Title: FIP200 Promotes Survival Through Autophagy of Metastatic Breast Cancer to the Brain

Background:

FIP200 functions to promote cell survival by promoting autophagy. Autophagy is a process in which during times of stress or starvation cells eat parts of themselves in order to gain the basic building blocks that will allow them to survive. FIP200 expression is necessary for this pro-survival process known as autophagy.

Progress:

Aim 1: Identify the minimal FIP200 sequence(s) required for autophagy.

As part of this aim we hypothesized that there would be increased FIP200 protein in the biopsies of metastatic breast cancer to the brain as compared to biopsies of primary breast cancer, as the cancer cells in the brain have to survive in a new and unfamiliar environment. Our unpublished results show that the expression of FIP200 is significantly increased in biopsies of metastatic breast cancer to the brain as compared to biopsies of primary breast cancer. These data suggest that the increased FIP200 in the metastatic breast cancer cells in the brain is promoting cancer cell survival likely through autophagy. To support our contention that there is increased autophagy occurring in metastatic breast cancer cells in the brain as compared to primary breast cancer cells, we examined the expression of a marker of autophagy. LC3 puncta, in these samples. We found that the percent of cancer cells with LC3 puncta was significantly increased, in the metastatic breast cancer cells in the brain as compared to the primary breast cancer cells. Importantly, within the group of patients with metastatic breast cancer to the brain, we found that patients with a higher expression of LC3 puncta in the metastatic cancer cells had a shorter survival. If cancer cells survive in tumors this typically correlates with a shorter patient survival.

We then asked whether these findings were also seen in a more homogeneous group of patients, so we separated out the patients that have triple negative metastatic breast cancer (estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative). In the patients with triple negative metastatic breast cancer, our unpublished results show that patients with a higher expression of the marker of autophagy (LC3 puncta) have a significantly shorter survival time. These data strongly support our studies of autophagy and FIP200 in metastatic breast cancer to the brain. Our data also suggest that identification of patients with metastatic breast cancer to the brain that have high expression of markers of autophagy is important, as these patients might benefit from therapy that would inhibit autophagy.

We have created an amino (N)-terminal fragment of FIP200 using PCR that functions as a dominant negative FIP200 (DN-FIP200) by inhibiting the pro-survival or pro-autophagy function of FIP200. Also, we have further truncated this amino-terminal fragment of FIP200 into smaller fragments and characterized the ability of these smaller fragments to inhibit autophagy when expressed in breast cancer cells.

Aim 2: Determine the effects of intra-arterial injection of brain-trophic breast cancer cells expressing DN-FIP200 into nude mice.

Based on the studies, in Aim 1, we choose to express one of the truncated amino-terminal FIP200 fragments in the brain trophic breast cancer cells and the parental breast cancer cells. We created the FIP200 construct by PCR and added a FLAG tag to the amino-terminus such that we could detect this molecule in cells. We also transfected these cells with a luciferase construct such that the tumor cells can be detected with Bioluminescent imaging after injection into the mouse model. We are currently selecting cells that have high level expression of both constructs (amino-terminal-FIP200 and luciferase) and expanding these cells. This requires repeated sterile sorting of the cell populations to obtain enough cells for the mouse experiment. Also, we are creating control cells that have high levels of luciferase and an empty vector (without the FIP200 construct). After intra-arterial injection of the breast cancer cells into mice, we will monitor the development of metastases over time by Bioluminescent Imaging.

Publications:

Nooshin Hashemi-Sadrei, Gaelle Muller-Greven, Ilya Ulasov, Erinn Downs-Kelly, Monica Burgett, Robert Weil, Lingling Du, Thomas G. Budd, Richard A Prayson, Niladri Kar, Jill Barnhiltz-Sloan, Amy Nowacki, Ruth Keri and **Candece L. Gladson**. Markers of Autophagy in Metastatic Breast Cancer to the Brain, Manuscript to be submitted.