Dependency of Aggressive Breast Cancer on Autophagy for Survival
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Introduction
Despite intense efforts breast cancer remains a major threat to women of all ages and race. Studies have found that non-Hispanic white women have the highest incidence rates but African American women have a higher mortality rate. Triple negative (TN) breast cancer is devoid of estrogen, progesterone and HER2 receptors and is known as the most aggressive subtype of breast cancer. Major challenges are heterogeneous cell types, their plasticity and resistance to available treatment options, tumor recurrence and metastasis. Currently, Chemotherapy is the only treatment option available. Surprisingly, aggressive breast cancers become addicted to key signaling pathways for their survival. Our lab has found that an aggressive triple negative (TN) subtype of breast cancer, frequently diagnosed in young African American (AA) women, express high levels of Bax, a pro-apoptotic molecule. We also found dependency of these cells on autophagy, which removes defective cellular organelles to increase their survival. We examined whether the inhibition of autophagy via chloroquine diphosphate salt treatment in TN breast cancer (HCC70) increased apoptosis, a programmed cell death. Autophagy levels as well as the stability of the key players were compared in cancer cells and mammary epithelial cells (MEC).

Methods
Cell Culture: Mammary epithelial cells were cultured in epithelial cell growth media and cell growth kit from ATCC. HCC70 cells were cultured in RPMI with 10% FBS, L-glutamine, and HEPES. Cells (2.0x10^6/ml) were prepared with matrigel (1:1) and injected subcutaneously in dorsolateral side of mice. Xenografts were grown for several weeks after which mice were euthanized and the tumors were removed for analysis.

Western blots: For in vivo, xenografts grown in different time points were homogenized by using T-PER buffer. For in vitro, the cells were lysed by using M-PER buffer. The proteins were transferred on PVDF membranes, which were blocked and probed for autophagy markers (ATG3, ATG7, Bc12, LC3, and Beclin), apoptosis markers (Bax and Casp3), and house keeping protein (β-Actin).

Histochemical Analysis: Breast cancer cells were fixed in 2.5% glutaraldehyde for three hours. The cells in Figure 1 (A) were stained in toluledine and analyzed under bright field microscope at 100X magnification. In Figure 1 (B and C) the cells were further embedded in paraffin and were prepared for analysis under electron microscope.

Results
Increased autophagy in African American triple negative breast cancer cell compared to mammary epithelial cells. Inhibition of autophagy in AA cell increased apoptosis.

Conclusion
Increased autophagy in African American triple negative breast cancer cell compared to mammary epithelial cells. Inhibition of autophagy in AA cell increased apoptosis.

Future Work
We are examining small molecule inhibitors that could inhibit autophagy in African American breast cancer cell lines. We are screening small molecule inhibitor with reduced in vivo toxicity to inhibit autophagy in African American triple negative breast cancer cells.

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