2020

BPTF Enhances Chemotherapy Induced Cytotoxicity

Valentina Posada

BPTF Enhances Chemotherapy Dependent Cytotoxicity

Valentina Posada[1], Liliya Tyutyunyk-Massey[2], David Gewirtz[3], and Joseph Landry[3]

Departments of Biology[1], College of Humanities and Science[1] Department of Pharmacology and Toxicology[2], Massey Cancer Center[2], Department of Human Molecular Genetics[3] , Virginia Commonwealth University School of Medicine[3]

Introduction

There are approximately 170,000 cases of Triple-Negative Breast Cancer (TNBC) in the United States. TNBC treatment is inefficient due to infrequent response to immunotherapies, and because of its resistance to common cancer chemotherapies.

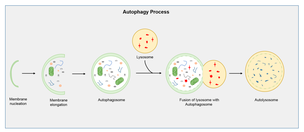
The nucleosome remodeling factor (NURF) is a chromatin remodeling complex composed of 3 core subunits with the BPTF being essential for function. BPTF is commonly over-expressed in cancers and has shown to be associated with increasing tumor growth and developing a malignant phenotype. These functions suggest that NURF may act as a suppressor for the anti-tumor immune response. Furthermore, in response to chemotherapies cancer cells often undergo the process of Autophagy. In this process, cells consume their cellular components to conserve energy and repair damage. Autophagy is regulated by many different proteins with one of them being ATG5. ATG5 is a protein that is involved in the extension of the isolation membrane into an autophagosome.

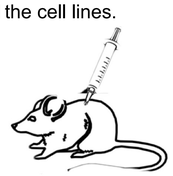
- ***Aim 1: Determine if BPTF-KD TNBCs are sensitive to chemotherapies.*** It is expected that cells with BPTF-KD will have sensitivity to some chemotherapies.
- ***Aim 2: To investigate the role of autophagy in the sensitization of BPTF-KD cells to chemotherapies.*** It is expected that BPTF-KD cells will have an enhanced sensitivity to Doxorubicin through the blockade of autophagy by ATG5 KD. 4T1 TNBC cells with BPTF and ATG5-KD will be transplanted into mice and tumor weight will be measured to see the response of the cells to chemotherapy after autophagy inhibition in vivo.
- ***Aim 3: Determine the possible molecular consequences of treating BPTF-KD cells with chemotherapies.*** Natural Killer (NK) cells will be depleted in mice to investigate role in sensitization to therapies.

Ultimately this thesis' goal is to develop strategies combining NURF targeted therapies with breast cancer chemotherapies to improve clinical outcomes.

Methodology

- Short hairpin RNA was used for BPTF and ATG5-KD which was confirmed through Western Blot.
- Clonogenic survival assays were used to see the effects chemotherapies on the BPTF-KD cells as well to investigate the roll of autophagy in plated cells.
- Acridine staining was used to test for enhanced levels of autophagy in BPTF-KD cells
- Clonogenic survival assays were used to measure toxicity of chemotherapies to BPTF-KD cells.
- Blockade of autophagy after ATG5-KD was measured by LC3B-I conversion to LC3B-II.
- Syngeneic mouse models were used to determine sensitization to Doxorubicin with BPTF-KD as well as with ATG5-KD in vivo.
- Mass spectrometry was used to measure eicosanoid abundance in culture media.
- Reverse ELISA was used to analyze PGE2 concentration in all the cell lines.
- rt-PCR was used to analyze cytokine gene expression data.





Results 1

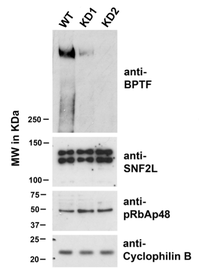


Figure 1

Figure1 shows BPTF-KD (KD1 and KD2) in the 4T1 mouse TNBC cell line. It also shows no reductions in any of the other subunits. Cyclophilin B is used as loading control.

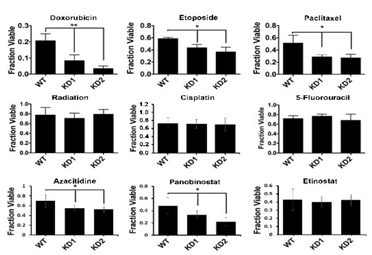


Figure 2

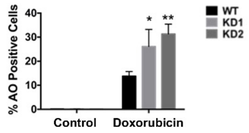


Figure 3

Figure 2 *shows sensitization to different types of chemotherapies in the WT, KD1 and KD2 cell lines. The highest sensitization can be seen in Doxorubicin as it has the smallest number of viable cells in KD1 and KD2 cell lines.*

Figure 3 *Acridine Orange staining shows enhanced autophagy in cells treated with chemotherapies in comparison to controls.*

Results 2

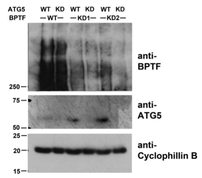


Figure 4

Figure 4 *shows ATG5-KD. Cyclophilin B is shown as the loading control and BPTF-KD is also shown for KD1 and KD2 cell lines.*

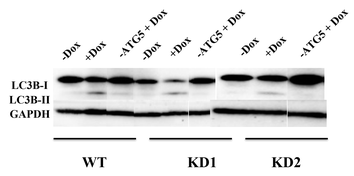


Figure 5

Figure 5 *shows functional blockade of autophagy through LC3B-I conversion to LC3B-II. GAPDH is used as loading control.*

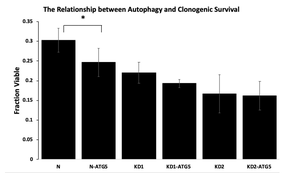


Figure 6

Figure 6 *clonogenic survival assays suggest that autophagy is cytoprotective in WT cells and nonprotective in BPTF-KD cells.*

Results 3

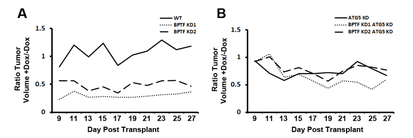


Figure 7

Figure 7 *shows results from syngeneic mouse models. It shows sensitization to Doxorubicin in BPTF-KD cells, but no sensitization in BPTF-WT cells that were ATG5-KD.*

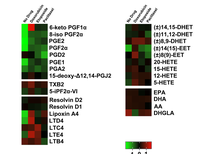


Figure 9

Figure 9 *reductions in PGE2 (known NK cell inhibitor) seen in BPTF-KD cells treated with chemotherapy*

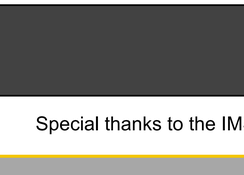


Figure 8

Figure 8 *shows that when we KD Natural Killer (NK) cells in mice they lose that initially seen sensitization to Doxorubicin.*

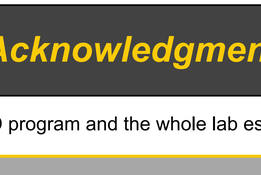


Figure 10

Figure 10 *shows a visible reduction in PGE2 when autophagy was blocked in the BPTF-KD cells.*

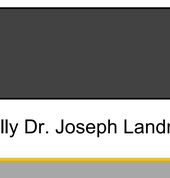


Figure 11

Figure 11 *shows increase in COX 1 and COX 2 expression in Doxorubicin treated cell lines. COX 2 expression is higher in ATG5-KD cells.*

Discussion

- ***Aim 1 results: Sensitivity is enhanced to select chemotherapies when NURF is depleted with BPFT-KD.*** Results also show enhanced autophagy in BPTF-KD cells treated with Doxorubicin. Doxorubicin had the lowest amount of viable cells.
- ***Aim 2 results: ATG5 KD increased sensitization to Doxorubicin in BPTF-WT cells but not BPTF-KD cells. This suggests that the autophagy is cytoprotective in the WT cells and nonproductive in the BPTF KD cells.*** BPTF-KD cell lines (KD1 & KD2) showed sensitization to Doxorubicin in syngeneic mouse models. Mouse models also showed sensitization in ATG5-KD cell lines with BPTF-WT but not the cell lines with BPTF-KD.
- ***Aim 3 results show that when Natural Killer cells are depleted in mice, the initial sensitization is lost. This could be explained by cytokine or metabolite secretion to the tumors or by altered gene expression.*** The metabolomics screen showed reductions in PGE2 in the BPTF-KD cells treated with chemotherapies. This explains improvements in tumor growth seen in the mice since PGE2 is a known NK cell inhibitor. rt-PCR analysis of cytokine gene expression in the BPTF-KD and the ATG5-KD cells showed no significant expression in any of the cytokines within the cell lines. The PGST1 and PGST2 gene expression data suggests that COX 2 is regulated by autophagy and autophagy is important for PGE2 secretion with or without Doxorubicin.

Acknowledgments

Special thanks to the IMSD program and the whole lab especially Dr. Joseph Landry