Detection of Bax𝛗2 Positive Macrophage in Breast Tissues

Anam Adil[*,1], Sana Basheer, Dr. Jialing Xiang [*]

[*]Department of Biological Sciences, Illinois Institute of Technology, Chicago, IL 60616, USA

I. ABSTRACT

BaxQ2 is an isoform of the Bax gene and functions as a tumor suppressor. Its gene products can cause programmed cell death. In previous studies, the lab found that BaxQ2 is found in cells throughout various human organs [2]. Based on the morphological structure of these cells, it is hypothesized that some of these cells may be macrophages. This project focuses on identifying whether BaxQ2 positive macrophages are found in breast tissue. Six different patient cases with varying grades of cancer were looked at and their cancerous tissue was compared to their normal tissue. A process known as Immunofluorescence Staining uses certain antibodies to test for certain antigens (markers) in a sample of tissue. Co Immunostaining was used to stain for two different markers. In this case, each tissue slide is stained with antibodies against macrophages and BaxQ2. We found that normal breast tissue had a greater amount of BaxQ2 in comparison to cancer adjacent tissue, stage 1, 2, and 3 malignant tumors. From the data, it was also analyzed that almost all macrophages were BaxQ2 positive, however not all BaxQ2 positive cells were macrophages. This suggests that in addition to its apoptotic function, BaxQ2 positive macrophage may play a role in immune response. Analyzing the amount of BaxQ2 present in different types of tissue could also serve as a prognosis marker for cancer patients. In addition, it can be explored if cancer cells expressing BaxQ2 are more sensitive to chemotherapy treatments.

II. INTRODUCTION

In 2019, the estimated leading site of new cancer diagnoses in females will be breast cancer. The death rate due to this carcinoma declined by 40% from 1989 to 2016 [1]. This progress reflects improvements in new therapies and treatments that are being made continuously. The investigation of BaxQ2 in breast tissue can hopefully contribute to this trend. The female breast is primarily composed of fat cells known as adipose tissue. Breast cancer generally arises in the terminal lobule ducts. This is the gland that produces milk in nursing women. It begins when cells in the breast begin to grow out of control, spread when they get into the blood or lymph system and then are carried to other parts of the body. The stage of the breast cancer is determined by its characteristics: the size of the tumor, whether it has spread, and the number of lymph nodes affected. Based on the tissue, one can identify the stage depending on the quantity of cells and the amount of collagen. Based on morphological structure, one can speculate what type of cell it is. Previously in the lab, colon tissue was stained for BaxQ2 [4]. Macrophage-like cells were evident in the images. One of the ongoing projects in the lab is to confirm whether some macrophage-like cells are in fact macrophage. This project aims to study this in healthy and cancerous breast tissue.

II. METHODS

Female Invasive breast ductal carcinoma tissue microarray containing six cases and quadruple cores per case was obtained from Biomax as shown in Figure 1. Patient information is specified in Table 1. Tissue slides were baked prior to starting.

Figure 1. BR251c - Female invasive breast ductal carcinoma tissue microarray

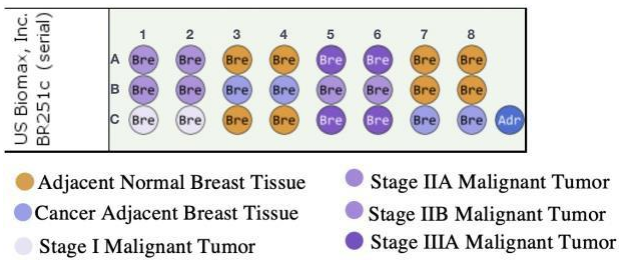


Table 1. Patient Samples

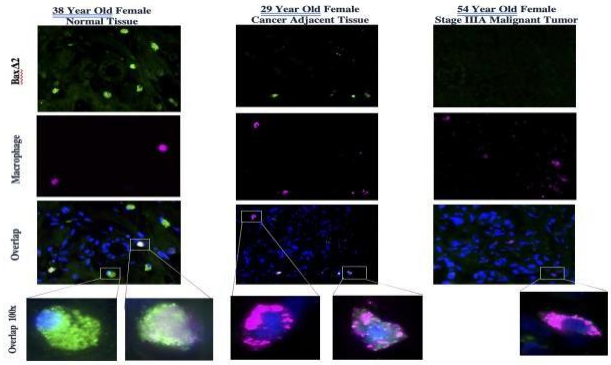
Case	Age (Years)	Grade	Tissue Pathology
1	40	2	Stage IIB Malignant Tumor
2	54	2	Stage IIIA Malignant Tumor
3	54	2	Stage IIB Malignant Tumor
4	29	2	Stage IIA Malignant Tumor
5	38	3	Stage 1 Malignant Tumor
6	38	3	Stage IIIA Malignant Tumor

Immunohistochemistry, Paraffin-Fixed Tissue: Fluorescence.

The tissue slide was deparaffinized using xylene, ethanol, and PBS buffer solution. The antigen was retrieved by heating the slide in a sodium citrate buffer. The slide was washed again with a PBS buffer. It was then treated with the Baxi'. 2 (2D4) mouse monoclonal primary antibody. The slide was incubated in 3% BSA in PBST blocking buffer for 2 hours at room temperature in an incubation chamber. The primary antibody was prepared in a 1:100 dilution. 200 uL of the antibody dilution was placed into each chamber and gently pressed on the tissue slide, top side down. The slide was then incubated overnight in a damp chamber in a 4ºC refrigerator. The slide was then washed 4 times with alternating 1X PBS and TBST for 10 minutes each. The slide was then treated with CD68 rabbit monoclonal primary antibody. The antibody was prepared in a 1:100 dilution. The slide was incubated for 1 hour at room temperature. The slide was then washed 4 times with alternation 1X PBS and TBST for 10 minutes each. The following steps were done under foil, protected from light. The secondary antibodies, an anti-mouse fluorescent marker at 488 nm and an anti-rabbit fluorescent marker at 647, were prepared in a 1:200 dilution in PBS. The slides were incubated in 200uL of the antibody and buffer solution. The slide was incubated for 1 hour in a damp chamber at room temperature. It was washed 4 times with alternation 1X PBS and TBST for 10 minutes each. The nuclei were stained by incubating the slide in 200 ul DAPI prepared in a 1:1000 dilution in PBS for 5 minutes. It was rinsed with 1X PBS once. The slide was then mounted using Fluoromount. The tissue slide was visualized using a Keyence BZ-X710 fluorescence microscope.

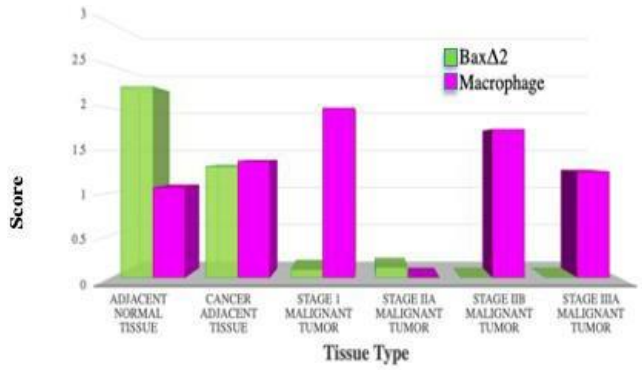
III. RESULTS AND DISCUSSIONS

Figure 2. BaxA2 and Macrophage Fluorescence in Varying Tissue Types



The images shown in Figure 2 were captured on a Keyence BZ-X710 fluorescence microscope. The green channel was used to visualize Baxi'. 2, while the far-red channel was used to identify macrophages. An overlap between the two markers was indicated by a white signal. The nuclei were seen using the blue channel.

Figure 3. Quantification of BaxA2 positive cells and macrophages in varying tissue types



The findings in Figure 3 display the quantification of Baxi'. 2 positive cells and macrophages present in a tissue. A scoring system based on a scale from 0-3 was used. This scale has been established by previous researchers in this lab [3]. A score of 0 is representative of 0-1% cells being positive. A score of 1 is 1-3%, 2 is 4-6%, and 3 for >10%.

Majority of Baxi'. 2 was detected in normal tissue, but not in cancerous tissue. Some of the cells found in the breast were macrophage marker positive. Since CD68 can also be positive for other types of cells, future work in the lab must

be done to confirm whether these cells are in fact macrophage. Also, if they are macrophages, then we need to study the function of BaxQ2 in macrophage.

IV. REFERENCES

[1] Cancer Statistics, 2019. Published early online January 8, 2019 in *CA Cancer Journal for Clinicians*. First author Rebecca L. Siegel, MPH, American Cancer Society, Atlanta, Ga

[2] Haferkamp, B., Zhang, H., Lin, Y., Yeap. X, Bounce, A., Sharpe, J., Xiang, J. (2012). BaxQ2 is a novel Bax isoform unique to microsatellite unstable tumors. *J. Biol. Chem. 287*(41), 347229.

[3] Mañas A, Davis A, Lamerand S, Xiang J (2018). Detection of pro-apoptotic BaxQ2 proteins in the human cerebellum. Histochem Cell Biol. 2018 Jul;150(1):77-82. doi: 10.1007/s00418-0181669-6

[4] Zhang H, Lin Y, Manas A, Zhao Y, Denning MF, Ma L, Xiang, J. (2014) BaxQ2 promotes apoptosis through caspase-8 activation in microsatellite unstable colon cancer. *Mol. Cancer Res.*
12(9), 1225-23.