Identification of ICAM-1 Positive Cells in BRCA1 Deficient Tumors as an Olaparib Resistant Sub-Population

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Abstract

Breast cancer is the most diagnosed and second most deadly cancer among women, with more than 276,000 cases estimated to be diagnosed in 2020. The BRCA1 (abbreviation for BReast CAncer 1) gene plays a role in preventing the development of breast cancer and is known as a tumor suppressor gene as it slows down cell division and repairs DNA mistakes. Mutations in the BRCA1 gene are the most important known inherited risk factor for breast cancer. Intercellular Adhesion Molecule 1 (ICAM-1) is a protein in humans encoded by the ICAM1 gene. Previous data in our lab has shown that there is a distinct sub-population of ICAM-1 expressing cells in BRAC1 deficient breast tumors. ICAM-1 is found on the surface of cells and can therefore be used to identify cells that express it. Flow cytometry is a technique used to identify and sort cells using surface proteins. In these studies, we utilize flow cytometry to separate cells from BRCA1 deficient breast tumors into populations of ICAM-1 positive and negative cells. We hypothesized that these ICAM-1 positive and negative cells would have different malignant phenotypes and treatment responses. We found that the ICAM-1 positive cells expressed lower levels of the protein E-Cadherin. We also found that ICAM-1 positive cells were more resistant to treatment with Olaparib, a therapy used in the treatment of BRCA1 deficient breast cancer. In conclusion, our results indicate that ICAM-1 expression demarcates a distinct sub-population of tumor cells with different drug resistant properties.

Introduction

ICAM-1 expressing cells in BRCA1 deficient tumor cell populations are generally considered to have an Epithelial-to-Mesenchymal Transition (EMT). This transition is involved with tumor progression by which epithelial cells lose give up their polarity and ability to adhere to other cells. Instead, they gain different properties that transition them into mesenchymal stem cells. EMT has been associated with sub-populations of tumor cells with aggressive traits. A sub-population of cells in BRCA1-null tumors exhibit EMT gene expression patterns and higher levels of a cell surface marker ICAM1. This experiment began with a group of BF3M cells and with the goal of sorting them into three groups: an unsorted group, an ICAM-1 positive group and an ICAM-1 negative group. The sort was made possible by fluorescence-activated cell sorting (FACS) through flow cytometry.

Methods

Several experiments were conducted using specific techniques to yield experimental results. The main techniques used in this project were flow cytometry, Western blots and alamarBlue assays.

- **Flow cytometry** – Flow cytometry is a technique used to analyze and separate individual cells based on their surface expression of ICAM-1. Figure 1 depicts the collection process of flow cytometry when the cell suspension is inserted into the stream at the beginning, then is hit with the laser one at a time as light is scattered and ending with the suspension being passed to the collection tube. As light is scattered, the light from the laser produces a fluorescence emission which is processed through the signal processor in the cytometer. Flow cytometers are efficient in measuring large numbers of parameters in a matter of seconds.
- **Western Blot** – The Western blot is an analytical technique used to detect specific proteins in a tissue sample. The three main steps of the Western blot include the separation, the transfer and the protein detection. During separation, gel electrophoresis is used to separate proteins by molecular weight. In the transfer step, the separated proteins are transferred to a solid membrane. Proteins are detected using antibodies specific to the target protein. Lastly to detect proteins, the solid membrane is blocked, incubated with a primary antibody, secondary antibody and a substrate then the target proteins are ready to be detected using chemiluminescence. The proteins of interest can be visualized via digital imaging equipment.
- **AlamarBlue assay** – The last set of experiments that were performed included the alamarBlue assay, which is a specific assay used to measure cell proliferation and viability in cell lines. The protocol for this assay includes adding the alamarBlue to the cell line suspended in medium in microplate wells, incubating for a certain time period, then reading the absorbance in a spectrophotometer. To analyze the results, the absorbance units and the drug dosage are plotted on a graph to yield quantitative results.

Summary

Using flow cytometry, we were able to sort an ICAM-1 positive and negative population from the BF3M cells as depicted in figures 2a-2d. After flow cytometry sorting, the ICAM-1 positive cells maintain a higher expression level. After sorting ICAM-1 positive cells by flow cytometry, we used Western blotting analysis to characterize the cells. The ICAM-1 positive cells did indeed show high ICAM-1 expression and less E-Cadherin expression, an epithelial marker, indicating that they have undergone EMT. When the cells were treated with a cancer therapy during the cell viability assay, the ICAM-1 positive population was less vulnerable to treatment with Olaparib for both doses. This indicates that this population of cells is indeed more drug resistant.

Acknowledgements

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Figures

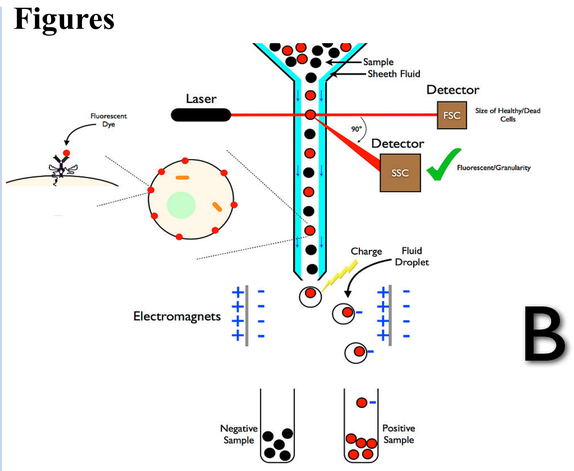
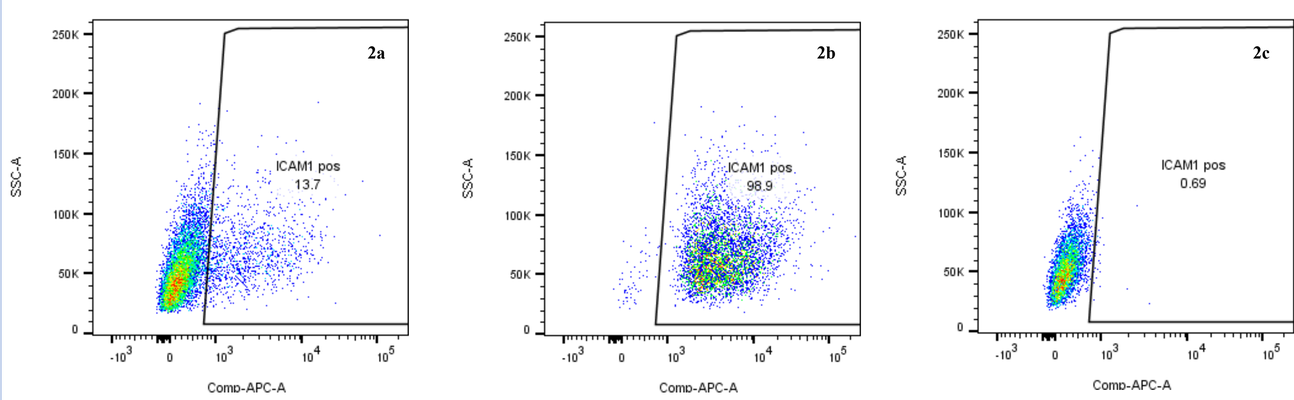


Figure 1. Schematic of the inside of the flow cytometer.



Figures 2a, 2b and 2c. Figure 2a is a graph that depicts the ICAM-1 expression in the total population of BF3M cells. Each dot on the graph represents a single cell. ICAM-1 expression is the parameter on the horizontal axis. Figure 2b is a graph resulting from analyzing the cells sorted into the ICAM-1 positive population. Figure 2c is a graph resulting from analyzing the cells sorted into the ICAM-1 negative population.

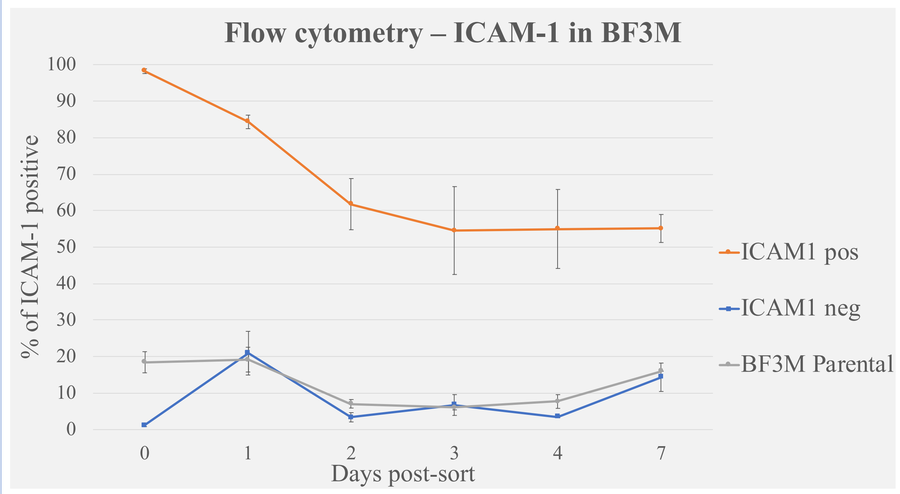


Figure 2d. Line graph depicting the expression of ICAM-1 positive, negative and parental sub-populations of BF3M cells over 7 days after the flow cytometry sorting.

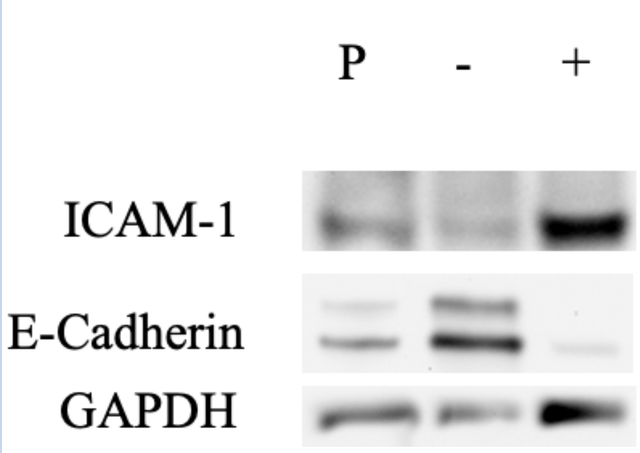


Figure 3. Protein blots as a result of the Western blot showing the levels of protein expression for the target proteins ICAM-1, E-Cadherin and GAPDH (glyceraldehyde 3-phosphate dehydrogenase). GADPH is used a loading control. ICAM-1 expression in ICAM-1 positive cells appears high while E-Cadherin expression appears low.

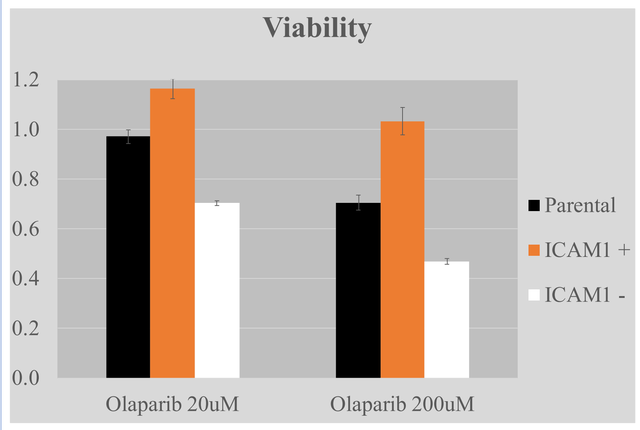


Figure 4. Bar graph that depicts the alamarBlue stained cells that are metabolically active and therefore considered viable. The bars represent the alamarBlue content in the Olaparib-treated cells normalized to the control-treated cells. ICAM-1 positive cells have increased viability while the negative cells have decreased viability.