Examination of Effects of PFKFB4 Inhibition on HER2+ Breast Cancer Cells

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Introduction

Breast cancer is a leading cause of cancer-related deaths in the female population with an estimated ~42,000 deaths in 2019 alone [1]. As a result, the development of treatment options that target various vulnerabilities linked to divergent breast cancer subtypes is crucial. Human epidermal growth factor receptor-2 (HER2) positive breast cancer is one subtype that makes up 25-30% of all reported breast cancer diagnoses [2]. It develops when mutations emerge on the HER2 gene, leading to receptor overexpression and, in turn, increased breast cell proliferation [2]. HER2+ breast cancer cells have been demonstrated to show a glycolytic phenotype with higher glucose utilization and lactate accumulation [2].

The enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4) is responsible in part for the increased glycolytic flux within cancerous cells and thus, has been deemed a necessity for neoplastic growth [3]. PFKFB4 synthesizes the strong effector, fructose-2,6-bisphosphate (F26BP), which allosterically activates PFK-1, a key enzyme in the glycolytic cascade [3]. Previous studies have looked into the effects of PFKFB4 inhibition on cancer cell viability and have found that inhibition leads to apoptosis in certain lung cancer cell lines [3].

In this study, we sought to examine the expression of PFKFB4, HER2, and β-actin in each of the three HER2+ breast cancer cell lines and then exposed these cell lines to a PFKFB4 inhibitor and measured the effects on cell growth.

Objective

- Determine the amount of PFKFB4 and HER2 that exists in each sample by Western Blotting
- Determine how PFKFB4 inhibition affects cell growth in three different HER2+ breast cancer cell lines

Methods

Cell Culture:
- Breast cancer cell lines SKBR3, ZR-75-1, and T47D were obtained and plated. The ZR-75-1 and T47D lines were cultured in RPMI Medium, while SKBR3 cells grew in McCoy’s 5A Modified Medium.
- The three cell lines were incubated at 37℃ for a period of 48 hours and then treated with escalating concentrations of the PFKFB4 inhibitor.
- Cells were lifted with trypsin following a period of 72 and 120 hours of treatment. Cells were then counted manually under the microscope and data was recorded.

Western Blot:
- SKBR3, ZR-75-1, and T47D cells were lifted with trypsin and lysed. Each specimen was then prepared for a bicinchoninic acid (BCA) assay to determine its protein concentration.
- A 4-15% gel was loaded with equal amounts of protein in a 1:1 ratio of protein solution to Laemmli buffer.
- The protein was then transferred to a membrane, which was then blocked and incubated with either HER2 or PFKFB4 primary antibody overnight or with β-actin for 1 hour.
- The membrane was washed with TBST, shaken in its respective secondary for an hour, and washed again.
- Images were then developed in the dark room and blots scanned.

Results

A. 

Figure A shows results of the Western Blots that depict the expression of PFKFB4, HER2, and β-actin in each of the three cell lines.

B. 

Figure B shows the results of exposure of these selected HER2+ breast cancer cell lines to escalating concentrations of the PFKFB4 inhibitor for a period of 120 hours.

C. 

Figures C-E represent the inhibition of growth that occurred in cell lines SKBR3 (C.), ZR-75-1 (D.), and T47D (E.) following 120 hours of exposure to increasing concentrations of PFKFB4 inhibitor.

Conclusion

- Based on our Western blot, SKBR3 cells showed the highest expression of HER2 of the three examined cell lines and T47D cells showed the lowest expression.
- Based on our Western blot, PFKFB4 was expressed in all three examined HER2+ cell lines. Further studies are needed to confirm the relative PFKFB4 expression in the cell lines.
- Across all three HER2+ breast cancer cell lines, inhibition of PFKFB4 led to decreased cell proliferation as the concentration of the inhibitor was increased.
- In these preliminary experiments, exposure to the PFKFB4 inhibitor appeared to have greater effects on the growth of SKBR3 and ZR-75-1 cells than it had on T47D cells.

Future Directions

- We will further examine PFKFB4 expression in these cells and the effects of the PFKFB4 inhibitor on these and other HER2+ breast cancer lines to fully evaluate the effects of the inhibition.
- We will conduct additional testing to determine whether the PFKFB4 inhibitor affects glycolysis and ATP production in the examined breast cancer cell lines.
- We plan to conduct further experimentation using cell lines from other breast cancer subtypes e.g. ER- PB and triple negative lines to determine how PFKFB4 inhibition affects their growth.

Acknowledgements

Many thanks to the University of Louisville Cancer Education Program NIH/NCI (R25-CA134283) for funding this research.

References