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, 4]. 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 in HER2+ breast cancer cells and determine the effect of PFKFB4 inhibition on these cells. We examined expression of PFKFB4 in 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°C for a period of 48 hours and then treated with escalating concentrations of a PFKFB4 inhibitor and returned to the incubator.

•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.

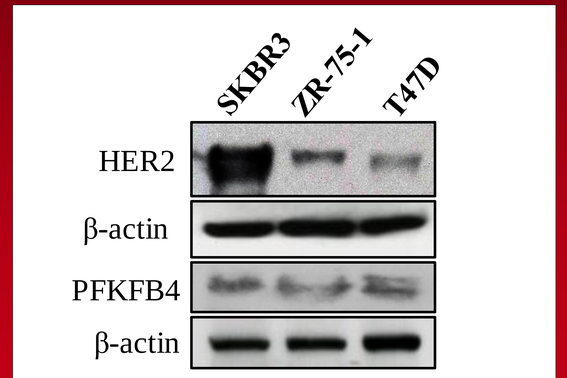


Figure A shows results of the Western Blots that depict the expression of PFKFB4, HER2, and β-actin in each of the three cell lines.

B.

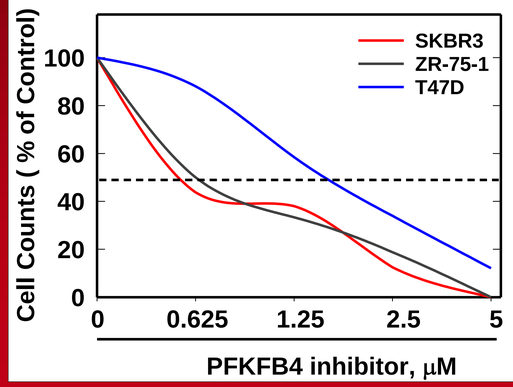
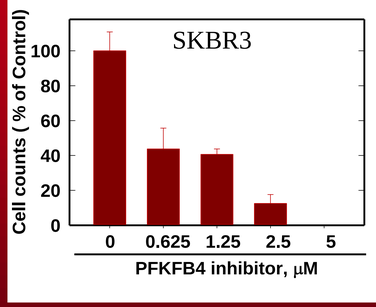
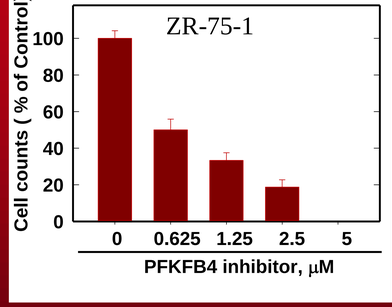


Figure B shows the results of exposure of these selected HER2+ breast cancer cell lines to escalating concentrations of the PFKFB4 inhibitor for a period of 120 hours.

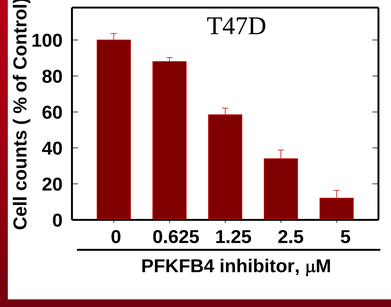
C.



D.



E.



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 inhibitor.
- 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+. PR+ and triple negative lines to determine how PFKFB4 inhibition affects their growth.

Acknowledgements

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References

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