THE EFFECT OF PFKFB INHIBITION ON CELL SURVIVAL IN LUNG CANCER

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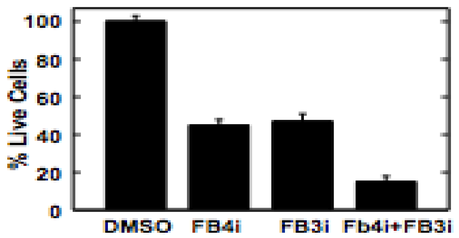
Introduction

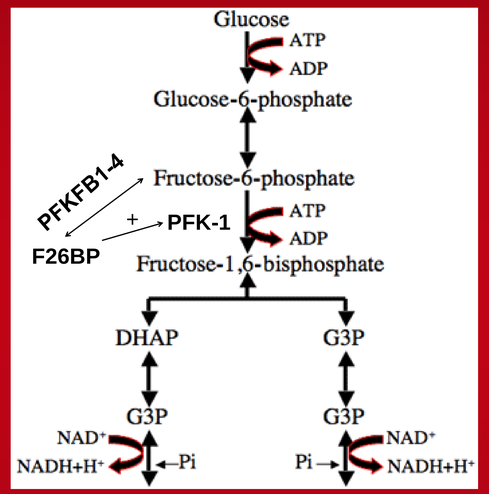
Converting fructose-6-phosphate (F6P) to fructose-1,6-bisphosphate (F16BP) through phosphofructokinase-1 (PFK-1) is an important rate limiting reaction in glycolysis. Fructose-2,6- bisphosphate (F26BP) is produced by the 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase enzymes (PFKFB 1-4) and is a strong activator of the PFK-1 enzyme. In previous studies from our laboratory, we have found high levels of the PFKFB4 enzyme in many cancer cell types and found that decreasing levels of PFKFB4 will decrease F26BP and cancer cell growth. The PFKFB3 enzyme is often simultaneously expressed with PFKFB4 in cancer cells and inhibition of this enzyme is also shown to decrease the growth of cancer cells. Previous data from our laboratory has shown that simultaneous administration of PFKFB4 (FB4i) and PFKFB3 (FB3i) inhibitors causes a marked decrease in live cell numbers (% live cells relative to DMSO, 1μM FB4i and FB3i used). Based on this result, we wanted to find out if the decrease in cell numbers may be caused by apoptosis.



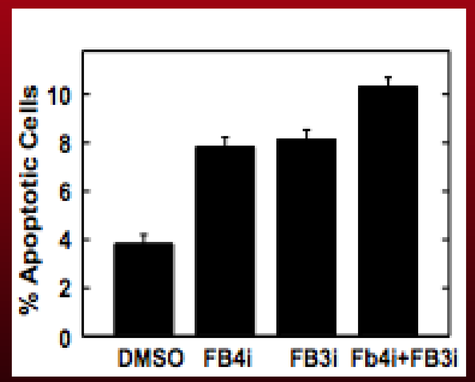
The goal of our study was to examine the effects of inhibiting PFKFB4 and PFKFB3 simultaneously on apoptosis.

Methods and Results

We plated H460 lung cancer cells in 6 well plates and treated the cells with several concentrations of a PFKFB4 inhibitor (FB4i) and a PFKFB3 inhibitor (FB3i).
After 72 hours of growth, we examined the cells for apoptosis using Annexin V and propidium iodide staining and flow cytometry.



The PFKFB enzymes produce F26BP which activates PFK-1.



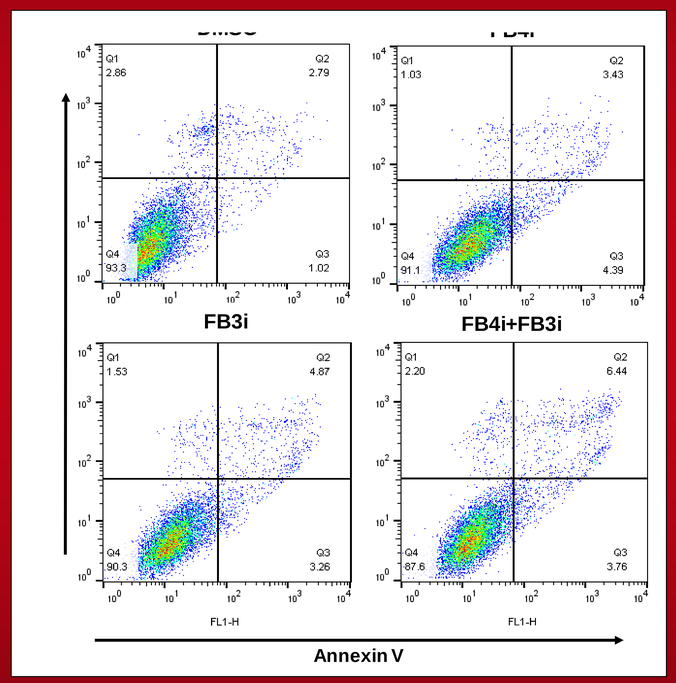


Figure 1. We treated H460 cells with 1μM of FB4i, FB3i or of FB4i+FB3i and examined effects on apoptosis by flow cytometry.

Conclusions

We found that treating the cells with a combination of FB4i and FB3i caused a moderate increase in apoptosis.

Future Directions and Clinical Impact

Future directions include studying effects of PFKFB4 and PFKFB3 inhibition further.

We will compare the effect of inhibition of the isoforms PFKFB 4 and 3 on cell cycle progression.

Learning the effects of inhibiting PFKFB3 and PFKFB4 on cell death can lead to new treatments for cancer.

Acknowledgements

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