6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 4 (PFKFB4) Inhibition in Lung Cancer

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INTRODUCTION

- The Warburg effect is a well-studied phenomenon where an increased rate of glycolysis is observed in cancer cells, even in the presence of oxygen.
- The generation of fructose-1,6-bisphosphate (F1,6 BP) from fructose-6-phosphate (F6P) by phosphofructokinase 1 (PFK1) is a critical regulatory step in the glycolytic process.
- Fructose 2,6-bisphosphate (F2,6BP) is a powerful allosteric activator of PFK1 and is the product of four bifunctional 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatases (PFKFB1-4).
- The PFKFB4 enzyme has been found to be upregulated in cancer cells and play an important role in regulating their glycolysis and growth.

Figure 1

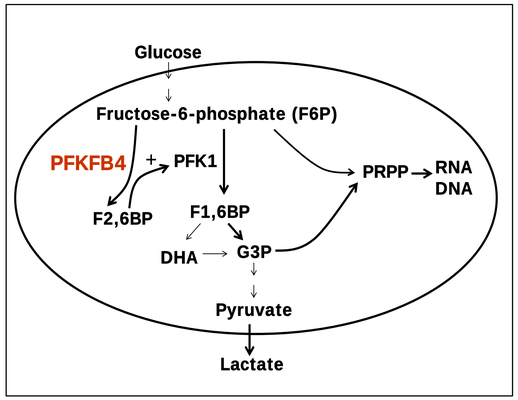

Figure 1: Schematic showing the steps of glycolysis and production of F2,6BP by PFKFB4

HYPOTHESIS

- We hypothesize that the use of a small molecule inhibitor of PFKFB4 in lung cancer cells will:
  - decrease the rate of glycolysis and ATP production
  - arrest the progression of the cell cycle in lung cancer cells.

METHODS

- Expression of PFKFB4 was confirmed in a series of non-small cell lung cancer cell lines (H23, H460, H1299, H1437 and PC9).
- Cell lines were plated and exposed to increasing concentrations of a PFKFB4 inhibitor (FB4i).
- Glycolysis assay: cells treated for 24 and 48 hours pulsed with tritiated glucose for one hour, then media from the cells collected and placed in open tubes in vials containing water. Vials were incubated at 37°C and tritiated water produced by glycolysis diffused into water in vials and was measured on a scintillation analyzer.
- ATP levels: treated cells were lysed and ATP determined by a bioluminescence assay and read in a luminometer.
- Cell cycle analysis: treated cells were collected and fixed in 70% ethanol, then treated with DNA-binding dye and analyzed by flow cytometry.

RESULTS

Figure 2

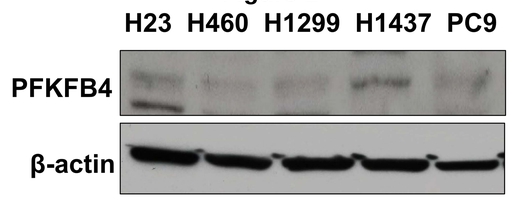

Figure 2: Western Blot analysis of PFKFB4 in five lung cancer cell lines

Figure 3

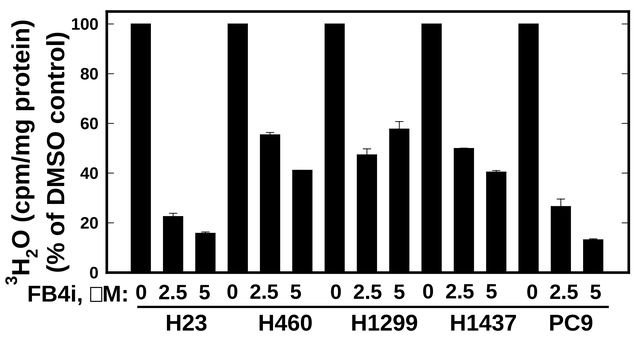

Figure 3: Graph showing the effect of PFKFB4 inhibition on glycolysis (by production of $^3H_20$ from ^3H-glucose) compared to DMSO control in five lung cancer cell lines.

Figure 4

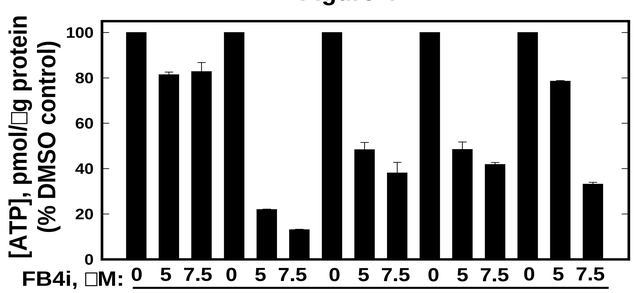

Figure 4: Graph showing the effect of PFKFB4 inhibition on ATP production compared to DMSO control in five lung cancer cell lines.

Figure 5

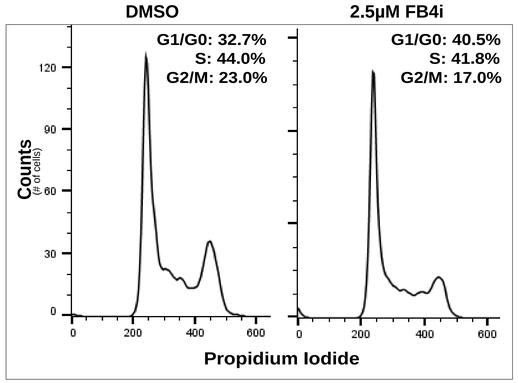

Figure 5: Graph showing distribution of cells in the cell cycle (by propidium iodide staining) in H460 cells treated with DMSO +/- 2.5μM FB4i for 24 hours.

RESULTS

Figure 6

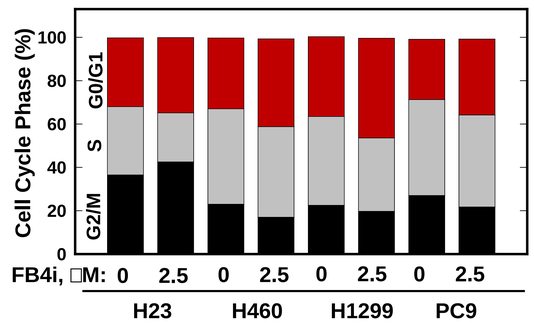

Figure 6: Graph showing the percentage of cells in G0/G1, S, and G2/M for four lung cancer cell lines that were treated with DMSO +/- 2.5μM FB4i for 24 hours.

CONCLUSIONS

- Small molecule inhibition of PFKFB4 decreased the rate of glycolysis and production of ATP.
- PFKFB4 inhibition arrested progression of the cell cycle in G0/G1 phase.
- Our data suggest that PFKFB4 inhibition is an effective mechanism for inhibiting glycolysis and growth in a variety of lung cancer cell lines carrying several oncogenic mutations
- Glycolytic inhibition could be a novel and effective treatment for lung cancer.

FUTURE STUDY

- Future studies will investigate:
  - effectiveness of PFKFB4 inhibition *in vivo*
  - synergistic capabilities with other metabolic inhibitors

ACKNOWLEDGEMENTS

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