Resistance mechanisms to PI3K-mTOR inhibition in NSCLC

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Introduction

Non-small cell lung cancer (NSCLC) is a leading cause of cancer mortality globally, having a 5 year survival rate of less than 15%. PI3K-mTOR signalling has been implicated in various hallmarks of cancer and this pathway is often dysregulated in different cancers, including NSCLC. Efforts to therapeutically target the PI3K-mTOR pathway have shown limited clinical efficacy, however the inevitable emergence of drug resistance inhibits a durable response to treatment. In this study a cell line model of acquired resistance to a phase II PI3K-mTOR inhibitor GDC-0980 was established following several months of chronic treatments with IC50 drug concentrations. The sensitivity of the established GDC-0980 resistant cells (H1975GR) to other PI3K-mTOR inhibitors was interrogated using another clinically relevant PI3K-mTOR dual targeting inhibitor, BEZ235. IL-6/STAT3 overexpression in cancer stimulates angiogenesis, migration, invasiveness, cytokine signalling and notable drug resistance. The IL-6/STAT3 pathway has been shown to contribute to PI3K/ mTOR signalling and in this study a potential role in drug resistance mechanisms to PI3K-mTOR blockade are investigated. Additionally, a comparative study investigating short term and long term chronic exposure of BEZ235 and IBL-301 (a novel PIM/PI3K/mTOR inhibitor) for effects on cell viability/proliferation and downstream signalling pathways is ongoing.

Methods

- The sensitivity of GDC-0980 resistant cells , H1975GR, to PI3K-mTOR inhibitor BEZ235 following a 72 hour treatment was compared to the sensitivity of the age-matched parent cells H1975P using a Cell Titre Blue cell viability assay (n=3).
- Alterations to the mRNA expression profile of GDC-0980 resistant cells, H1975GR versus age-matched parent cells H1975P were examined using an IL-6/STAT3 signalling-specific RT2 gene profiler array (n=1). This array contains 84 key genes involved in the activation and downstream effects of IL6/STAT3 signalling. Selected genes from the array were validated by SYBR-based qPCR and western blot analysis (n=3-4).
- BEZ235 and IBL-301 drug dose responses were measured in two wild-type NSCLC cell lines (H1975 and H1838) by CellTitre Blue assay.
- Alterations to protein expression of H1975 and H1838 cells were measured by Western blotting analysis.

Development of PI3K/mTOR inhibitor-resistant cell line model:

- Figure 1: GDC-0980 resistant cells, H1975GR, are also resistant to another PI3K-mTOR inhibitor BEZ235. (A) A cell line model of acquired drug resistance to PI3K-mTOR inhibition was generated following several months of chronic treatment with GDC-0980. At month 6 IC50 values determined by EdU cell proliferation assay were 2.94µM and 0.89µM for H1975GR and H1975P respectively. (B) Similarly, cell viability dose response curves generated by the Cell Titre Blue assays indicated an increased resistance of H1975GR to the PI3K-mTOR inhibitor BEZ235 compared to H1975 following 72 hour treatment (IC50: 186.44µM vs. 25.86µM; n=3). (C) H1975GR had a significant increase in cell viability compared to H1975P at a number of drug doses (*p<0.05, paired student t-test, n=3).

Interrogation of IL-6/STAT3 signalling in PI3K/mTOR inhibitor resistance:

- Figure 2: the gene expression profile of H1975GR was analysed using an IL-6/STAT3 pathway array. mRNA expression of IL-6/STAT3 pathway-related genes was compared between H1975GR and age-matched H1975P. 22 genes involved in molecular functions of IL-6/STAT3 signalling were found to be differentially expressed between the two cell lines. As indicated on scatter plot, a number of genes altered by ≥2-fold in the array were chosen for further validation by qPCR.

Effect of triple targeting PI3K/mTOR/PIM kinase inhibitor IBL-301 on NSCLC in vitro:

- Figure 5: PI3K/mTOR inhibitor BEZ235 and triple targeting PI3K/mTOR/PIM inhibitor IBL-301 demonstrate dose dependent effects on cell viability in NSCLC cell lines H1975 and H1975GR.

Conclusion

Our group has developed a PI3K-mTOR inhibitor resistant NSCLC cell line model that demonstrates acquired resistance to both GDC-0980 and BEZ235. This indicates the utility of this model to interrogate resistance mechanisms to other PI3K-mTOR inhibitors and is not limited to just GDC-0980. This study identifies alterations in the IL-6/STAT3 signalling pathway contributing to resistance to PI3K-mTOR inhibition and these data may provide novel effective multi-targeted therapeutic strategies for lung cancer patients. A novel PI3K/mTOR/PIM inhibitor IBL-301 has shown promising in vitro data that warrant further investigation as a therapeutic strategy for NSCLC.