Identification of Molecular Targets of BEZ-235 in Pancreatic Cancer Cell Line AsPC-1

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ABSTRACT

Pancreatic cancer is one of the deadliest types of cancers with a relatively high mortality rate. With many cancers and diseases there are common risk factors that include tobacco smoking, age, and genetics among others. Abrupt regulation and expression of key signaling pathways that control cell growth, division and survival are common in Pancreatic cancer. PI3 kinase pathway is one of the important molecular targets for various candidate chemotherapeutics. In this study, we have analyzed the effects of PI3 Kinase pathway inhibitor Bez235 on the growth, survival and death of Pancreatic cells. Cell survival/death was analyzed with XTT assay after 72 hours of drug addition. It was found that there is a significant reduction in cell survival at 5 micromolar concentration of Bez235. We have further isolated RNA from treated and control cells using Trizol method. cDNA was synthesized from the RNA using reverse transcriptase enzyme. We have further diluted the cDNA and used it for real time PCR with SYBR green. We have analyzed the various genes involved in apoptosis and metastasis. Bez235 increases the apoptosis as indicated by decrease in Bcl2 expression. There is no significant change in the expression of metastasis related gene N-Cadherin in Bez235 treated cells.

INTRODUCTION

Pancreatic cancer is one of the deadliest types of cancers with a relatively high mortality rate. With many cancers and diseases there are common risk factors that include tobacco smoking, age, and genetics among others. It is one of the leading causes of cancer related deaths with over 250,000 new cases annually and a very poor five-year survival rate. The five-year survival rate is very low, with a rough percentage of 6% survival rate and roughly 331,000 deaths per year. Due to the poor prognosis, there is a continuous search for new/combinational chemotherapeutics against Pancreatic cancer.

Abrupt regulation and expression of key signaling pathways that control cell growth, division and survival are common ion Pancreatic cancer. PI3 kinase is one of the important molecular targets for various candidate chemotherapeutics. This pathway is involved in the cell growth, survival and cell division and it is considered as major chemotherapeutic target in different cancers. In this study, we have analyzed the effects of PI3 Kinase pathway inhibitor BEZ235 on the survival and apoptosis related genes in Pancreatic cancer cells.

METHODS

Cell Culture: Human prostate cancer cell line LNCaP was purchased from ATCC. Cells were grown in DMEM medium supplemented with 10% Fetal Bovine Serum and Pen/Strep. Antimycotic solution was also added to control fungal contamination.

XTT Assay:

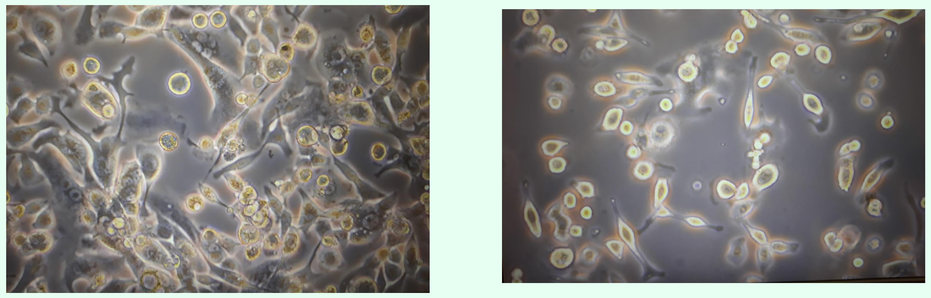
Cells were treated with 5 micromolar of BEZ235 in 96 well plate. Untreated (Control) and treated cells were incubated for 72 hours, and then XTT reagent was added. Measurements were taken three and five hours after the addition of XTT substrate.

METHODS

Real time PCR analysis:

Cells were grown in 6 well plates and treated with BEZ 235. Total RNA was isolated from the cells using Trizol method. Briefly, supernatant was removed from the wells and Trizol was added to the wells to isolate RNA. cDNA was prepared using reverse transcriptase. Realtime PCR was performed using SYBR Green. Beta actin was used as endogenous control. Relative expression was calculated using delta delta Ct method

RESULTS



(a) Untreated AsPC-1 cells *(b) AsPC-1Treated with BEZ235*

Fig. 1. Qualitative assessment of untreated (DMSO only) and Bez235 treated AsPC-1 cells

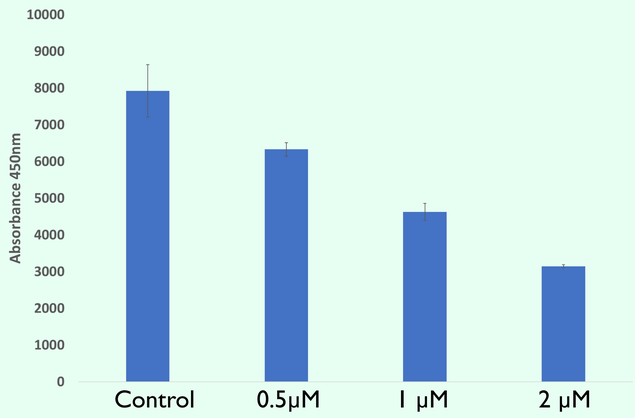


Fig.2. Cell viability of AsPC-1 with different concentrations of Bez235 as analyzed by XTT assay

RESULTS

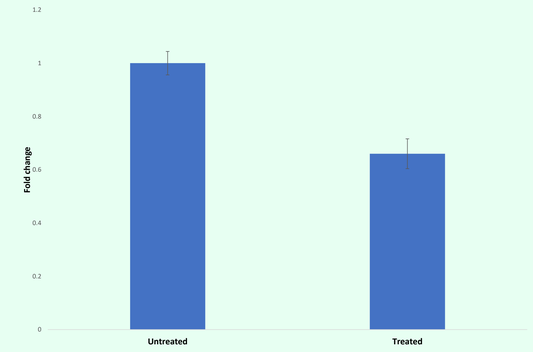


Fig. 3a. Bcl2 expression in untreated and Bez 235 treated cells

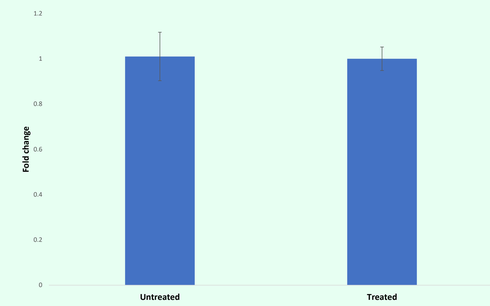


Fig. 3b. N-Cadherin expression in untreated and Bez235 treated AsPC-1 cells

CONCLUSIONS AND FUTURE WORK

- Bez235 inhibits the cell viability of AsPC-1 cells in dose dependent manner.
- There is significant change in Bcl2 expression in Bez235 treated cells as compare to control cells.
- There is no significant changes in N-Cadherin levels in treated and more genes will be screened for apoptosis and PI3 Kinase pathway.

References:

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