Analysis of apoptosis related genes in LNCaP cells treated with PI3 Kinase inhibitor BEZ-235

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ABSTRACT

Prostate cancer is the most common type of non-skin cancer found in and it is the second leading cause of cancer death in men in Western societies. PI3K (phosphoinositide 3-kinase)/mTOR (mammalian target of rapamycin)/AKT pathway is one of the main molecular pathways that is abruptly active in the prostate cancer. In the present study, we have analyzed the effect of BEZ235 on the growth on prostate cancer cell line LNCaP by using XTT assay. We have found that 1 micromolar concentration of BEZ 235 was quite potent on LNCaP cells. We have further treated the cells with BEZ235 and isolated RNA for quantitative gene expression analysis. BEZ 235 enhances the apoptosis of LNCaP cells as analyzed by apoptosis markers. We are further analyzing the expression of the genes involved in PI3 kinase pathways impacted by BEZ235.

INTRODUCTION

Prostate cancer is the most common type of non-skin cancer found in men and it is the second leading cause of cancer death in men in Western societies. In the United States, there are an estimated 191,930 new cases projected for 2020. Prostate cancer makes up 1 in 5 new cancer diagnosis. The prostate gland is only found in men and it is located between the bladder and the penis. When prostate cells mutate, they begin to divide rapidly and can lead to cancer. Recent research found that androgen receptors play a large role in the health of the prostate gland. Androgens receptors and signaling are required for a normal prostate to develop and function properly. These receptors have been found to mutate and feed into tumor growth in the prostate. Further, various key Cell signaling pathways play major role in the development of Prostate cancer. PI3K (phosphoinositide 3-kinase)/mTOR (mammalian target of rapamycin)/AKT (protein kinase B) pathway is one of the main molecular pathways that is abruptly active in the prostate cancer. In the present study, we have analyzed the effect of PI3 Kinase pathway inhibitor BEZ235 on the cell viability of prostate cancer cell line LNCaP. We have also analyzed the expression of apoptosis related genes in the drug treated and control (untreated cells).

Aims of the present study:

1. To analyze the effect of BEZ235 on the viability of prostate cancer cell line LnCAP cells.
2. To analyse the comparative expression of the genes involved in the apoptosis in control and BEZ 235 treated LnCAP 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.

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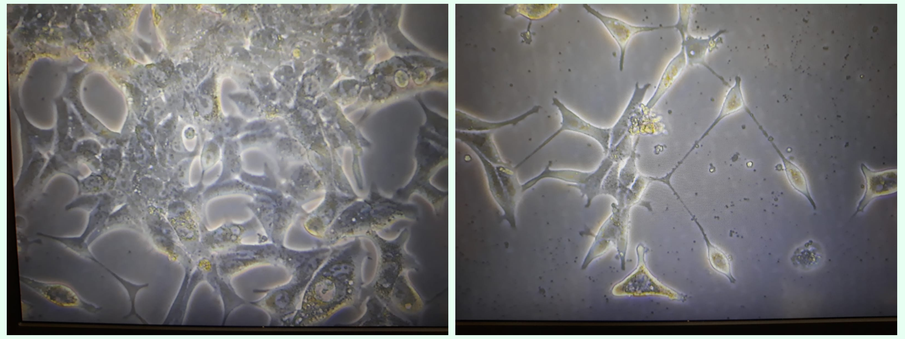
METHODS

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) LnCAP (untreated) *(b) LnCAP treated with BEZ235*

Fig. 1. ***Prostate cancer cell line LNCaP ; Untreated (a) Treated with BEZ235 (b)***

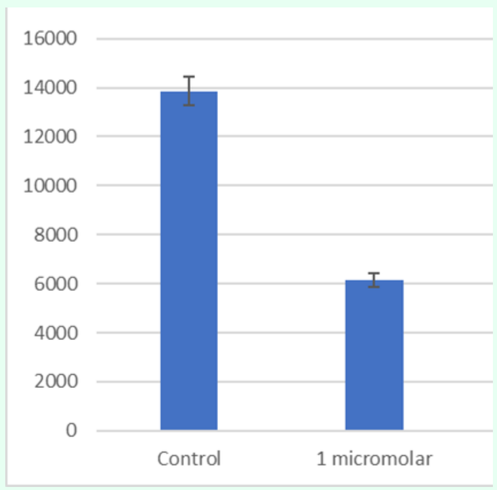


Fig.2. Cell Viability analysis using XTT assay

RESULTS

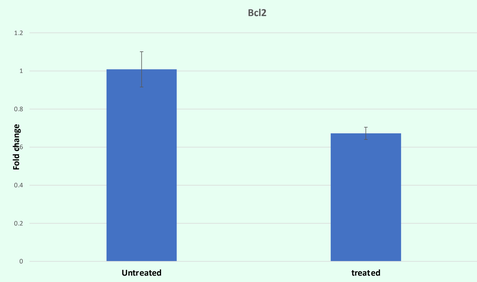


Fig. 3a. Comparative expression of Bcl2 gene in control cells and Bez treated cells

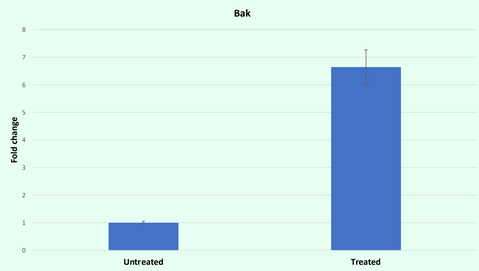


Fig. 3b. Comparative expression of Bak gene in control cells and Bez treated cells

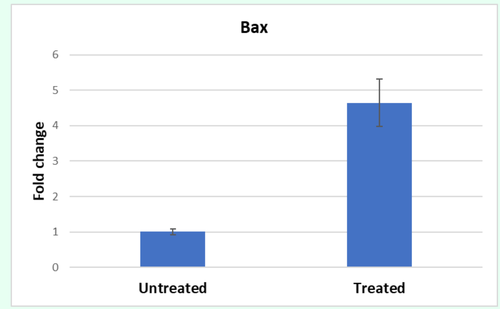


Fig. 3c. Comparative expression of Bax gene in control cells and Bez treated cells

CONCLUSIONS AND FUTURE WORK

- 1µM BEZ235 inhibits about 50% viability of LNCaP cells.
- Bcl2 expression is decreased, where as Bak and Bax expression is increased in BEZ235 treated cells that indicates induction of apoptosis in LNCaP cells.

References: Steele, C. B., Li, J., Huang, B., & Weir, H. K. (2017). Prostate cancer survival in the United States by race and stage (2001-2009): Findings from the CONCORD-2 study. Cancer, 123(24), 5160–5177. doi: 10.1002/cncr.31026.

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