

Anti-proliferative Activity of NVP-BEZ235, a Dual Inhibitor of PI3K and mTOR, in Colorectal Cancer Cells

Deok Bae Park, Youngki Lee

Department of Histology, Jeju National University School of Medicine, Jeju, Korea

(Received November 30, 2013; Revised December 7, 2013; Accepted December 14, 2013)

Abstract

In colorectal cancer diverse mutations of genes involved in signal transduction pathways for cell survival and proliferation are observed, which have been the therapeutic targets for improvement of malignancies. The present study was undertaken to investigate whether NVP-BEZ235 (BEZ235), a dual inhibitor of PI3K/mTOR, shows any anti-proliferative effect on colorectal cell lines harboring KRAS, and/or, PIK3CA, and/or BRAF mutations and to determine whether these mutational status affects the sensitivity of colorectal cancer cells to BEZ235. BEZ235 treatment induced growth inhibition in all four cell lines tested. HCT116 cell line with both KRAS and PIK3CA mutation, and LoVo cell line with KRAS mutation showed higher IC₅₀ values (643±19 nM and 663±7 nM, respectively) at 3 days post-treatment. On the other hand, HCT15 cell line harboring the same mutational status as HCT116 with KRAS and PIK3CA mutation, and HT29 cell line with BRAF mutation were more sensitive to BEZ235 (IC₅₀ values: 25±7 nM and 213±40 nM, respectively). At suboptimal concentrations of BEZ235 (50 nM), HCT116 and LoVo cell lines were less responsive comparing with the HCT15 and HT29 cell lines, whereas a higher doses of BEZ235 showed a similar response of growth inhibition in all four cell lines tested except HCT15. These studies provide the preclinical rationale for evaluating the efficacy of BEZ235. (J Med Life Sci 2013;10(2):179-183)

Key Words : NVP-BEZ235, PI3K, MTOR, KRAS, BRAF, colorectal cancer

Introduction

KRAS, BRAF and PIK3CA are frequently mutated in colorectal cancer (CRC), approximately 40%, 15% and 17%, respectively^{1,2}. These mutations result in aberrant activation of the RAS/RAF/MEK and phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathways³. Both the RAS/RAF/MEK and PI3K/Akt/mTOR pathways are the chief mechanisms for regulation of cell survival, proliferation, differentiation, tumor growth and metabolism and have been employed as a potential therapeutic target in human cancers including CRC^{4,5}.

NVP-BEZ235 (BEZ235) is a novel and orally available dual inhibitor of both PI3K and its downstream mTOR. The compound potently and reversibly inhibits both class I PI3K and mTOR kinase catalytic activity by competing at their ATP-binding site⁶. Several in vitro and in vivo studies have

shown the anti-proliferative and anti-tumor activities of BEZ235 in diverse human cell lines and animal models^{7,8}. In terms of the relationship between mutation status and sensitivity to BEZ235, however, there are some conflicting and controversial reports. Whereas initial studies suggested that the effect of BEZ235 may be confined to tumors harboring mutations in PIK3CA^{9,10}, others show no differential efficacy of BEZ235 against PIK3CA wild type and mutant cell lines¹¹.

In the present study to re-evaluate and extend these findings, 4 human CRC cell lines having different mutational status are selected and screened for their sensitivity of growth inhibition to BEZ235 treatment. The result shows that BEZ235 has highly growth inhibitory activity in colorectal cancer cell lines harboring KRAS and/or PIK3CA, or BRAF mutants. Moreover, there is a differential sensitivity to BEZ235 at suboptimal concentration (50nM), while stronger inhibition of cell growth is observed at relatively higher concentrations (800nM) in all four CRC cell lines tested.

Correspondence to : Youngki Lee
Department of Histology, Jeju National University School of
Medicine, 102 Jejudaehakno, 690-756, Jeju, Korea
E-mail: yklee38@juju.ac.kr

Materials and methods

Cell lines and compounds

The human colorectal cancer cell lines, HCT116, HCT15 and HT29 were obtained from the American Type Culture Collection (Manassas, VA) and LoVo from the DSMZ (Braunschweig, Germany). These cell lines were grown in a monolayer culture in DMEM (Sigma) supplemented with 10% fetal bovine serum and 1% streptomycin/penicillin, and cells were maintained at 37°C in a humidified atmosphere consisting of 5% CO₂ and 95% air. Cells have been regularly tested for mycoplasma and kept free of this contamination by treating 5 µg/ml of Plasmocin (InvivoGen). NVP-BEZ235 (BEZ235) was purchased from LC Laboratories (Woburn, MA). BEZ235 was dissolved in dimethyl sulfoxide (DMSO, Sigma) to a concentration of 2 mM and stored at -20°C. DMSO was added to the culture medium of control groups and the final DMSO concentration was always kept below 0.1%.

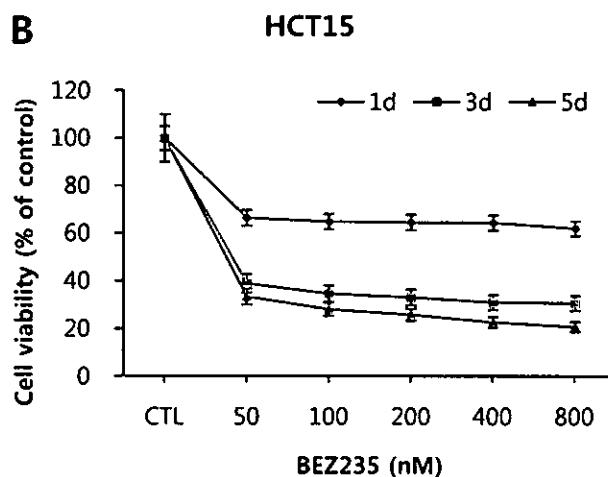
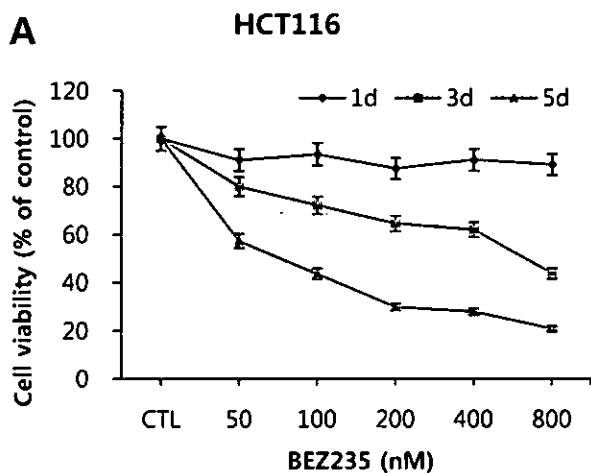
Determination of sensitivity to BEZ235

Colorectal cancer cells were seeded into 24-well plates at different initial densities (HCT116: 2×10^4 cells/well, HCT15: 1×10^4 cells/well, HT29: 3×10^4 cells/well, and LoVo: 1×10^5 cells/well) according to the different rates of cell growth. After 24 hours, cells were treated with 0, 50, 100, 200, 400 and 800 nM of BEZ235 for 1d, 3d and 5d. Medium containing BEZ235 was changed every 24 hours. Cell viability after BEZ235 treatment was assessed by incubating

with MTT reagent for 3 hours at 37°C, followed by solubilization of the formazan crystal with propanol for 30 minutes. Absorbance was measured at 570 nm with a microplate analyzer. All the experiments were performed triplicate.

Results

To determine the effect of in vitro BEZ235 treatment on cellular viability, four human CRC lines (HCT116, HCT15, HT29 and LoVo) were treated with various concentrations of BEZ235 (50–800nM) for 1d, 3d, and 5d, and cellular viability was assessed using MTT assay. All four cell lines tested in the present study responded well to the BEZ235 treatment. However there is a differential sensitivity to BEZ235 treatment in both cell line specific and BEZ235 dose-dependent manner. HCT116 cell line with both KRAS and PIK3CA mutation, and LoVo cell line with KRAS mutation showed higher IC₅₀ values (643 ± 19 nM and 663 ± 7 nM, respectively) at 3 days post-treatment. On the other hand, HCT15 cell line harboring the same mutational status as HCT116 with KRAS and PIK3CA mutation, and HT29 cell line with BRAF mutation were more sensitive to BEZ235 (IC₅₀ values: 25 ± 7 nM and 213 ± 40 nM, respectively) (Fig. 1). In respect to dose-dependent sensitivity of these cell lines to BEZ235 treatment, at suboptimal concentration of BEZ235 (50 nM) HCT116 and LoVo cell lines were less responsive as compared with the HCT15 and HT29 cell lines, whereas at higher concentration (800 nM) HCT116, HT29 and LoVo but HCT15 give a similar sensitivity (Fig. 2).



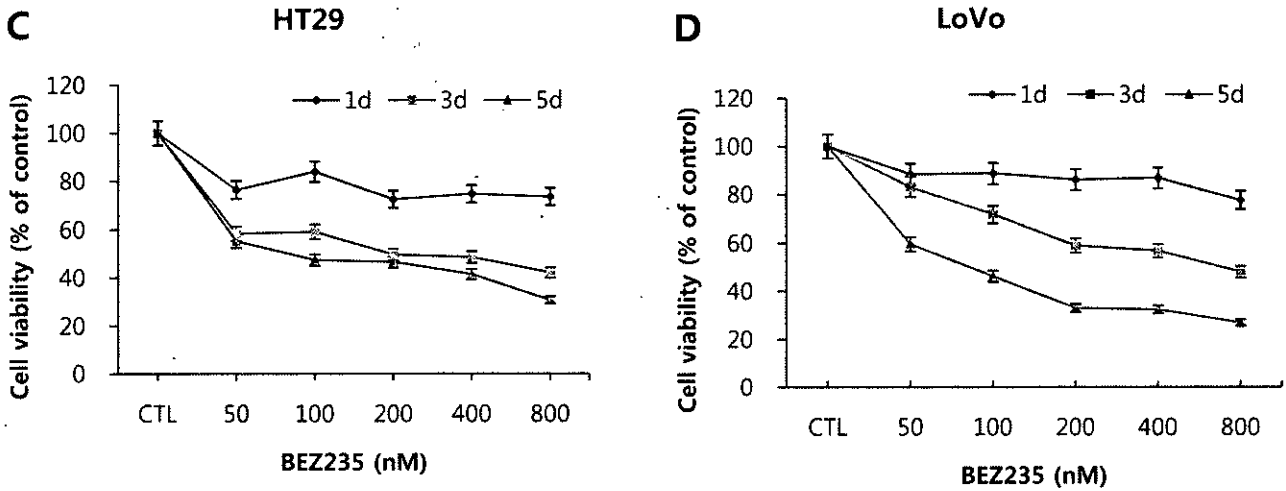


Figure 1. A differential sensitivity of colorectal cancer cell lines to increasing doses of NVP-BEZ235 incubated for 1 d, 3 d and 5d. Cell viability was determined by MIT assay. HCT15 (B, $IC_{50} = 25 \pm 7$ nM) and HT29 (C, $IC_{50} = 213 \pm 40$ nM) are more sensitive than HCT116 (A, $IC_{50} = 643 \pm 19$ nM) and LoVo (D, $IC_{50} = 663 \pm 7$ nM) at 3 d post-treatment. The data was normalized to the value of control cell. Points, mean (n = 3 experiments); bars, SE.

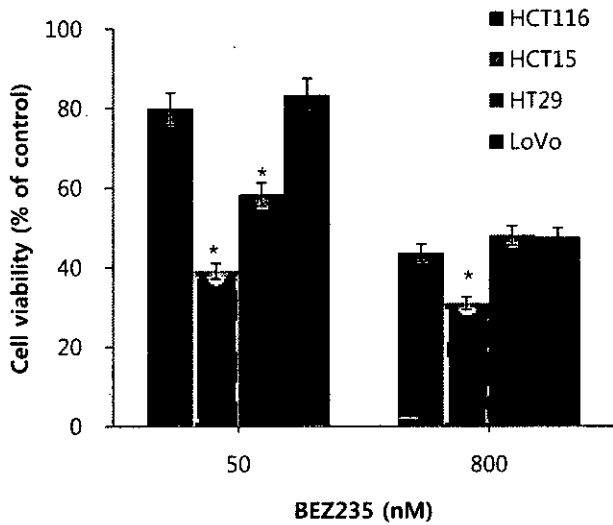


Figure 2. Dose-dependent differential sensitivity of colorectal cell lines to 72 h NVP-BEZ235 treatment. Cell viability was determined using MIT assay. HCT15 and HT29 are more sensitive than HCT116 and LoVo to BEZ235 treatment at suboptimal concentration (50 nM), HCT15, HT29 and LoVo but HCT116 give a similar sensitivity at higher concentration (800 nM). The data was normalized to the value of control cell. Points, mean (n = 3 experiments); bars, SE. *, $P < 0.001$.

Discussion

The present study investigated the growth inhibitory effect of BEZ235, a dual inhibitor of PI3K and mTOR, in four CRC cell lines with different mutational status. It revealed that HCT116 (both PIK3CA and KRAS mutation) and LoVo (KRAS mutation) are less sensitive to BEZ235 comparing with HT29 (BRAF mutation) and HCT15 (both PIK3CA and KRAS mutation). Moreover, at suboptimal dose of BEZ235 (50 nM) it shows the differential responses of growth inhibition irrespective of mutational status, while there is a strong growth inhibitory effect at higher concentration of BEZ235 (800 nM) in all four cell lines tested here.

It has been known that cell survival, proliferation, tumor growth, cell metabolism and cell motility are regulated by RAS/RAF/MEK/ERK and RAS/PI3K/Akt/mTOR signaling pathways. The activation of these pathways occurs after ligands binding to their receptor, which successively triggers a chain of downstream signaling events. Abnormal activation of these pathways occurs in many human cancers due to mutations at the upstream membrane receptors, RAS and BRAF as well as PI3K, PTEN and Akt. For these reasons, inhibitors for the components of RAS/RAF/MEK/ERK and RAS/PI3K/Akt/mTOR cascades have been developed for the therapeutic intervention of human malignant cancers¹². For example, compounds blocking RAS, RAF, MEK, PI3K, Akt, mTOR and some downstream targets have been developed

and many are currently in clinical trials. However, due to cross activation and signaling convergence between these two pathways the oncogenic activation of RAS/RAF/MEK/ERK and RAS/PI3K/Akt/mTOR signaling can facilitate the development of resistance to therapeutics targeting only one pathway³⁹.

In CRC, oncogenic mutations of KRAS, BRAF, and PIK3CA are frequently found and result in the aberrant activation of signaling cascades, predominantly RAS/RAF/MEK/ERK and RAS/PI3K/Akt/mTOR pathways. NVP-BEZ235 is a dual inhibitor of pan-class I PI3K and mTOR kinase which has been proved to have anti-proliferative and anti-tumor activity in a number of human cancer cells³⁷ including CRC cell lines and xenograft mouse models⁴⁰. Some studies have suggested that PIK3CA mutant cancers show the increased sensitivity to PIK3CA inhibitor therapy⁴¹ but others have demonstrated the comparable efficacy of BEZ235 against both PIK3CA mutant and wild type human cell lines¹¹. In addition, a group has showed the differential sensitivity of human CRC cell line to BEZ235 depending on the mutational status⁴², while other does not give any relationship between mutational status and sensitivity of CRC cell lines to BEZ235 treatment¹¹. In the present study, HCT116 and HCT15 CRC cell lines have the same mutations of PIK3CA and KRAS but the growth inhibitory effect of BEZ235 treatment is much more greater in HCT15 than in HCT116 ($IC_{50}=25\pm 2$ nM versus 643 ± 33 nM) at 3 days post-treatment. The concurrent mutations of KRAS and PIK3C lead to additive activation of PIK/Akt/mTOR signaling pathway. BEZ235 has been known to inhibit mTOR only at a low concentration (<100 nM) but dual PI3K/mTOR blockade is observed at relatively higher concentration (>500 nM)¹¹. Thus, treatment of higher concentration of BEZ235 could abolish the enhanced PIK/Akt/mTOR pathway, giving a similar sensitivity in these two cell lines, but at low concentration HCT15 cells are more sensitive than HCT116 cells to BEZ235 treatment. In the present, the reason for this differential sensitivity at low concentration of BEZ235 between two cell lines with the same mutational status is unknown and may result from cell lineage-specific effects¹⁵.

BRAF mutations were not thought to frequently occurred in human cancer and more attention was paid to KRAS mutations which are frequently observed in human CRC (about 40%). KRAS mutations activate both RAF/MEK/ERK and PI3K/AKT/mTOR pathways. However, recent studies have shown that BRAF is frequently mutated in certain types of cancer including CRC (5 to 20%). HT29 (BRAF mutant) and LoVo (KRAS mutant) cell lines show similar

sensitivity to BEZ235 treatment especially at higher concentration, implying the almost complete inhibition of PI3K and mTOR with BEZ235.

In summary, this study was undertaken to delineate the effect of BEZ235 treatment on the survival of four CRC cells harboring different mutational status. It shows the dose- and time-dependent growth inhibition of all four cell lines following BEZ235 treatment. However, BEZ235 treatment results in only partial growth inhibition even at higher concentration in all four CRC cell lines (approximately 60% inhibition at 800 nM) regardless the status of their mutations. This may be due to cross talk and signal convergence between RAS/RAF/MEK/ERK and RAS/PI3K/Akt/mTOR pathways. Therefore to enhance the efficacy of therapeutic intervention in the future clinical trials, further studies are required by employing inhibitors of RAS/BRAF/MEK/ERK pathway in combination with BEZ235.

Acknowledgement

This research was supported by the 2013 scientific promotion program funded by Jeju National University

References

- 1) Laurent-Puig, P., Cayre, A., Manceau, G., Buc, E., Bachet, JB., Cecomte, T., et al. Analysis of PTEN, BRAF, and EGFR status in determining benefit from cetuximab therapy in wild-type KRAS metastatic colon cancer. *J Clin Oncol* 2009;27:5924-30.
- 2) Baba, Y., Noshio, K., Shima, K., Hayashi, M., Meyerhardt, JA., Chan, AT., et al. Phosphorylated Akt expression is associated with PIK3CA mutation, low stage, and favorable outcome in 717 colorectal cancers. *Cancer* 2011;117:1399:1408.
- 3) Mendoza, MC., Er, EE., Blenis J. The Ras-ERK and PI3K-mTOR pathways: cross-talk and compensation. *Trends in Biochemical Sciences* 2011;36(6):320-327.
- 4) Rodriguez-Viciana, P., Warne, PH., Dhand, R., Vanhaesebroeck, B., Gout, I., Fry, MT., et al. Phosphatidylinositol-3-OH kinase as a direct target of ras. *Nature* 1994;370:527-532.
- 5) Kato, S., Iida, S., Higuchi, T., Ishikawa, T., Takagi, Y., Yasuno, M., et al. PIK3CA mutation is predictive of poor survival in patients with colorectal cancer. *Int J Cancer* 2007;121:1771-1778.
- 6) Maira, S., Stauffer, F., Brueggen, J., Furet, P., Schnell, C., Fritsch, C., et al. Identification and characterization of

- NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. *Mol Cancer Ther* 2008; 7(7):1851-1863.
- 7) Serra, V., Markman, B., Scaltriti, M., Eichhorn, JA., Valero, V., Guzman, M., et al. NVP-BEZ235, dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. *Cancer Res* 2008;68(19):8022-8030.
 - 8) Kim, A., Lee, J., Lee, S., Kim, C., Lee, S., Jang, W., et al. Coexistent mutations of KRAS and PIK3CA affect the efficacy of NVP-BEZ235, a dual PI3K/mTOR inhibitor, in regulating the PI3K/mTOR pathway in colorectal cancer. *Int J Cancer* 2013;133:984-996.
 - 9) Di Nicolantonio, F., Arena, S., Tabernero, J., Grosso, S., Molinari, F., Macarulla, T., et al. Deregulation of the PI3K and KRAS signaling pathways in human cancer cells determines their response to everolimus. *J Clin Invest* 2010;120:2858-2866.
 - 10) Tanaka, H., Yoshida, M., Tanimura, H., Fujii, T., Sakata, K., Tachibana, Y., et al. The selective class I PI3K inhibitor CH5132799 targets human cancers harboring oncogenic PIK3CA mutations. *Clinical Cancer Research* 2011;17:3272-3281.
 - 11) Roper, J., Richardson, MP., Wang, W., Richard LH., Chen, W., Coffee, E., et al. The dual PI3K/mTOR inhibitor NVP-BEZ235 induces tumor regression in a genetically engineered mouse model of PIK3CA wild-type colorectal cancer. *PLoS ONE* 2011;6(9):1-10.
 - 12) Chappell, WH., Steelman, LS., Long, JM., Kempf, RC., Abrams, SL., Franklin, RA., et al. RAS/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR inhibitors: Rationale and importance to inhibiting these pathways in human health. *Oncotarget* 2011;2:135-164.
 - 13) Mendoza, MC., Emrah Er, E., Blenis, J. The Ras-ERK and PI3K-mTOR pathways: cross talk and compensation. *Trends in Biochemical Sciences* 2011;36(6):320-329.
 - 14) Samuels, Y., Diaz, LA., Schmidt-Kittler O., Cummins, JM., Delong, L., Cheong, I., et al. Mutant PIK3CA promotes cell growth and invasion of human cancer cells. *Cancer Cell* 2005;7:561-573.
 - 15) McCubrey, JA., Steelman, LS., Chappell, WH., Abrams, SL., Wong, EWT., Chang, F., et al. Roles of Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. *Biochimica et Biophysica Acta* 2007;1773:1263-1284.