Abstract

Colorectal cancer is the third leading cause of death due to cancer in both men and women with significant geographical, racial and ethnic variation in its pattern and rate of incidence. Aberrant activation of phosphotidylinositol 3-kinase (PI3K) pathway effectors has been observed in many cancers including colorectal cancer. As cancers develop due to multiple genetic alterations it is seldom possible to treat them with monotherapy. Combination therapy has been the standard care in cancer treatment. Therefore, the anticancer effects of the combination of limonene which is a natural monoterpene and NVP-BEZ235, a dual inhibitor of PI3K/mammalian target of rapamycin (mTOR) pathway were investigated in colorectal cancer cell lines. In vitro anticancer assays such as cell viability, colony formation assay, cell migration, cell cycle analysis, apoptosis, caspases estimation and Western blot analysis for key phosphorylated proteins of PI3K pathway were performed to determine the combination effects on cells. Results showed that both drugs exhibited dose and time dependent cytotoxicity. Drug combination exhibited additive, inhibitory effects on both colony formation and cell migration, corroborating with the profound anti-tumor and anti-metastatic activity respectively. Induced the cells in G1 phase arrest with concomitant decrease in S phase and G2 phase cells. The drug combination increased the activities of caspases-3 and -9 and expression of pro-apoptotic BAD and BAX with concomitant decrease in anti-apoptotic Bcl-2. However, p53 appeared to have minimal role in apoptosis at least in cells having wild type p53. Drug combination is more effective in inhibition of phosphorylation of PI3K, mTORC1 and mTORC2 than individual treatments. The inhibition of mTORC1 and mTORC2 was more sensitive than PI3K to the combination. In conclusion, the results indicate that limonene and BEZ combination demonstrated anticancer effects and are mediated by down regulation of PI3K/mTOR signaling and induction of apoptosis by mitochondrial death pathway.