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STR1720, a potential sensitizer for radiotherapy and cytotoxicity effects of NVB-BEZ235 in metastatic breast cancer cells

Daryoush Fatehi[a], Amin Soltani[b], Mahdi Ghatrehsamani[b,*]

[a] Department of Medical Physics, Faculty of Medicine, Shahrekord University of Medical Sciences, Shahrekord, Iran
[b] Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

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ABSTRACT

Background: Chemo-radio therapy (CRT) resistance is a main barrier in treating the triple negative breast cancer (TNBC). The success of conventional treatment may be ameliorated by elevating the responsiveness of the cancer cells to CRT. NVP-BEZ235 as a PI3K/AKT/mTOR dual inhibitor has been shown promising results in treating breast cancer cells. However, potential radiation-sensitizing effect of NVP-BEZ235 in TNBC remained unclear. In addition, SIRT-1 activation state and environmental cytokine were identified as being responsible for cancer cells responses to CRT. Herein, we investigate the role of interleukin 6 (IL-6) as a tumor environmental cytokine and SIRT1 in the effectiveness of NVP-BEZ235 plus radiotherapy.

Material and methods: TNBC cells were pre-treated with/without IL-6 and were exposed to single and combination of SRT1720 (SIRT1 activator)/EX-527 (SIRT1 inhibitor) and/or NVP-BEZ235 and/or gamma radiation. The effect of our treatments on cellular growth was determined by MTT and the cellular death and CSCs percentage were determined by Flow cytometry. Senescence detection kit was used to assay the effect of our treatments on cellular senescence induction.

Results: Activation of SIRT1 via SRT1720 increased the efficacy of CRT in TNBC cells, especially when IL-6 exists in tumor microenvironment. Additionally, IL-6 pre-treatment followed by exposure to SRT1720 and NVP-BEZ235 significantly increased sensitivity of the cancer stem cells to radiation ($p < 0.05$).

Conclusion: Our result shows that combination of NVP-BEZ235 and SRT1720 may effectively improve late stage breast cancer cells therapeutics approach. Activation of SIRT1 and STAT3 in resistance breast cancer cells improves the in-vitro therapeutic efficacy of CRT.

1. Introduction

Breast cancer (BC) is the major causes of cancer-related death among women. Between the various BC kinds, Triple-negative breast cancer (TNBC) is known to be extremely metastatic and resistance to conventional therapies [1]. Therefore, rate of death and metastasis is higher in women with TNBC [2]. Thus, it is crucial to find new strategies to treat this type of cancer. Several studies have indicated that activation of phosphatidylinositol 3-kinase (PI3K)/AKt/Mammalian target of rapamycin (mTOR) signaling via SIRT1 plays a major role in cancer cells growth and metastasis [3,4]. Activation of PI3K leads to Phosphatidylinositol (3,4,5)-trisphosphate (PIP3) generation which is required for the activation of AKT. AKT acts as a proto-oncogene and mediates mTOR activation, which is common in BC leads to tumor progression, resistance and survival [3]. In addition, many reports proposed that PI3k/AKT/mTOR activation mediates cancer progression and resistance to CRT [5]. It was shown that activation of PI3k/AKT/mTOR pathway increases cancer stem cells (CSCs) phenotypes [6]. CSCs are major reason for tumor metastasis, progression and resistance to combined treatment regimen including CRT [7]. Further evidence linking SIRT1 expression with PI3k/AKT activation. SIRT1 is a NAD-dependent histone deacetylase that induces deacetylation and activation of AKT as well as PI3k. A number of trials have demonstrated that SIRT1 promotes cancer progression by activating AKT [8,9]. These trials indicated that elevated activation of PI3k/AKT or expression of SIRT1 increase resistance to CRT. Activation of SIRT1 [10], similar to PI3K/AKT, increases tumor metastasis and CSCs phenotype in TNBC [11]. Based on the findings of these trials, application of NVP-BEZ235 in combination with SIRT1 activator/inhibitor can reveal promising outcomes for treatment of patients with TNBC. Despite the PI3k/AKT

Abbreviations: TNBC, triple negative breast cancer; IL-6, interleukin 6; BC, breast cancer; PI3K, phosphatidylinositol 3-kinase; mTOR, Mammalian target of rapamycin; CSC, cancer stem cell; CRT, chemo-radio therapy

* Corresponding author at: Cellular and Molecular Research Center, Shahrekord University of Medical Sciences, PO Box: 88155-571, Shahrekord, Iran.
E-mail address: ghatreh.m@SKUMS.ac.ir (M. Ghatrehsamani).

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and SIRT1 activation, tumor environmental cytokine has major effects on the cellular mechanisms of the action. Interleukin 6 (IL-6) is a well-known tumor environmental cytokine modulates various cellular processes, such as apoptosis via activation of SIRT1. Likewise, Maria et al. have reported that IL-6 directly promote PI3K/AKT activation [12]. In another study, Seyung et al. reported the IL-6 impress CSCs induction and resistance to apoptosis in TNBC cells [12]. These observations suggest that IL-6, SIRT1 and PI3k/AKT/mTOR play a crucial role in TNBC metastasis and resistance to CRT. Although, NVP-BEZ235 as a PI3k/AKT/mTOR inhibitor, has shown promising results; but, efficacy of the drug in combination with radiotherapy and its relation with SIRT1 activation state remains unclear. In the current study, we investigated the effect of NVP-BEZ235 as a survival factor inhibitor and SIRT1 activation/inhibition in the presence of IL-6 plus radiotherapy in TNBC.

2. Material and methods

2.1. Cell culture

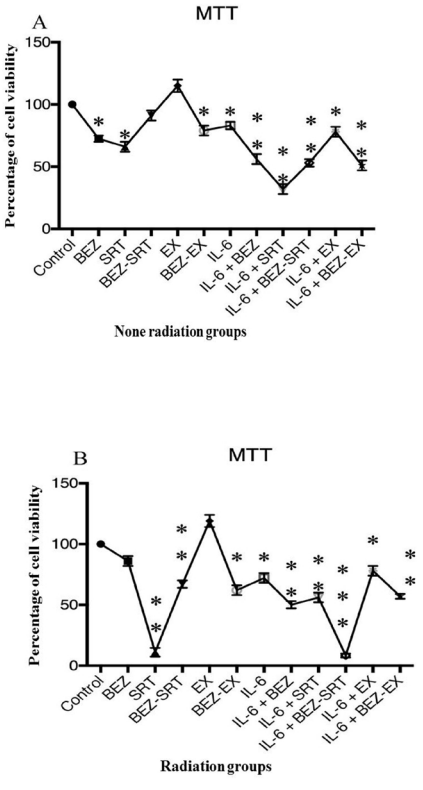
The Human breast cancer cell line, MDA-MB231 was purchased from the Pasteur Institute (IRAN) and the cells were cultured in complete RPMI 1640, supplemented with 10% heat- inactivated FBS, 1% pen/strep, in 75 ml flasks at 37 ˚C and 5% CO_2. The cells were cultured with/without 20 ng/ml IL-6 [13] (eBioscience, San Diego, CA, USA) for 2 weeks. Then, the cultures were exposed to 75 nM NVP-BEZ235 (Novartis Pharma, Basel, Switzerland) and/or 2 µM SRT1720 (SIRT1 activaor)/EX-527 (SIRT1 inhibitor) (Selleckchem, Houston, TX, USA) for 24 h (Fig. 1). All mentioned steps were done for gamma irradiation groups separately, but after 24 h of single and combinatorial drugs exposure, the flasks were irradiated with 2 Gy of gamma rays. The cultures were irradiated with 6 MV photons under sterile condition at room temperature. The radiation source was Linac installed in Parsian Hospital, Shahrekord, Iran, with the following parameters: total dose: 2 Gy, dose rate: 1 Gy/min, medium energy: 6 MeV, distance between center of the source and center of the sample containers: 80 cm.

2.2. Cell viability assay

10,000 numbers of IL-6 treated/untreated cells were seeded in the 4 different 96-well plates with 100 µl of culture medium. Afterwards, all defined treatments were done similarly for 24 h. To investigating the effects of our treatment with gamma irradiation, 2 of the 96-well plates (IL-6 treated/untreated) were gamma irradiated. Subsequently, after performing all treatments, 10 µl of MTT reagent (5 mg/ml of PBS) was added to each well, incubated at 37C for 3 h. For color development, DMSO was added to solubilize formazan crystals. Absorbance at 590 nm was measured using ELISA microplatereader.

2.3. Flow cytometry

Cells were trypsinized with 0.05% trypsin 0.25% EDTA and harvested. To analyzing the percentage of CSCs, phycoerythrin conjugated anti CD44 (ebioscience) and FITC conjugated anti CD24 (ebioscience)



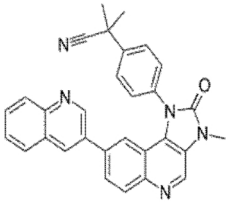
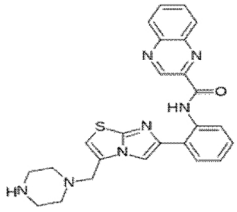
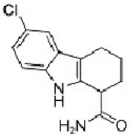
Name	Chemical structure	Target
NVP-BEZ235	C30H23N5O 	Inhibitor of PI3K and mTOR
SRT1720	C25H23N7OS 	Selective activator of SIRT1
EX-527	C13H13ClN2O 	selective inhibitor of SIRT1

Fig. 1. SRT1720 treatment sensitized MDA-MB231 cells to radiation exposure.
MDA-MB231 cells were first treated with/without the IL-6 (for 14 d) and then exposed to SRT1720 or EX-527 and/or NVP-BEZ235 (for 24 h) followed by 2 Gy gamma radiation and then cell viability was assayed. The mean percentage of cell viability ± SD (n = 3) is shown before radiation (A) and after radiation (B). The table shows the chemical structure of compounds. [Mean ± SD, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; compared to the control group (control A = no treatment, control B = only irradiated culture)]. BEZ; NVP-BEZ235, SRT; SRT1720, EX; EX-527.