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Research paper

MCL-1 is the key target of adjuvant chemotherapy to reverse the cisplatin-resistance in NSCLC

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ABSTRACT

Cisplatin is one of the most effective chemotherapeutic agents for the treatment of lung cancer. However, the acquired resistance occurred in cancer cells limits the clinical application of cisplatin. MCL-1, which is an important member in the pro-survival Bcl-2 family, plays a critical role in multidrug resistance (MDR). The aim of the present study is to investigate the value of Pan-Bcl-2 inhibitor as sensitizer for the chemotherapy of cisplatin-resistant non-small cell lung cancer (NSCLC) cells. We found the obatoclax but not the ABT-737 significantly decreased the IC50 (half maximal inhibitory concentration) of cisplatin in cisplatin-resistant NSCLC cells. Furthermore, we demonstrated that the mechanism of obatoclax-promoted cell death induced by cisplatin was dependent on the inhibition of MCL-1, which couldn't be inhibited by ABT-737 but is the target of obatoclax. Moreover, inhibition of MCL-1 recovered the function of NOXA and BAK in cisplatin-resistant NSCLC cells, leading to the promotion of mitochondrial apoptosis induced by cisplatin. Interestingly, our date indicated the obatoclax also reversed the cross-resistance in cisplatin-resistant NSCLC cells. Therefore, we demonstrated that the targeted therapy with MCL-1 inhibitors, such as obatoclax, may represent a novel strategy for cancer therapy.

1. Introduction

Lung cancer represents one of most leading causes of cancer-related death worldwide, and more than 80% of all lung cancer cases are diagnosed as NSCLC (Siegel et al., 2013; Juergens and Brahmer, 2007). Unfortunately, the 5-year overall survival rate is only about 15% because of the poor prognosis for NSCLC (Ettinger et al., 2015; Henschke et al., 2006). Therefore, there is an urgent need to elucidate the underlying mechanisms and identify new therapeutic targets for NSCLC.

Cisplatin (CDDP) is one of the first-line chemotherapeutic agents for cancer treatment, which displays a great deal of clinical activity for the treatment of a wide variety of solid tumors (Sui et al., 2015; Naka et al., 2015). CDDP kills tumor cells by interaction with DNA to form DNA adducts, leading to lethal DNA damage which induces the activation of DNA damage-mediated apoptotic program (Kartalou and Essigmann, 2001). However, the repeated clinical medication of CDDP usually induced the chemoresistance for tumor cells to evade the apoptosis program by CDDP treatment (Subhash et al., 2015; Hu et al., 2015). Therefore, there is a pressing need to explore the molecular mechanisms of CDDP-resistance and take efficient drug combination to impair the chemoresistance in cancer therapy.

Obatoclax and ABT-737 belong to the synthetic compounds called BH3-mimetics. Both of them act as Bcl-2 inhibitors by binding to the BH3 cleft of pro-survival Bcl-2 family proteins (such as Bcl-xl, Bcl-w, Bcl-2, and MCL-1) (Khaw et al., 2011; Harnett et al., 2015). This interaction leads to the inactivation of pro-survival Bcl-2 family proteins and the subsequent activation of pro-apoptotic Bcl-2 family proteins (such as NOXA, BIM, BAK), which finally promotes the programmed cell death (Bodur and Basaga, 2012). ABT-737 has been reported to selectively inhibit the function of Bcl-xl, Bcl-w, and Bcl-2 but not the MCL-1 (van Delft et al., 2006). In contrast, obatoclax, which is known as a pan-Bcl-2 inhibitor, differed from the ABT-737 by inhibiting all pro-survival Bcl-2 family proteins including MCL-1 (Albershardt et al., 2011). Despite the previous studies have proved that the BH3-mimetics are safe and efficient in treating multiple cancers according to the date of clinical trials (Vela and Marzo, 2015), the valid experimental

Abbreviations: NSCLC, non-small cell lung cancer; MCL-1, Myeloid cell leukemia-1; IC50, half maximal inhibitory concentration; CDDP, cisplatin; MMP, mitochondrial membrane potential; CPR-A549, cisplatin resistant A549; DMEM, Dulbecco's modified Eagle medium; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; cyto-C, cytochrome C; AIF, apoptosis inducing factor; Smac/DIABLO, second mitochondria-derived activator of caspases/direct IAP-binding protein with low PI; PARP, poly(ADP-ribose) polymerase; JC-1, 5,5′,6,6′-Tetrachloro-1,1′,3,3′-tetraethyl-imidacarbocyanine; PI, Propidium Iodide; MOMP, mitochondria outer membrane permeability; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; DOX, doxorubicin; 5-FU, 5-fluorouracil.

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data of combination treatment with BH3-mimetics and chemotherapeutic agents are limited. In the present study, we studied the value of BH3-mimetics as sensitizers for chemotherapy in NSCLC. Our results indicated that the pro-survival Bcl-2 family protein MCL-1 may be the key target for reversing the cisplatin-resistance.

2. Materials and methods

2.1. Cell culture and cisplatin resistance paradigm

Human NSCLC cell line A549 was purchased from the American Type Culture Collection. Cells were incubated in DMEM (Gibco, USA) supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco) at 37 °C in a humidified 5% CO_2 incubator. CDDP resistant A549 (CPR-A549) was established by continuous exposure to CDDP as previously described (Ou et al., 2015). Since the CPR-A549 cells were established successfully, they were always cultured in DMEM medium (Gibco) with 5 μM CDDP (Sigma-Aldrich, USA). Importantly, before the experiments were performed, CPR-A549 cells were moved to the CDDP-free medium for 2 weeks.

2.2. Transfection

For silence of MCL-1, NOXA, and BAK, the MCL-1-specific siRNA, NOXA-specific siRNA, BAK-specific siRNA and the negative control RNA were purchased from Qiagen (USA). For enforced expression of MCL-1, the open reading frame of human MCL-1 was cloned in a pcDNA3.1 vector (Invitrogen, USA). For transfection of these siRNA

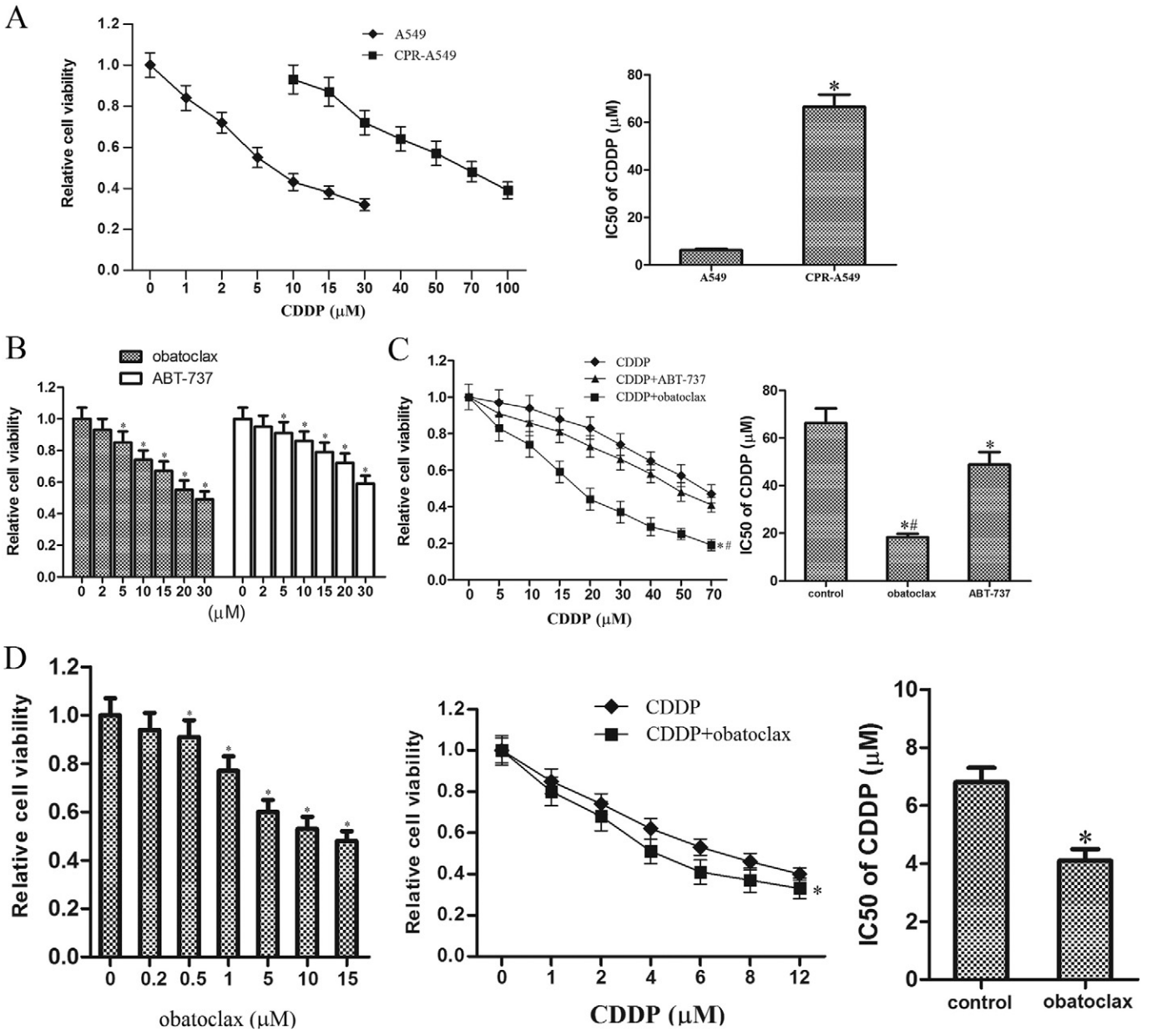

Fig. 1. Pan-Bcl-2 inhibitor obatoclax decreased the IC50 of CDDP in CPR-A549 strongly. (A) A549 and CPR-A549 cells were treated with various concentrations of CDDP for 48 h. MTT assay was then used to evaluate the sensitivity of A549 and CPR-A549 cells to cisplatin. The IC50 of CDDP was calculated according to the survival curves. $^{*}P < 0.05$ vs. A549 cells. (B) CPR-A549 cells were treated with various concentrations of obatoclax or ABT-737 for 48 h. The cytotoxicity of obatoclax and ABT-737 was then evaluated by MTT assay. $^{*}P < 0.05$ vs. untreated group. (C) MTT assay was used to evaluate the synergism of obatoclax (2 μM) and ABT-737 (5 μM) to CDDP treatment in CPR-A549. The IC50 was calculated according to the survival curves. $^{*}P < 0.05$ vs. CDDP alone treatment group, $^{\#}P < 0.05$ vs. CDDP plus ABT-737 treatment group. (D) A549 cells were treated with different concentrations of obatoclax for 48 h to determine the optimal dose for combination. The synergism of obatoclax (0.5 μM) to CDDP was evaluated by MTT assay in A549 cells. $^{*}P < 0.05$ vs. obaloclax-free group.