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Original Articles

The positive inotropic agent DPI-201106 selectively reverses ABCB1-mediated multidrug resistance in cancer cell lines

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ABSTRACT

The overexpression of ABCB1 in cancer cells is a major factor contributing to the development of multidrug resistance (MDR) and treatment failure in cancer patients. Therefore, re-sensitization of MDR cancer cells to anticancer drugs remains an important aspect in chemotherapy. The progress in developing clinically applicable synthetic inhibitors of ABCB1 has been slow, mostly due to complications associated with intrinsic toxicities and unforeseen drug-drug interactions. Here, we explored the drug-repositioning approach for cancer therapy by targeting ABCB1-mediated MDR in human cancer cells. We found that DPI-201106, a positive inotropic agent, selectively inhibits the drug efflux function of ABCB1, and in doing so, re-sensitizes ABCB1-overexpressing MDR cancer cells to conventional anticancer drugs. Furthermore, the ATPase activity of ABCB1 and docking analysis of DPI-201106 in the drug-binding pocket of ABCB1 were determined to confirm the interaction between DPI-201106 and ABCB1 protein. In summary, we revealed an additional action and a potential clinical application of DPI-201106 to reverse ABCB1-mediated MDR in human cancer cells, which may be beneficial for cancer patients who have developed multidrug resistance and no longer respond to conventional chemotherapy, and should be further investigated.

1. Introduction

The occurrence of multidrug resistance (MDR) remains a major obstacle to successful cancer chemotherapy [30]. The overexpression of ATP-Binding Cassette (ABC) transporter ABCB1 (P-glycoprotein/MDR1) often contributes, at least in part, to the MDR phenotype in tumors that results in treatment failure and cancer relapse [15,43]. ABCB1 is a typical mammalian ABC transporter protein, composed of two transmembrane domains (TMD) and two nucleotide-binding domains (NBD) that utilizes energy derived from ATP hydrolysis to actively transport a wide range of therapeutic agents that are structurally and mechanically unrelated, out of cancer cells [1,16]. As a result, the intracellular concentration and cytotoxicity of ABCB1 substrate drugs are significantly reduced in these ABCB1-overexpressing cancer cells, rendering chemotherapy ineffective [43]. Therefore, it is not surprising that the overexpression of ABCB1 has been linked to the development of MDR phenotype in blood cancer and solid tumors [27,31,33,36,40,47]. Moreover, ABCB1 is highly expressed in cells forming the blood-brain and blood-tissue barrier sites, capable of altering the absorption, distribution, metabolism, and elimination of most drugs, thereby affecting the therapeutic outcome [9,29]. For these reasons, modulating the function and/or protein expression level of ABCB1 has clinical importance.

At present, direct modulation of the drug efflux function of ABCB1 is

Abbreviations: MDR, multidrug resistance; ABC, ATP-binding cassette; FCS, fetal calf serum; CCK-8, Cell Counting Kit-8; IMDM, Iscove's Modified Dulbecco's Medium; MTT, 3-(4,5-dimethylthiazol-yl)-2,5-diphenyllapatinibrazolium bromide; Vi, sodium orthovanadate; RF, resistance-factor; RR, relative-resistance

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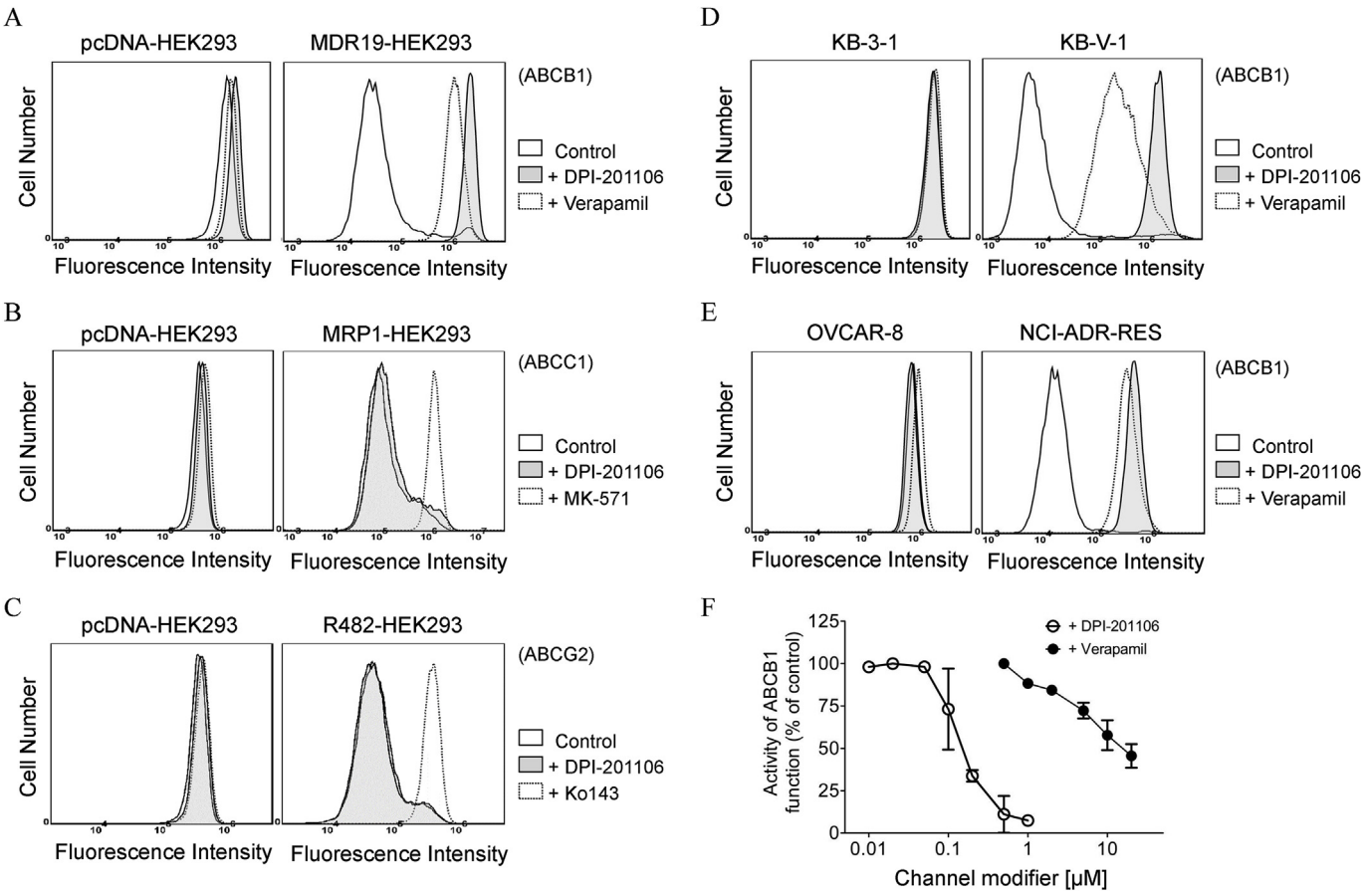

Fig. 1. DPI-201106 selectively inhibits ABCB1-mediated drug efflux. The accumulation of fluorescent calcein in HEK293 cells (A and B, left panels), HEK293 cells transfected with human ABCB1 (A, right panel) or human ABCC1 (B, right panel), human KB-3-1 epidermal cancer cells (D, left panel) and ABCB1-overexpressing KB-V-1 cancer cells (D, right panel), as well as in human OVCAR-8 ovarian cancer cells (E, left panel) and ABCB1-overexpressing NCI-ADR/RES cancer cells (E, right panel), or fluorescent PhA in HEK293 cells (C, left panel) and HEK293 cells transfected with human ABCG2 (C, right panel), was measured in the absence (solid lines) or presence of 500 nM DPI-201106 (shaded, solid lines) or 20 μM verapamil, a reference inhibitor for ABCB1 (A, D and E, dotted lines), 25 μM MK-571, a reference inhibitor for ABCC1 (B, dotted lines) or 1 μM Ko143, a reference inhibitor for ABCG2 (C, dotted lines), and analyzed immediately by flow cytometry as described previously [52] Representative histograms of three independent experiments are shown. (F) The concentration-dependent inhibition of ABCB1-mediated calcein-AM efflux by DPI-201106 (empty circles) or verapamil (filled circles) was determined in ABCB1-transfected MDR19-HEK293 cells. Values are presented as mean ± SD calculated from at least three independent experiments.

still considered by many to be the most effective approach to re-sensitize MDR cancer cells to chemotherapeutic agents [50]. The basic concept is to use a compound that has the ability to transiently block the function of ABCB1 at non-toxic concentrations, thus potentiating the efficacy of co-administered anticancer drugs in ABCB1-overexpressing MDR cancer cells [42,43]. Unfortunately, there is currently no synthetic reversing agent that can be applied clinically to treat MDR cancer, mostly due to complications associated with selectivity, high toxicity and unforeseen drug-drug interactions [42]. Consequently, instead of developing novel synthetic compounds, many research groups, including our own, have adopted the drug repurposing (drug repositioning) approach and examined the chemosensitization effect of therapeutic agents with known pharmacological and toxicological profiles on MDR cancer cells [42].

In the present study, we investigated the potency and selectivity of DPI-201106 on ABCB1-mediated MDR in cancer cells. DPI-201106 is a positive inotropic agent that has been used frequently as a standard cardioselective modulator of voltage-gated sodium channels (VGSCs) [12,13,28,32,34,49], and in patients who have undergone coronary arterial bypass grafting (CABG) [12]. DPI-201106 has also been proposed as a treatment option for patients with heart failure [14,20,26]. Our data demonstrated that DPI-201106 is capable of inhibiting the transport function of ABCB1, enhancing drug-induced apoptosis and reversing MDR in ABCB1-overexpressing cancer cells at non-toxic nanomolar concentrations. More importantly, we found that DPI-201106 selectively interacts with ABCB1 as a high-affinity substrate similar to cyclosporine A or as a modulator compared to ABCC1 and ABCG2.

2. Materials and methods

2.1. Chemicals

RPMI medium, Iscove's modified Dulbecco's medium (IMDM), Dulbecco's Modified Eagle's medium (DMEM), fetal calf serum (FCS), Phosphate-buffered saline (PBS), trypsin-EDTA, penicillin and streptomycin were purchased from Gibco, Invitrogen (CA, USA). DPI-201106 was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Tools Cell Counting (CCk-8) Kit was purchased from Biotools Co., Ltd (Taipei, Taiwan). Verapamil, MK-571, Ko143 and all other chemicals were purchased from Sigma (St. Louis, MO, USA), unless stated otherwise. Annexin V: FITC Apoptosis Detection Kit was purchased from BD Pharmingen (San Diego, CA, USA).

2.2. Cell culture conditions

The human KB-3-1 epidermal cancer cell line and the ABCB1-overexpressing variant KB-V-1, as well as pcDNA3.1-HEK293, ABCB1-