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CEP-33779 antagonizes ATP-binding cassette subfamily B member 1 mediated multidrug resistance by inhibiting its transport function

Shang-jun Tang [a,b], Li-kun Chen [b], Fang Wang [b], Yun-kai Zhang [d], Zhen-cong Huang [b], Kenneth Kin Wah To [c], Xiao-kun Wang [b], Tanaji T. Talele [d], Zhe-sheng Chen [d], Wei-qiang Chen [a,**], Li-wu Fu [b,*]

[a] Department of General Surgery, Chen Xing Hai Hospital, Guangdong Medical College, Zhongshan, China
[b] State Key Laboratory of Oncology in South China, Cancer Center of Sun Yat-Sen University, Guangzhou, China
[c] School of Pharmacy, Chinese University of Hong Kong, New Territories, Hong Kong, China
[d] Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John's University, Queens, NY 11439, USA

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ABSTRACT

The overexpression of ATP-binding cassette (ABC) transporters often leads to the development of multidrug resistance (MDR), which is the major factor contributing to the failure of chemotherapy. The objective of this study was to investigate the enhancement of CEP-33779, a small-molecule inhibitor of Janus kinase 2 (JAK2), on the efficacy of conventional chemotherapeutic agents in MDR cells with overexpression of P-glycoprotein (ABCB1), multidrug resistance-associated protein 1 (ABCC1) and breast cancer resistance protein (ABCG2). Our results showed that CEP-33779, at nontoxic concentrations, significantly sensitized ABCB1 overexpressing MDR cells to its anticancer substrates. CEP-33779 significantly increased intracellular accumulation and decreased the efflux of doxorubicin by inhibiting the ABCB1 transport function. Furthermore, CEP-33779 did not alter the expression of ABCB1 both at protein and mRNA levels but did stimulate the activity of ABCB1 ATPase. CEP-33779 was predicted to bind within the large hydrophobic cavity of homology modeled ABCB1. In addition, the down-regulation of JAK2 by shRNA altered neither the expression of ABCB1 nor the cytotoxic effect of chemotherapeutic agents in ABCB1-overexpressing cells. Significantly, CEP-33779 enhanced the efficacy of vincristine against the ABCB1-overexpressing and drug resistant KBv200 cell xenograft in nude mice. In conclusion, we conclude that CEP-33779 enhances the efficacy of substrate drugs in ABCB1-overexpressing cells by directly inhibiting ABCB1 transport function. The findings encouraged to further study on the combination therapy of CEP-33779 with conventional chemotherapeutic agents in ABCB1 mediated-MDR cancer patients.

Abbreviations: ABC, ATP-binding cassette; ABCC1, multidrug resistance-associated protein 1; ABCG2, breast cancer resistance protein; CSCs, Cancer stem cell; DOX, doxorubicin; FTC, fumitremorgin C; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Rho123, Rhodamine 123; MDR, multidrug resistance; JAK2, Janus kinase 2; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; P-gp/ABCB1, P-glycoprotein; STAT3, Signal transducer and activator of transcription 3; TKI, tyrosine kinase inhibitor; Vi, vanadate; VRP, verapamil; VCR, vincristine.

* Corresponding author. State Key Laboratory of Oncology in South China, Cancer Center of Sun Yat-Sen University, Guangzhou 510060, China. Tel.: +86 (20) 873 431 63; fax: +86 20 873 431 70.
** Corresponding author. Department of General Surgery, Chen Xing Hai Hospital, Guangdong Medical College, Zhongshan 528415, China. Tel.: +86 (0760) 222 874 02; fax: +86 760 222 878 08.
E-mail addresses: tangshangjun1119@126.com (S.-j. Tang), chenlk@sysucc.org.cn (L.-k. Chen), wangfang0203@163.com (F. Wang), helloyunyun@live.com (Y.-k. Zhang), zc271211033@163.com (Z.-c. Huang), kennethto@cuhk.edu.hk (K.K.W. To), wxk198708@163.com (X.-k. Wang), talelet@stjohns.edu (T.T. Talele), chenz@stjohns.edu (Z.-s. Chen), cwq20138@tom.com (W.-q. Chen), Fulw@mail.sysu.edu.cn (L.-w. Fu).

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1. Introduction

Chemotherapy is an important therapeutic strategy for cancer. However, multidrug resistance (MDR), the ability of cancer cells to acquire resistance to a broad spectrum of structurally and functionally unrelated anticancer drugs, remains one of the primary obstacles to successful cancer chemotherapy [1]. A number of cellular and molecular alterations may contribute to the development of the MDR phenotype. One of the most common causes of MDR results from the overexpression of ATP-binding cassette (ABC) transporters, which actively extrude a variety of chemotherapeutic drugs out of the cancer cells, thereby decreasing the intracellular drug accumulation and resulting in drug resistance [2,3]. Currently, in the human genome, 49 different members of ABC transporter family have been identified and classified into seven subfamilies (A–G) based on the sequence similarities as well as structural organization [4]. Among which, the ATP-binding cassette subfamily B member 1 (ABCB1/P-gp), subfamily C member 1 (ABCC1/MRP1) and subfamily G member 2 (ABCG2/BCRP) play a major role in producing MDR in cancer cells [5,6].

Using the energy provided by ATP hydrolysis, these ABC transporters actively pump out a wide range of anticancer drugs from the inside of cancer cells, thereby attenuating their cytotoxic actions [7]. ABCB1, a 170 kD membrane glycoprotein, is over-expressed in a broad range of human solid tumors and hematologic malignancies, such as liver, colon, kidney and pancreas cancers [8]. It can pump out a wide spectrum of compounds including vinca alkaloids, epipodophyllotoxins, taxanes and some tyrosine kinase inhibitors (TKIs) [9,10]. ABCC1, a 190 kD transmembrane protein, confers resistance to anthracyclines, vinca alkaloids, epipodophyllotoxins, camptothecins and methotrexate [11]. In contrast to ABCB1, ABCG2, a 72 kD transmembrane protein, is a half transporter that consists of only one transmembrane domain with six helices and one ATP-binding site, conferring resistance to mitoxantrone, indolocarbazole, topoisomerase I inhibitors and anthracyclines, as well as fluorescent dyes such as Hoechst 33342 [12].

A logical strategy to overcome ABC transporters mediated MDR is to develop inhibitors to prevent the efflux of anticancer drugs from the cancer cells [13]. TKIs are a novel class of anticancer agents, functioning by competing with ATP for binding to the ATP sites of the catalytic domain of tyrosine kinase. Interestingly, it has been reported that several TKIs, such as gefitinib [14], lapatinib [15], apatinib [16] and sunitinib [17] could interact with specific ABC transporters, thereby inhibiting those drug transport activity and enhancing the anticancer efficacy of conventional chemotherapeutic agents. Thus, it is possible that TKIs could be used as promising MDR inhibitors in cancer cells.

CEP-33779 is a novel, highly selective and orally bioavailable, ATP-competitive and small-molecule Janus kinase 2 (JAK2) inhibitor with a favorable preclinical profile [18]. Fueled by previous encouraging sensitized effect of TKIs to MDR cancer cells, we hypothesized that CEP-33779 may inhibit the function of specific ABC transporters and help circumventing anticancer drugs resistance. Therefore, in this study, we conducted experiments to determine whether CEP-33779 could potentiate the efficacy of specific conventional antineoplastic drugs through interaction with ABCB1, ABCC1 and ABCG2 *in vitro* and *in vivo*.

2. Materials and methods

2.1. Chemicals and reagents

CEP-33779, whose molecular structure was shown in Fig. 1B, was purchased from Selleck Chemicals (Houston, TX, USA) and prepared as a 50 mM stock solution in DMSO (Sigma-Aldrich, St. Louis, MO, USA) for *in vitro* studies. Dulbecco's modified Eagle's medium (DMEM) and RPMI-1640 were purchased from Gibco BRL (Gaithersburg, MD, USA). Monoclonal antibodies against ABCB1 (sc-55510) was purchased from Santa Cruz Biotechnology, Inc. (California, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) antibody was purchased from Kangchen Co. (Shanghai, China). Phosphorylated-STAT3 (#9145), STAT3 (#4904) and JAK2 (#3230) antibody were purchased from Cell Signaling Technology, Inc. (Danvers, MA). SYBER Green was product of Invitrogen (Minneapolis, USA). Four individual GV112 lentiviral shRNAs targeting JAK2 were obtained from Genechem (Shanghai, China). The shRNA sequences were as follows: JAK2 shRNA I, 5′-CAGTTTGAAGAGAGACATT-3′; JAK2 shRNA II, 5′-AGAATTAG-CAAACCTTATA-3′; JAK2 shRNA III, 5′-TTTGTCTTTCGTGTCATTA-3′; JAK2 shRNA IV, 5′-CTGACCCTAAATAATACAT-3′ and control shRNA, 5′-TCTCGCTTGGGCGAGAGTAAG-3′. 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT), fumitremorgin C (FTC), MK-571, verapamil (VRP), vincristine (VCR), doxorubicin (DOX), rhodamine 123 (Rho123), cisplatin and other chemicals were purchased from Sigma Chemical Co (St. Louis, MO, USA).

2.2. Cell lines and cell culture

The human oral epidermoid carcinoma cell line KB and its vincristine-selected ABCB1-overexpressing derivative KBv200 were gifts from Dr. Xu-Yi Liu (Cancer Hospital of Beijing, Beijing, China) [19]. The following cell lines were gifts from Dr. S.E. Bates (National Cancer Institute, NIH, Bethesda, MD, USA): human breast carcinoma cell lines MCF-7 and its doxorubicin-selected ABCB1-overexpressing derivative MCF-7/adr [20], human colon carcinoma cell lines S1 and its mitoxantrone (MX)-selected ABCG2-over-expressing derivative S1-Mi-80 [21], human leukemia cell lines HL60 and its doxorubicin-selected ABCC1-overexpressing derivative HL60/adr [22], human primary embryonic kidney cell line HEK293 and its pcDNA3.1, ABCB1 stable gene-transfected cell line HEK293/ABCB1 (cultured in medium with 2 mg/ml G418) [23]. Cell lines used in this study were thawed from early passage stocks and were passaged for less than 6 months. Cell lines were periodically monitored for mycoplasma by Hoechst staining. All cell lines were cultured in DMEM or RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and with 1% antibiotic solution (penicillin-streptomycin). All cells were grown in drug free culture medium for more than 2 weeks before assay.

2.3. Experimental animals

Athymic nude mice (BALB/c-nu/nu), 5–6 weeks of age and weighing 18–20 g, were obtained from the Center of Experimental Animals, Sun Yat-Sen University (Guangzhou, China). All animals received sterilized food and water. All animal care and experimental procedures have been approved by the ethics committee for animal experimentation and were carried out in accordance with the guidelines on animal care and experiments of laboratory animals (Center of Experimental Animals, Sun Yat-Sen University, China), and conformed to the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.4. Cell cytotoxicity assay

The MTT assay was done as previously described to assess the sensitivity of cells to drugs [24]. The concentration required to inhibit cell growth by 50% (IC_{50}) was calculated from survival curves using the Bliss method [25]. The degree of resistance was estimated by dividing the IC_{50} for the MDR cells by that of the parental sensitive cells. 10 μM VRP, 50 μM MK571 and 2.5 μM FTC