PBK/TOPK inhibitor OTS964 resistance is mediated by ABCB1-dependent transport function in cancer: in vitro and in vivo study

Yuqi Yang1, Qiu-Xu Teng1, Zhuo-Xun Wu1, Jing-Quan Wang1, Zi-Ning Lei1,2, Sabrina Lusvarghi3, Suresh V. Ambudkar3, Ning Ji1,4* and Zhe-Sheng Chen1*

**Highlights**

- ABCB1 overexpression significantly desensitized both drug-selected and gene-transfected cells, which overexpress ABCB1, to OTS964 and that this drug resistance can be antagonized by verapamil, a known ABCB1 inhibitor. Consistently, a similar trend was observed in tumor-bearing mice.
- OTS964 stimulated ATPase activity of ABCB1 and upregulated expression levels of ABCB1, resulting in induced resistance to other ABCB1 substrate-drugs, such as paclitaxel.
- OTS964 received a comparable affinity score and can dock into the substrate-binding site of human ABCB1 protein.

**Keywords:** T-LAK cell-originated protein kinase (TOPK), PDZ-binding kinase (PBK), OTS964, ATP-binding cassette sub-family B member 1 (ABCB1), Multidrug resistance (MDR)

**Aim**

Accumulating reports have suggested that acquired drug resistance is linked to ATP-binding cassette sub-family B member 1 (ABCB1) overexpression. OTS964 is a potent inhibitor targeting to PDZ-binding kinase (PBK)/T-lymphokine-activated killer cell-originated protein kinase (TOPK). In present study, we aimed to explore the relationship between ABCB1 transporter and the regulation of OTS964 efficacy.

**Results interpretation and discussion**

By means of MTT-based cell viability assay, we examined the susceptibility of OTS964 to cells overexpressed ABCB1 and found that the effectiveness of OTS964 was restricted in both drug-selected and gene-transfected cells, which overexpress ABCB1, compared to those of the corresponding parental cells (Fig. 1A-C and Table S1). Interestingly, an ABCB1 inhibitor verapamil (VPL) can re-sensitize the acquired resistance to OTS964 and restore the efficacy of OTS964 to similar level as drug-sensitive cells do. Upon ABCB1 gene knockout, the cell viability curves of drug-resistant cells were overlapping with those of the parental cells, and SW620/Ad300 cells became more sensitive to OTS964 after ABCB1 knock-out (Fig. 1C-D and Table S2). Meanwhile, doxorubicin (DOX), a verified ABCB1 substrate, was used as a reference to compare the degree of reduced efficacy caused by ABCB1-overexpressing (Fig. S1 and Table S1-S2).
we previously reported that OTS964 is a substrate-drug of ABCG2 [1], cells transfected with both transporters were used to confirm (Fig. S2). The cytotoxic activity of OTS964 was limited in B1/G2 cells relative to that in parental PEL cells, and that this effect can be partially re-sensitized by a known inhibitor of ABCB1 or ABCG2. Hence, we hypothesized that ABCB1 overexpression is possible to confer drug resistance to OTS964. Several mechanistic studies were subsequently performed to examine its possible mechanism.

To evaluate ABCB1-mediated transport function, a [3H]-PTX accumulation assay was conducted. As the

---

Fig. 1 A D Cytotoxic activity of OTS964 in drug-selected, gene-transfected, or gene-knockout cells and their respective parental cells. The concentration-response curves and IC_{50} values for OTS964 with or without a verified ABCB1 inhibitor in A) KB-C2 and KB-3-1, B) HEK293/ABCB1 and HEK293/pcDNA3.1, C) SW620/Ad300 and SW620, and D) SW620/Ad300-ABCB1ko and SW620-ABCB1ko cells. The GraphPad software [log (inhibitor) vs. response] was used to fit nonlinear regression and to calculate IC_{50} values. Each dot is expressed as mean ± SD from a representative of three independent experiments. *p<0.05 versus the respective control group. E) Effects of OTS964 on transport function mediated by ABCB1. The intracellular accumulation of [3H]-PTX in E) KB-C2 and KB-3-1 and F) HEK293/ABCB1 and HEK293/pcDNA3.1 cells after 2 h of pretreatment with vehicle, OTS964, or VPL. Data are expressed as mean ± SD from a representative of three independent experiments. *p<0.05 versus the respective control group. G) Effects of OTS964 on ATPase activity mediated by ABCB1. Concentration of OTS964 was plotted against basal level (without OTS964) of ABCB1 ATPase activity. Data are expressed as mean ± SD from a representative of three independent experiments. H) Overview of PTX and the best-scoring pose of OTS964 in the drug binding pocket of ABCB1 protein. PTX and OTS964 are displayed as colored sticks, blue: PTX; red: OTS964. I) Details of interactions between OTS964 and ABCB1 binding pocket. Predicted bonds are displayed as colored dash lines: hydrogen bond: yellow; pi−pi stacking: blue; cation−pi interaction: green. J) 2D OTS964-ABCB1 interaction. Important amino acids are displayed as colored bubbles (green: hydrophobic; blue: polar; red: positively charged). Predicted bonds are displayed as colored lines: green line: pi−pi stacking; purple line with arrow: hydrogen bond; red line: cation−pi interaction.
cells were incubated with OTS964 for a short time, we supposed that OTS964 is unlikely to affect cell viability or other cellular functions, even though high concentrations were used. The results showed that, after co-treatment of PTX with high concentration (3 μM) of OTS964, an increased amount of PTX was detected in drug resistance cells but not in their corresponding parental cells (Fig. 1E and F). This effect may be the results of a high concentration of OTS964 competing with another substrate-drug PTX for transport function mediated by ABCB1, and thus leading to an enhanced intracellular accumulation of PTX. This assumption is further elucidated in our in silico molecular docking analysis.

As ABCB1 is an ATP-dependent transporter, stimulated ATP hydrolysis is generally coupled to substrate transport mediated by ABC transporters [2, 3]. To this end, an ATPase assay was performed to determine whether OTS964 can stimulate ABCB1 ATPase activity. We found that OTS964 concentration-dependently stimulates the vanadate-sensitive ABCB1 ATPase activity (Fig. 1G). The ATPase activity reached a peak of 164.3% of basal activity. Additionally, tepotinib, as an inhibitor of ABCB1 ATPase activity [4], can antagonize the stimulated ATPase activity and restore the ABCB1 ATPase activity to basal level. These results demonstrated that OTS964 may interact with the drug-binding domain of ABCB1 protein and behave as a substrate-drug of ABCB1 transporter.

The in silico molecular docking analysis is widely applied in the field of structural molecular biology as an efficient tool to predict ligand-protein interactions [5, 6]. In accordance with stimulatory results from ATPase assay, the docking simulation was conducted in the substrate-binding pocket (6QEX) of ABCB1. OTS964 received a docking score of −7.2 kcal/mol. Moreover, our results showed that OTS964 is positioned in transmembrane region via hydrophobic interaction with amino acid residues and stabilized by pi-pi stacking interaction and cation-pi interaction formed with amino acid residues (Fig. 1H-J). To further validate the possibility that OTS964 may be a human ABCB1 substrate, we analyzed a verified ABCB1 substrate PTX under the same parameters (Fig. S3). Above results thereby demonstrated that OTS964 interacts with ligand-binding cavity of ABCB1 and behaves as a substrate for ABCB1 transporter.

Mechanistic studies have indicated that some chemosensitizers interact with the substrate-binding site of the transporter and compete with anticancer drugs for trans- portation, and thereby these modulators are themselves transported [7–9]. This enables a substrate-drug transported by ABCB1 can reposition as a competitive inhibitor to antagonize ABCB1-mediated drug resistance. To this end, a reversal study was conducted to evaluate whether OTS964 can be repurposed as a ABCB1 modulator. Low concentrations (5 and 10 nM), assuming non-toxic concentrations, of OTS964 were selected to rule out the possibility of additive toxicity. The results showed that OTS964 at low concentrations failed to restore drug sensitivity to ABCB1 substrate-drugs in drug-selected and gene-transfected ABCB1-overexpressing cells (Fig. 2A-B and Table S3). However, non-toxic OTS964 treatment promoted PTX resistance in resistant KB-C2 cells without affecting the PTX sensitivity in parental KB-3-1 cells. Consistently, a similar trend was observed in OTS964-treated ABCB1-transfected HEK293 cells. Moreover, it was found that the efficacy of non-substrate drug cisplatin (CDDP) was not significantly affected in cells treated with OTS964, suggesting that this effect may be specific to ABCB1. Of note, the exact nature of interaction between OTS964 and ABCB1 transporter,
Fig. 2 (See legend on previous page.)
namely, whether OTS964 acts as a transportation substrate or a competitive inhibitor, is dependent on the concentration used, the assay being used, and the varying activities of the transporters in each cell type. Therefore, our results do not warrant further testing of OTS964 as a chemosensitizer.

Since amplifying the ABCB1 drug efflux pump could be a possible mechanism for acquired drug resistance, a Western blot analysis was conducted to further evaluate the mechanism of action. Attempts to simulate the clinical settings and circumvent the off-target toxicity, we selected low concentration, assuming negligible cytotoxicity, for up to 72 h of exposure and high concentration, approached IC50 value, for 24 h of exposure. The results showed that, within 72 h, a low concentration (5 nM) of OTS964 did not significantly change the ABCB1 protein level in drug-resistant cells overexpressed ABCB1 (Fig. 2C-D). After 40 nM of OTS964 incubated for 24 h, significantly stimulated ABCB1 protein expression was found in ABCB1-overexpressing cells. Given that ABCB1 protein upregulation could be in consequence of ABCB1 gene amplification, we postulated that ABCB1 mRNA level could be increased by OTS964. A qRT-PCR analysis was subsequently carried out. Remarkably upregulated ABCB1 mRNA level was observed in resistant cells treated with OTS964 at low concentration (5 nM) with 72 h of incubation and at high concentrations (20 nM or 40 nM) with 24 h of incubation (Fig. 2C-D). Notably, there is a discrepancy between mRNA abundances and protein abundances especially for cells with 5 nM OTS964 treatment. Indeed, it has been shown that mRNA transcript abundances only partially, but not strongly, correlate with protein abundances, and the squared Pearson correlation coefficient is approximately 0.40 [10]. As above discrepant results are thought to be determined by cofactors, a detailed mechanism of this discrepancy and upregulation remains to be investigated further. Together, enhanced ABCB1 expression at protein and mRNA levels is an underlying reason for drug resistance induced by OTS964.

Combined the results from Western blot and qRT-PCR, we postulated that stimulated ABCB1 protein and mRNA allow a more functionable ABCB1 transporter to pump out its substrates, resulting in a decreased sensitivity to drugs transported by ABCB1. To further examine this assumption, a 72-h accumulation assay was conducted using the same concentrations as in reversal study, and the amounts of [3H]-PTX in both intracellular and extracellular space were quantified. Consistent with the cytotoxicity results, OTS964 at low concentration induced a reduction of PTX intracellular accumulation and an enhanced level of extracellular PTX in drug-resistant cells (Fig. 2E). Moreover, this effect was not observed in corresponding drug-sensitive cells. As ABCB1 protein expression is not significantly changed within 72 h exposure of OTS964 at low concentration, we postulated that OTS964 at negligible toxic concentrations may affect the transport function without altering the protein expression level. This hypothesis should be further verified. However, we noticed that, combined the results from 4-h accumulation assay and Western blot analysis, OTS964 at high concentration competitively inhibits PTX efflux leading to more PTX accumulating in drug-resistant cells, but paradoxically promotes ABCB1 expression at both transcriptional and translational levels. Therefore, we postulated that OTS964 at approached IC50 concentrations may have off-target activities in addition to its interaction with ABCB1 transporter, which needs to be collaborated further. It is also possible that OTS964 may induce a conformational change upon binding with ABCB1 protein, consequently preventing PTX binding and thus resulting in a competitive inhibitory of PTX efflux after short time exposure to OTS964. By contrast, after 72 h of OTS964 treatment, the stimulated ATPase activity, which would supply energy for efflux function, enables more PTX being pumped out from drug-resistant cells. This hypothesis remains to be further validated in the future.

Subsequently, to further translate our in vitro findings into in vivo evaluation, athymic nude mice were implanted with SW620 and its ABCB1-overexpressing SW620/Ad300 cells to establish tumor xenograft model. As a result, the tumor growth was significantly suppressed in OTS964-treated mice bearing SW620 tumors compared to the control group (Fig. 2F). By contrast, in SW620/Ad300 tumor-bearing mice, OTS964 alone treatment did not induce significant reduction in tumor size relative to those in the control group (Fig. 2G). These results suggested that the efficacy of OTS964 is restricted by ABCB1-mediated drug resistance. An immunohistochemistry analysis of ABCB1 from tumor xenograft model was also performed. Consistent with the results from Western blot, the expression level of ABCB1 protein was induced in OTS964-treated SW620/Ad300 tumor sections (data not shown). Interestingly, the combination of OTS964 and VPL significantly inhibited the tumor growth in SW620/Ad300 xenograft model compared to the control group, which, at least in part, verified that the limited efficacy of OTS964 is induced by ABCB1 overexpression (Fig. 2G). Given that toxicity is a major concern for any chemotherapeutic agent, the body weight of mice was used as an indicator of tolerability throughout the course of this study. Our results showed that no remarkable weight loss was caused by OTS964 either applied alone or used in combination with VPL (Fig. 2H). Also, the number of WBCs and platelets in all
groups was within the normal range and without marked changes (Fig. 2I-J).

Collectively, this article presents in vitro and in vivo evidence that OTS964 is susceptible to ABCB1-mediated drug resistance and provides important indications for follow-up clinical use of OTS964.

Abbreviations
ABC transporter: ATP-binding cassette transporter; ABCB1: ATP-binding cassette sub-family B member 1; CDDP: Cisplatin; COL: Colchicine; DOX: Doxorubicin; MDR: Multidrug resistance; PBK: PDZ-binding kinase; PTX: Paclitaxel; qRT-PCR: Quantitative real-time PCR; TOPK: T-lymphokine-activated killer cell-originated protein kinase; VCR: Vincristine; VPL: Verapamil; WBCs: White blood cells.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12943-022-01512-0.

Acknowledgements
The first author (YY) thanks the supports from Department of Pharmaceutical Sciences, St. John’s University (New York, United States) as a doctoral fellowship. All authors are grateful to Dr. Shin-Ichi Akyama (Kagoshima University, Kagoshima, Japan) for providing KB-3-1 and KB-C2 cell lines. Thanks are given to Drs. Susan E. Bates and Robert W. Robey (NCI, NIH, Bethesda, MD) for kindly providing SW620 and SW620/Ad300 cell lines. The authors also thank Drs. Robert W. Robey and Michael M. Gottesman (NCI, NIH, Bethesda, MD) for providing the PEL and B1/G2 cell lines.

Authors’ contributions
Conceptualization: YY, N.J., and Z.-S.C. Methodology: YY, Q.-X.T., Z.-X.W., and J.-Q.W. Resources: Z.-N.L., S.L., and S.V.A. Writing-Original Draft: Y.Y. Writing-review and editing: YY, N.J., and Z.-S.C. Supervision: N.J., and Z.-S.C. All authors discussed the results and implications and developed the manuscript at all stages. The author(s) read and approved the final manuscript.

Funding
The work was partially supported by Department of Pharmaceutical Sciences, St. John’s University and National Natural Science Foundation of China (No. 81873901). S.L. and S.V.A. were supported by the Intramural Research Program, National Institutes of Health, National Cancer Institute, Center for Cancer Research.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethic approval and consent to participate
The animal study was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of St. John’s University.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Pharmaceutical Sciences, College of Pharmacy and Health Sciences, St. John’s University, Queens, New York City, NY 11439, USA.

References
1. Yang Y, Wu ZX, Wang JQ, Teng QX, Lei ZN, Lusvarghi S, et al. OTS964, a TOPK inhibitor, is susceptible to ABCG2-mediated drug resistance. Front Pharmacol. 2021;12:620874.
2. Ambudkar SV, Dey S, Hrycyna CA, Ramachandra M, Pastan I, Gottesman MM. Biochemical, cellular, and pharmacological aspects of the multidrug transporter. Annu Rev Pharmacol Toxicol. 1999;39:361–98.
3. Ambudkar SV, Kimchi-Sarfaty C, Sauna ZE, Gottesman MM. P-glycoprotein: from genomics to mechanism. Oncogene. 2003;22:7468–85.
4. Wu ZX, Teng QX, Cai CY, Wang JQ, Lei ZN, Yang Y, et al. Tepotinib reverses ABCB1-mediated multidrug resistance in cancer cells. Biochem Pharmacol. 2019;166:120–7.
5. Ferreira RJ, Bonito CA, Cordeiro M, Ferreira MJ, Dos Santos D. Structure-function relationships in ABCG2: insights from molecular dynamics simulations and molecular docking studies. Sci Rep. 2017;7:15534.
6. Morris GM, Lim-Wilby M. Molecular docking. Methods Mol Biol. 2008;443:365–82.
7. Eadie LN, Hughes TP, White DL. Interaction of the efflux transporters ABCB1 and ABCG2 with imatinib, nilotinib, and dasatinib. Clin Pharmacol Ther. 2014;95:294–306.
8. Nanayakkara AK, Folliot CA, Chen G, Williams NS, Vogel PD, Wise JG. Targeted inhibitors of P-glycoprotein increase chemotherapeutic-induced mortality of multidrug resistant tumor cells. Sci Rep. 2018;8(1):1–8.
9. Eckford PD, Sharmo PJ. ABC efflux pump-based resistance to chemotherapy drugs. Chem Rev. 2009;109:2989–3011.
10. Vogel C, Marcotte EM. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. Nat Rev Genet. 2012;13:227–32.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more biomedcentral.com/submissions