MET inhibitor tepotinib antagonizes multidrug resistance mediated by ABCG2 transporter: In vitro and in vivo study

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Received 27 September 2021; received in revised form 19 November 2021; accepted 29 November 2021

KEY WORDS
MET inhibitor; Tepotinib; Multidrug resistance; ABCG2 transporter; Reversal agent; Combination treatment; Chemotherapy; In vivo study

Abstract
Overexpression of ABCG2 transporter in cancer cells has been linked to the development of multidrug resistance (MDR), an obstacle to cancer therapy. Our recent study uncovered that the MET inhibitor, tepotinib, is a potent reversal agent for ABCB1-mediated MDR. In the present study, we reported for the first time that the MET inhibitor tepotinib can also reverse ABCG2-mediated MDR in vitro and in vivo by directly binding to the drug-binding site of ABCG2 and reversibly inhibiting ABCG2 drug efflux activity, therefore enhancing the cytotoxicity of substrate drugs in drug-resistant cancer cells. Furthermore, the ABCB1/ABCG2 double-transfected cell model and ABCG2 gene knockout cell model demonstrated that tepotinib specifically inhibits the two MDR transporters. In mice bearing drug-resistant tumors, tepotinib increased the intratumoral accumulation of ABCG2 substrate drug topotecan and enhanced its antitumor effect. Therefore, our study provides a new potential of repositioning tepotinib as an ABCG2 inhibitor and combining tepotinib with substrate drugs to antagonize ABCG2-mediated MDR.

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1. Introduction

Currently, chemotherapy and targeted therapy are two mainstream cancer treatment strategies. However, the development of drug resistance, which results in decreased or diminished therapeutic response, is one of the major challenges for cancer treatment. It is recognized that some ATP-binding cassette (ABC) transporters can render cancer cells multidrug resistance (MDR) phenotype and attenuate the efficacy of anticancer drugs. MDR is characterized as the acquired drug resistance of cancer cells to multiple anticancer drugs even though they have distinct chemical structures or mechanisms of action. The well-established, MDR-associated ABC transporters are ABCB1, ABCG2, and ABCC13. These transporters are membrane-bound efflux pumps that translocate their substrates against the concentration gradients by hydrolyzing ATP, decreasing substrate intracellular retention, therefore rendering cancer cells MDR phenotype. To dates, numerous clinically used chemotherapeutic drugs and tyrosine kinase inhibitors (TKIs) are recognized as substrate drugs of ABC transporters.

As one of the major MDR-related ABC transporters, ABCG2 is widely distributed in normal tissues including placenta, prostate, liver, and maintained the cellular homeostasis. Clinical studies suggested that ABCG2 is one of the key resistance factors of sorafenib, sunitinib and erlotinib, which affect to drug pharmacokinetics and pharmacodynamics. Consistent data have suggested that ABCG2 is one of the key resistance factors of cancer cells to multiple anticancer drugs even though they have distinct chemical structures or mechanisms of action. The well-established, MDR-associated ABC transporters are ABCB1, ABCG2, and ABCC13. These transporters are membrane-bound efflux pumps that translocate their substrates against the concentration gradients by hydrolyzing ATP, decreasing substrate intracellular retention, therefore rendering cancer cells MDR phenotype. To dates, numerous clinically used chemotherapeutic drugs and tyrosine kinase inhibitors (TKIs) are recognized as substrate drugs of ABC transporters.

Because ABC transporters are believed to mediate MDR in cancer, the research on combating MDR by targeting these ABC transporters is ongoing. One promising strategy is to combine substrate drugs with an inhibitor, which inhibits the drug efflux process, thereby increasing the intracellular drug level and enhancing the anticancer efficacy. However, unlike the extensive clinical development of ABCB1 inhibitors, no ABCG2 inhibitor has been subjected to clinical trials to date. Still, it is important to identify effective ABCG2 inhibitor candidates which allow future clinical investigations and predict potential drug–drug interactions. Tepotinib is developed mainly for non-small cell lung cancer (NSCLC) harboring METex14 mutations. It is currently approved for use in US and Japan for METex14-altered NSCLC patients. In addition, tepotinib is also under clinical investigation for hepatocellular carcinoma (NCT02115373, NCT01988493) and colorectal cancer (NCT04515394). Previously, we identified that tepotinib can specifically reverse ABCB1-mediated MDR by inhibiting the substrate efflux property. Moreover, the molecular docking analysis suggested that tepotinib has good binding affinity with the drug-binding site of ABCG2 transporter. Therefore, we stepped further to investigate the potential interaction of tepotinib with ABCG2.

In the present study, we revealed that tepotinib can potently antagonize ABCG2-mediated MDR both in vitro and in vivo. Therefore, combining tepotinib with ABCG2 substrate drugs may overcome MDR and achieve better anticancer effect in drug-resistant tumors.

2. Materials and methods

2.1. Reagents

Tepotinib was purchased from ChemieTek (Indianapolis, IN, USA). Ko143 (Enzo Life Sciences, Farmingdale, NY, USA) is a selective ABCG2 inhibitor. Cisplatin was dissolved in dimethyl formamide, all other drugs were dissolved in DMSO to a final concentration of 10 mmol/L as stock solution. All other reagents were acquired from Sigma Chemical Co. (St. Louis, MO, USA) unless stated otherwise.

2.2. Cell lines

The ABCG2-overexpressing drug-resistant NCI-H460/TPT10 cells were established previously by exposing NCI-H460 cells to topotecan. The ABCG2 gene knockout NCI-H460-KO and NCI-H460/TPT10-KO cell lines were constructed using CRISPR/Crispr-associated (Cas) 9 system. The ABCB1-overexpressing KB-C2 and ABCC1-overexpressing KB-CV60 cells were established by selecting KB-3-1 cells with colchicine or vincristine plus cepharanthine, respectively. HEK293/pDNA3.1 and HEK/ABCG2 were generated by transfecting the HEK293 cells with empty and ABCG2 expressing vector. The ABCB1 and ABCG2 co-expressed HEK293/B1G2 cells and the parental HEK293/PEL cells were maintained in Eagle’s minimum essential medium (EMEM).

2.3. Evaluation of anticancer drug cytotoxicity

The drug cytotoxicity was evaluated by the MTT colorimetric assay. The experiments were conducted using the protocol as previous described. The concentrations of tepotinib selected for combinational treatment were below IC20, where more than 80% of the cells remain viable. The AccuSkan™ GO UV/Vis Microplate Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) was used to measure the absorbance.

2.4. [3H]-Mitoxantrone accumulation and efflux assay

The accumulation and efflux of [3H]-mitoxantrone (Moravek Biochemicals, Inc., Brea, CA, USA) in parental and drug-resistant cells was measured in the absence or presence of tepotinib or Ko143 at 1 and 3 μmol/L, as previously described. See additional detailed methods in Supporting Information.

2.5. Evaluation of ABCG2 ATPase activity

The effect of tepotinib on the ATPase activity of ABCG2 transporter was determined using the established protocol. See additional detailed methods in Supporting Information.

2.6. Cellular thermal shift assay

The assay was performed as mentioned previously with modified protocol. NCI-H460/TPT10, KB-C2, and KB-CV60 cells were lysed by freezing—thawing using liquid nitrogen and 25 °C heat block for five times. The protein samples were collected by centrifuging the mixture at 15,000 rpm (Centrifuge 5420, Eppendorf, Hamburg, Germany) for 20 min. The samples were...
then incubated with 30 μmol/L of tepotinib or DMSO at room temperature for 30 min. Subsequently, equal amount of protein was aliquot and incubated at different temperatures for 3 min. Finally, the protein samples were subjected to Western blot analysis.

2.7. Immunoblotting analysis

The Western blot was performed as previously described27. See additional detailed methods in Supporting Information.

2.8. Immunofluorescence microscopy

The assay was performed as mentioned previously28. Cells were seeded in 24-well plates and incubated overnight. Subsequently, the cells were incubated with 3 μmol/L of tepotinib for up to 72 h. The antibodies used are ABCG2 antibody and Alexa Fluor 488 anti-mouse antibody with 1:1000 dilution (Cat# A-11001, Thermo Fisher Scientific Inc., Waltham, MA, USA). The nuclei were stained by DAPI solution. The images were captured using an EVOS FL Auto Imaging System (Thermo Fisher Scientific Inc., Rockford, IL, USA).

2.9. Molecular docking of tepotinib with human ABCG2 models

The tepotinib 3-D structure was constructed for docking simulation with human ABCG2 models. Human ABCG2 protein models 6VXI and 6ETI were obtained from RCSB Protein Data Bank. 6ETI has a co-crystallized inhibitor MZ2929 while 6VXI has a co-crystallized substrate mitoxantrone10. Docking calculations were performed in AutoDock Vina (version 1.1.2)30. Hydrogen atoms and partial charges were added using AutoDockTools (ADT, version 1.5.4). Docking grid coordinates were determined from the bound ligand mitoxantrone or MZ29 provided in 6VXI or 6ETI respectively. Receptor/ligand preparation and docking simulation were performed using default settings. The top-scoring pose (sorted by affinity score: kcal/mol) was selected for further analysis and visualization.

2.10. Tumor xenografts

Male athymic NCR nude mice (18–23 g, 5-week old) were obtained from the Taconic Farms. The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of St. John’s University (Protocol #1962), and the project was conducted in compliance with the Animal Welfare Act and other federal statutes. Mice were inoculated subcutaneously in the flanks with NCI-H460 or NCI-H460/TPT10 cells. The animals were weighed, and tumors were measured with a caliper every 3rd day before the treatment. Details about treatment and pharmacokinetic studies are described in additional methods provided in Supporting Information.

2.11. Statistical analysis

All calculation and statistical analyses were performed in GraphPad software (Prism 7.0). Data are expressed as mean ± standard deviations (SD). Statistical analysis was performed using a one-way ANOVA and a P value < 0.05 was considered statistically significant.

3. Results and discussion

In the present study, the MDR reversal effect of tepotinib (chemical structure given in Fig. 1A) was evaluated in cancer cells as well as in HEK293 cells that overexpress ABCG2 transporter. To determine the nontoxic concentrations for MDR reversal studies, the cytotoxicity profile was first evaluated in parental and ABCG2-overexpressing cells (Fig. 1B). Based on the IC_{20} values, 1 and 3 μmol/L of tepotinib, which did not significantly affect to cell viability, were selected to conduct the MDR reversal studies. A major finding of our study is that tepotinib can specifically sensitize ABCG2-overexpressing cells to ABCG2 substrate drugs mitoxantrone and topotecan, demonstrated by the decreased IC_{50} values of these substrate drugs in the drug-resistant cells. The calculated IC_{50} values and resistance fold are presented in Supporting Information Tables S1 and S2. As shown in Fig. 1C and D, NCI-H460/TPT10 cells were significantly resistant to mitoxantrone (90-fold) and topotecan (160-fold) as compared to the parental cells NCI-H460. In the combinational treatment, 1 and 3 μmol/L of tepotinib enhanced the cytotoxicity and decreased the resistance fold of mitoxantrone (12- and 3-fold) and topotecan (27- and 4-fold) in the drug-resistant NCI-H460/ TPT10 cells without affecting to the parental NCI-H460 cells. In addition, the reversal effect of tepotinib at 3 μmol/L was comparable to that of the positive ABCG2 inhibitor Ko143. To further evaluate whether the reversal effect of tepotinib is attributed to antagonizing the activity of ABCG2, we performed the combinational treatment in ABCG2 gene-knockout NCI-H460-KO and NCI-H460/TPT10-KO cells. As shown in Fig. 1C and D, when the ABCG2 gene was knockout from the drug-resistant NCI-H460/ TPT10 cells, the cells became sensitive to mitoxantrone and topotecan. Importantly, the MDR reversal effect of tepotinib was abolished in the ABCG2 gene-knockout cells.

In HEK 293 cells, tepotinib showed a similar cytotoxicity profile (Fig. 2A). Previously, we demonstrated that tepotinib is able to reverse ABCB1-mediated MDR27. In the ABCB1/ABCG2 double-transfected HEK293 cells, tepotinib was able to significantly decrease the resistance fold of doxorubicin (from 45- to 3.6-fold). Moreover, as shown in Fig. 2B, the reversal effect of tepotinib is stronger than verapamil, a known ABCB1 inhibitor (from 45- to 6.6-fold) or ABCG2 inhibitor Ko143 (from 45- to 9.4-fold), suggesting tepotinib may serve as a dual ABCB1/ ABCG2 inhibitor. Previous studies found that the primitive leukemic CD34+/38 cells express high levels of ABCB1, ABCB1, and ABCG231. The co-expression of MDR-related ABC transporters in cancer cells may require simultaneous modulation of multiple ABC transporters to achieve optimal inhibition and a better clinical outcome32,33. Our results confirm that tepotinib can effectively antagonize ABCB1- and ABCG2-mediated MDR within clinically reachable concentrations, proposing tepotinib as a candidate inhibitor of ABCB1 and ABCG2. The combination of tepotinib with chemotherapeutic drugs or TKIs that are substrates of ABCB1/ABCG2 may benefit a subset of cancer patients with MDR tumor expressing both ABCB1 and ABCG2 transporters.

We further examined the reversal effect of tepotinib in HEK293 cells overexpressing wide-type (WT) or mutant ABCG2 transporter. It is documented that R482 residue mutations of ABCG2 can produce distinct substrate recognition and transport capacity34,35. Some ABCG2 inhibitors may have selective MDR reversal effect towards ABCG2-WT, such as venetoclax36 and AC22037. As shown in Fig. 2C and D, similar to the observation in drug-resistant cancer cells, the sensitizing effect was demonstrated
in HEK293/ABCG2-WT as well as R482G and R482T mutant cells, suggesting the R482 mutations does not attenuate the MDR reversal effect of tepotinib. Finally, tepotinib did not affect to the IC50 of cisplatin, a non-substrate drug of ABCG2, in parental and drug-resistant cells (Supporting Information Fig. S1), confirming the MDR reversal effect is ABCG2-related.

Subsequently, experiments were performed to investigate the mechanism of tepotinib’s MDR reversal effect. Several potential mechanisms are proposed for ABCG2 inhibitor, including 1) directly inhibiting ABCG2 transporter from extruding the substrates, 2) translocating the transporter from cell membrane to cytoplasm, and 3) downregulating the protein expression of ABCG2. Therefore, we performed Western blot to evaluate if tepotinib affects to the protein expression level. As shown in Fig. 3A, tepotinib did not alter the expression level of ABCG2. Instead, cellular thermal shift assay results (Fig. 3B) showed that tepotinib treatment can stabilize ABCG2 protein against high temperatures compared to the solvent control DMSO. It is proposed that, upon heating, the target protein will unfold and precipitate, while a ligand engaged protein will require a higher temperature to unfold and precipitate. In the solvent control group, ABCG2 protein signal decreased in a temperature-dependent manner from 44 to 59 °C. When tepotinib was incubated with the protein samples, it induced ABCG2 thermal stability, suggesting a direct binding interaction between tepotinib and ABCG2 transporter. In agreement with previous report, tepotinib induced ABCB1 thermal stability (positive control) without affecting to the thermal profile of ABCC1 (negative control). Studies have suggested that MET signaling pathway is associated with cancer drug resistance by upregulating ABCG2 expression38,39. Therefore, we performed Western blot to investigate if the MDR reversal effect is associated with MET inhibition. As shown in

Figure 1  The effect of tepotinib on the cytotoxicity of anticancer drugs in ABCG2-overexpressing cancer cells. (A) The chemical structure of tepotinib. (B) Cell viability curves for non-small cell lung cancer NCI-H460, NCI-H460/TPT10, NCI-H460-KO and NCI-H460/TPT10-KO cancer cells. The effect of tepotinib on the cytotoxicity of mitoxantrone (C), and topotecan (D) in cancer cells. Data are expressed as mean ± SD from a representative of three independent experiments (n = 3). *P < 0.05 versus the corresponding control group.
Our results confirm that tepotinib did not affect its designated target MET and p-MET protein, as no significant difference was observed between the control group and the treatment groups. Collectively, these results suggest that the MDR reversal effect of tepotinib is unrelated to MET inhibition or ABCG2 downregulation. Since tepotinib also reversed ABCB1- but not ABCC1-mediated MDR, cells overexpressing ABCB1 or ABCC1 transporters were incubated with tepotinib for up to 3 days. The results confirmed that tepotinib did not affect the protein level of all three MDR-associated ABC transporters. The immunofluorescence assays show that tepotinib did not cause ABCG2 transporter internalization after 3 days treatment (Fig. S2C), suggesting these two mechanisms are unlikely to involve in the MDR reversal effect of tepotinib.

To this end, we conducted an ATPase assay to further validate whether tepotinib has direct interaction with ABCG2 transporter. It is suggested that certain ABCG2 inhibitors can either inhibit or stimulate the ABCG2 ATPase. Inhibiting the ATPase activity will attenuate the substrate efflux function since ABCG2 transporter requires ATP hydrolysis to facilitate the drug translocation. If an inhibitor stimulates the ATPase activity, it is possible that the inhibitor can bind to the drug-binding site of the transporter, preventing the binding and efflux of other substrate drugs.

**Figure 2** The effect of tepotinib on the cytotoxicity of anticancer drugs in HEK293 transfected cells. (A) Cell viability curves for HEK293/pcDNA3.1, HEK293/ABCG2-WT, HEK293/ABCG2-R482G and HEK293/ABCG2-R482T cells. (B) The effect of tepotinib on the cytotoxicity of doxorubicin in HEK293/B1G2 cells. The effect of tepotinib on the cytotoxicity of mitoxantrone (C) and topotecan (D) in HEK293/ABCG2 cells. Data are expressed as mean ± SD from a representative of three independent experiments (n = 3). *P < 0.05 versus the corresponding control group.
According to the results presented in Fig. 4A, tepotinib, in 0–40 μmol/L range, stimulated ABCG2 ATPase in a concentration-dependently manner with a maximum 7.6-fold stimulation at 20 μmol/L. The stimulatory effect of tepotinib reached EC_{50} at 1.23 μmol/L, which falls within the reversal concentrations used in the combinational treatments. Therefore, the results suggest that tepotinib may binds to the drug-binding site and hinder the substrate efflux function of the transporter.

Subsequently, substrate accumulation and efflux assays were performed to characterize the tepotinib–ABCG2 interaction in-depth. To determine whether the tepotinib–ABCG2 interaction is reversible or irreversible, [3H]-mitoxantrone accumulation assay was performed using ABCG2-overexpressing NCI-H460/TPT10 cells. As shown in Fig. 4B, when tepotinib was presented in the pretreatment and uptake buffer, an increased intracellular concentration of [3H]-mitoxantrone was observed. In contrast, when tepotinib was only presented in the pretreatment buffer, the intracellular level of [3H]-mitoxantrone remained unchanged. Therefore, the results indicate that tepotinib interact with ABCG2 transporter in a reversible manner.

The MDR reversal effect of tepotinib could result from the inhibition of ABCG2 efflux activity, leading to increased intracellular drug accumulation. Hence, we performed the [3H]-mitoxantrone accumulation assay using NCI-H460/TPT10 cells and HEK293/ABCG2-WT cells. As shown in Fig. 4C and F, the vehicle-treated drug-resistant cells demonstrated active drug efflux process as indicated by the decreased intracellular mitoxantrone accumulation. The incubation of drug-resistant cells with either tepotinib or Ko143 signifi cantly upregulated the intracellular level of [3H]-mitoxantrone. At 3 μmol/L, tepotinib restored the mitoxantrone accumulation level in drug-resistant cells to the similar extent observed in the parental cells. Because increasing mitoxantrone accumulation can be attributed to increased substrate influx and/or decreased substrate efflux, the [3H]-mitoxantrone efflux assay was carried out to further investigate this factor. As presented in Fig. 4D and E, the intracellular levels of mitoxantrone in NCI-H460 cells remained relatively constant throughout the 2 h incubation (from 100% to 80%) and none of the inhibitors altered [3H]-mitoxantrone accumulation level. In contrast, the intracellular level of mitoxantrone dropped significantly in NCI-H460/TPT10 cells (from 100% to 25%), suggesting a large portion of mitoxantrone was pumped out by ABCG2. Importantly, the efflux of mitoxantrone was significantly inhibited with 3 μmol/L of tepotinib or Ko143, while 0.1 μmol/L of tepotinib inhibited the efflux process to a lesser extent. Same trends were observed in HEK293/ABCG2-WT cells that tepotinib inhibited the efflux and increased the intracellular level of [3H]-mitoxantrone (Fig. 4G and H).

A recent study revealed that ABCG2 inhibitors such as Ko143 and tariquidar would tightly bind to the transmembrane domain of ABCG2, thereby blocking access for substrates15. To further illustrate the molecular mechanisms of action, we utilized the molecular docking analysis to predict the interaction between tepotinib and ABCG2 transporter. Docking analysis was performed on both inhibitor-bound (6ETI) and substrate-bound (6VXI) ABCG2 models. Our results show that tepotinib docked into the inhibitor binding site with a higher affinity score of –12.645 kcal/mol than substrate binding site which has a score of –10.254 kcal/mol. Details of ligand–receptor interaction were depicted in Supporting Information Figs. S3 and S4. Hydrophobic interactions are the primary factor that stabilized tepotinib to the ABCG2 inhibitor or substrate binding site. For inhibitor binding site, tepotinib is positioned and stabilized in the hydrophobic cavity formed by Phe431, Phe432, Tyr435, Asn436, Phe439, Thr542, Ile543 in chain A, and Phe431, Phe432, Thr435, Phe439, Ser440, Thr542 in chain B. Additionally, the piperidine group of tepotinib was stabilized by a hydrogen bond formed with Asn436 in chain A. For substrate binding site, tepotinib bound in the hydrophobic pocket formed by Phe432, Thr435, Asn436, Phe439, Val436, Met549 in chain A, and Thr435, Asn436, Phe439, Val442, Ser443 in chain B. The piperidine group of tepotinib was further stabilized by a hydrogen bond with Glu446 in chain B. Moreover, the binding position of tepotinib and substrate/inhibitor in ABCG2 have significant overlap. As a result, tepotinib potentially binds to both substrate and inhibitor binding sites, with stronger affinity to the inhibitor binding site of ABCG2 transporter. However, the cryo-EM structure of ABCG2 bound to tepotinib will be of important to confirm the interactions.

Collectively, the in vitro results suggest that tepotinib reverses ABCG2-mediated MDR primarily by inhibiting the efflux activity of
the transporter, thereby facilitating the accumulation of substrate drugs, and enhancing their cytotoxicity in drug-resistant cell lines. Based on the in vitro findings, we selected the tumor xenograft model to evaluate the MDR reversal effect in vivo. In the parental tumor-bearing mice (Fig. 5A–C), 30 mg/kg of tepotinib showed a moderate 27% inhibition ratio of tumor weight (IRW) and 14% inhibition ratio of tumor volume (IRV). In contrast, 3 mg/kg topotecan demonstrated a 67% of IRW and IRV, while the combinational treatment did not enhance its antitumor effect. In the drug-resistant tumor-bearing mice (Fig. 5D–F), the antitumor effect of topotecan was attenuated, with 50% IRW and 40% IRV. The combinational treatment resulted in a more significant antitumor effect than the single treatments, with 83% IRW and 88% IRV, which confirmed that tepotinib can antagonize ABCG2-mediated MDR and enhance the antitumor effect of topotecan.

To understand the pharmacokinetics of the drugs, HPLC analysis was applied to quantify the plasma and intratumoral concentrations of tepotinib and topotecan. The combinational treatment did not significantly alter the plasma concentrations of topotecan but increased the plasma concentrations of tepotinib (Supporting Information Fig. S5A and S5B). However, the tepotinib plasma concentration in combinational treatment decreased to the similar level of that in the single treatment at the end of 240 min evaluation. Previous studies revealed that the reference ABCG2 inhibitor Ko143 is unstable in rat plasma with complete degradation in 60 min, resulting in a relative short ABCG2 inhibition. In contrast, tepotinib is not a substrate of CYP3A4 and the plasma concentration of tepotinib was relative stable in our study, predicting a more optimal ABCG2 inhibitory effect. As shown in Fig. S5C, the intratumoral level of tepotinib was increased in drug-resistant tumors compared to the parental tumors. This data may explain the phenomenon that tepotinib achieved a stronger anticancer effect in drug-resistant tumors compared to the parental tumors. However, the reason for increased tepotinib accumulation remained unclear and should be further investigated. As shown in Fig. S5D, the topotecan...
concentration in drug-resistant tumors decreased by 40% compared to the parental tumors, suggesting ABCG2 actively extruded topotecan from the tumors. The combinational treatment did not significantly alter the topotecan level in parental tumors, but a 3-fold increase of topotecan concentration was observed in drug-resistant tumors. Hence, the data suggests that tepotinib can inhibit ABCG2-mediated substrate efflux, thereby facilitating the topotecan accumulation in the ABCG2-overexpressing tumors.

Finally, tepotinib was shown to be well-tolerated either as single treatment or as part of the combinational treatment since no obvious weight loss was observed (Fig. S5E). The hematological parameters were evaluated in nude mice receiving different treatments (Fig. S5F). The data show that both white blood cells and platelets counting were consistent between control and the treatment groups, suggesting tepotinib as an MDR reversal agent may not induce additional toxicity in vivo.

4. Conclusions

This study shows that tepotinib can reverse MDR by targeting the ABCG2 transporter both in vitro and in vivo. The MDR reversal mechanism of tepotinib is primarily attributed to the reversible inhibition of ABCG2 transporter efflux function, which restores the substrate accumulation in MDR cells. Moreover, tepotinib can effectively reverse MDR in cells overexpressing both ABCB1 and ABCG2 transporters. Therefore, tepotinib may be a candidate of MDR modulator for clinical setting, and the combination treatment may be beneficial to patients with high ABCG2/ABCB1-overexpressing tumors.

Acknowledgment

This study was supported by the Key Research and Development Program of Jiangsu Province (BE2020637, China), Wuxi double hundred young and middle-aged medical and health top-notch talent project (No. 2020014, China). The first author thanks the teaching assistantship and partial supports for the project from Department of Pharmaceutical Sciences, St. John’s University (New York, NY, USA). The authors would like to thank Dr. Shin-Ichi Akiyama (Kagoshima University, Japan) for the KB-3-1, KB-C2, and KB-CV60 cell lines. The authors would like to thank Dr. Susan E. Bates (Columbia University, New York, NY, USA) for kindly offering the NCI-H460, HEK293/pcDNA3.1 and HEK293/ABCG2 cell lines. The authors would also like to thank Drs. Robert W. Robey and Michael M. Gottesman (NCI, NIH, Bethesda, MD, USA) for providing the HEK293/PEL and HEK293/B1G2.

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Conflicts of interest
The authors declare no conflict of interest.

Appendix A. Supporting information
Supporting data to this article can be found online at https://doi.org/10.1016/j.appsb.2021.12.018.

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