1521-009X/48/10/972–979$35.00
Drug Metabolism and Disposition

https://doi.org/10.1124/dmd.120.000050
Drug Metab Dispos 48:972–979, October 2020

Special Section on Natural Products: Experimental Approaches to Elucidate Disposition Mechanisms and Predict Pharmacokinetic Drug Interactions — Minireview

The Effects of Traditional Chinese Medicine on P-Glycoprotein–Mediated Multidrug Resistance and Approaches for Studying the Herb–P-Glycoprotein Interactions

Yuhong Cao, Yiwei Shi, Ying Cai, Zhanying Hong, and Yifeng Chai

School of Pharmacy, Second Military Medical University, Shanghai Key Laboratory for Pharmaceutical (Chinese Materia Medica) Metabolites Research, Shanghai, China (Yu.C., Y.S., Yi.C., Z.H., Y.Ch.) and School of Pharmacy, Fujian University of Traditional Chinese Medicine, Fuzhou, China (Yi.C.)

Received March 30, 2020; accepted July 20, 2020

ABSTRACT

As a member of the ATP-dependent membrane transport proteins, P-Glycoprotein (P-gp) is known to pump substrates out of cells using an ATP-dependent mechanism. The overexpression of P-gp in tumor cells reduces the intracellular drug concentrations, which decreases the efficacy of extensive antitumor drugs and leads to multidrug resistance (MDR) clinically. The combination of anticancer drugs with P-gp inhibitor has been an attractive and promising strategy to reverse MDR in cancer treatment. However, nonspecific or nonselective distribution of P-gp inhibitors to nontarget organs is one of the most fatal shortcomings in clinical application. Thus, there is an urgent need for effective and nontoxic MDR reversal agents, particularly in P-gp–mediated MDR. Traditional Chinese medicine (TCM) natural products may prove less toxic for use in P-gp inhibition to promote MDR reversal. P-gp modulatory effects have been previously demonstrated using selected TCM, including the flavonoid, alkaloid, terpenoid, coumarin, and quinonoid compounds, and some Chinese medicine extracts. Moreover, the approaches for screening active components from TCM are necessary, and these approaches face challenges. At present, the approaches to study the interaction between TCM and P-gp are divided into in vitro, in vivo, and in silico methods. This review will provide an overview and update on the role of TCM in overcoming P-gp–mediated MDR and the approaches to study the interaction between TCM and P-gp.

SIGNIFICANCE STATEMENT

This review summarized some traditional Chinese medicines identified to have a modulatory effect on P-gp, including flavonoids, alkaloids, terpenoids, coumarins, quinonoid compounds, and some Chinese medicine extracts, and it introduced possible mechanisms. The approaches to study the interaction between TCM and P-gp are divided into in vitro, in vivo, and in silico methods.

Introduction

Molecular Structure of Human P-Glycoprotein. P-gp is the 170-kDa protein product of the human gene *MDR1* (Gottesman and Pastan, 1993; Schinkel, 1997). P-gp is an ATP-binding cassette (ABC) transporter, which uses the energy from ATP hydrolysis to pump substrates across the membrane. It consists of two transmembrane domains (TMDs) and two cytoplasmic nucleotide-binding domains (NBDs). P-gp comprises 1280 amino acids divided into two symmetrical halves with 43% sequence homology between the two halves (Chen et al., 1986). Each half contains six TMDs that are separated by a sequence of 75 amino acids that connect the C-terminal TMD with the N-terminal ATP-binding domain (Shustik et al., 1995) (Fig. 1).

Physiological Function of P-gp. P-gp is located in many physiological barriers, such as the blood–cerebrospinal fluid, blood-brain, and blood-testis barriers. P-gp plays a vital role in regulating the absorption and the efflux of various exogenous substances. P-gp is also present in multiple tissues and organs, including gut, kidney, liver, and placenta. Therefore, its activity is highly significant to the metabolism of numerous drugs (Hussaarts et al., 2019), and the behavior of drug absorption will be

This work was financially supported by the National Natural Science Foundation of China [NO. 81673386, NO. 81872829].

All authors were supported by their own institutions during the preparation of this manuscript and had no conflict of interest.

https://doi.org/10.1124/dmd.120.000050.

ABBREVIATIONS: ABC, ATP-binding cassette; ADR, Adriamycin; AKT, protein kinase B ATPase Adenosine triphosphatase; BCRP, breast cancer resistance protein; CR, coptidis rhizoma; GQ, 7-*O*-geranylquercetin; LC-MS/MS, liquid chromatography–tandem mass spectrometry; MDR, multidrug resistance; MSI, mass spectrometry imaging; NBD, nucleotide-binding domain; P-gp, P-glycoprotein; PI3K, phosphatidylinositol 3-kinase; PTX, paclitaxel; Rh123, rhodamine 123; TCM, traditional Chinese medicine; TMD, transmembrane domain.

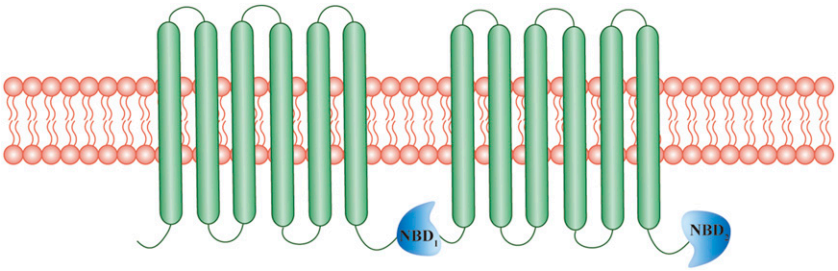

Fig. 1. Secondary structure models of P-gp.

influenced significantly through coadministration with P-gp inhibitor or inducer. Specifically, P-gp inhibitor may increase the bioavailability of drugs, whereas the inducer may reduce the drug absorption.

Models of P-gp–Mediated Substrate Efflux. Drug resistance mediated by P-gp depends on ATP hydrolysis, and the Adenosine triphosphatase (ATPase) activity of P-gp is stimulated by the transported drugs (Mollazadeh et al., 2018). P-gp inhibitors or substrates can enter the cell from the extracellular compartment to the cytosol via filtration, simple diffusion, or specialized transport. The first step of substrate efflux is the combination of P-gp and the substrates promoted by ATP-binding and hydrolys(Wang et al., 2017a)is. Vanadate trapping and photo-cleavage experiments showed that P-gp contains two active ATPase sites, but only one ATP is hydrolyzed at a time (Hrycyna et al., 1998). The hydrophobic "vacuum cleaner" model and the flippase model have been proposed to describe P-gp–mediated drug translocation (Binkhathlan and Lavasanifar, 2013). In the hydrophobic vacuum cleaner model, P-gp pulls its substrates from the lipid bilayer and pumps them out of the cell. The flippase model hypothesizes that P-gp "scans" the inner leaflet of the lipid bilayer and binds specific lipids and hydrophobic drugs before their extrusion by "flipping" the phospholipids from the inner to outer leaflets of the lipid bilayer. The flippase model and the hydrophobic vacuum cleaner model are not mutually exclusive (O'Brien et al., 2012), and the combination of the two models is illustrated (Fig. 2).

Multidrug Resistance and the Mechanisms Mediated by P-gp. MDR is common because of the overexpression of transporters on the tumor cell membranes. Several transporters belonging to the ABC pump family, such as P-gp and BCRP, are overexpressed in resistant tumor cells compared with normal cells (Robey et al., 2018). This induced membrane state determines the efflux of chemotherapeutic agents through a process in which they are captured into the bilayer by the transporter and then effluxed out of the cells. Consequently, the efficacy of antitumor therapy is lowered because of the insufficient drug concentration inside the tumor cells.

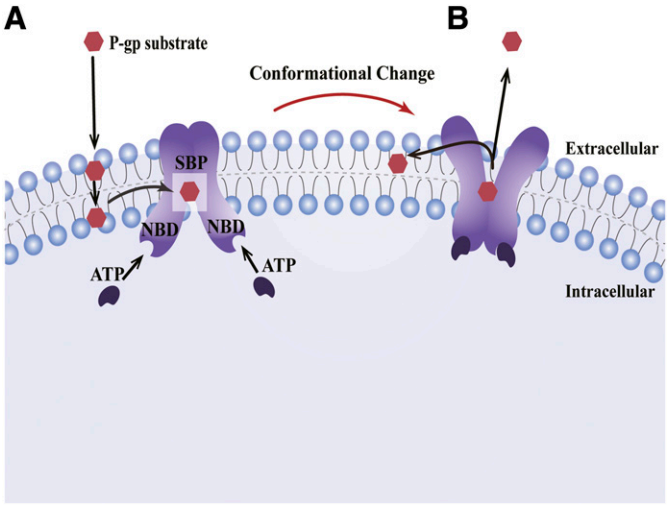

Fig. 2. Model of P-gp substrate transport. (A) Substrate-binding conformation. The molecule travels through one of two portals to enter the substrate-binding pocket (SBP) of P-gp. Substrate–P-gp interactions lead to the binding of two ATP molecules to the NBD. The binding of ATP to the NBDs causes dimerization of the NBDs. (B) This leads to a conformational change, resulting in an outward-facing configuration that presents the substrate and drug-binding site(s) to the outer leaflet/extracellular space. Above broken line = outer leaflet of phospholipid bilayer. Below broken line = inner (cytoplasmic) leaflet of phospholipid bilayer.

Role of P-gp in Cancer MDR. P-gp was first identified in the plasma membrane of mammalian cells that had been selected for resistance to drugs (Gottesman and Ling, 2006; Sharom, 2011). P-gp can intercept drugs before they reach their specific target in the cell by facilitating drug efflux and promoting MDR. Hence, it is always selected as the target for MDR reversal agents. The past three decades have witnessed the rapid development of strategies to reverse P-gp–mediated MDR. Many small molecular compounds were identified as P-gp inhibitors and can reverse P-gp–mediated MDR (Leopoldo et al., 2019; Dong et al., 2020). Many anticancer agents are P-gp substrates, such as vinca alkaloids (Gherbovet et al., 2016), anthracyclines, and taxanes (Li et al., 2019; Nguyen et al., 2020; Xie et al., 2020). The P-gp transport of anticancer agents can be inhibited with a competitive or noncompetitive mechanism of some small-molecule reversal agents. These reversal agents are coadministered with an anticancer drug to reduce its efflux, thus increasing its efficiency. Some compounds have shown promising experimental results in vitro. However, at present, there is no compound being approved for clinical use as a reversal agent (Kumar and Jaitak, 2019). Studies of certain reversal agents were terminated as a result of unacceptable toxicities in clinical trials or insufficient efficacy (Varma et al., 2003). Consequently, there remains a great challenge in searching for effective and less toxic agents to reverse tumor MDR.

Development of P-gp Inhibitors in MDR Modulation

To date, plenty of P-gp inhibitors have been found to modulate MDR activity in clinical trials. Based on their affinity with P-gp and different side effects, these inhibitors could be classified into first-, second-, and third-generation modulators. The first-generation modulators had unacceptable toxicities in clinical trials (Varma et al., 2003). The second-generation modulators are inhibitors with higher affinity and efficacy, lower toxicity, and absence of the intrinsic pharmacological activity that belonged to the analogs of the first generation. Most of them can be metabolized by the cytochrome P450 enzyme family and interact with chemotherapeutic drugs. When used together with anticancer drugs, their metabolism or interaction with the drugs leads to their unacceptable toxicity and, in turn, treatment failure (Kathawala et al., 2015). The third generation is more specific and effective in reducing pharmacokinetic interactions compared with the second generation (Mistry et al., 2001); some have been reported to increase the sensitivity of anticancer drugs in preclinical trials. However, most of these inhibitors still underperform in clinical trials because of diverse reasons, such as high toxicity. Examples of first-, second-, and third-generation P-gp inhibitors are shown in Table 1.

Although progress has been made in the field of MDR, a suitable P-gp inhibitor with significant MDR reversal and acceptable toxicity has yet to be identified. The main reasons are summarized as follows: firstly, P-gp is a complex protein owing to its plastic conformation (Ma et al.,

TABLE 1

Examples of P-gp multidrug resistance reversal agents

Generation	Representative Agent	Mechanisms of Action	Clinical Response	Reference
First	Verapamil; reserpine; quinine; tamoxifen; cyclosporine A; toremifene	Altering ATP hydrolysis pathway (tariquidar; verapamil; cyclosporine A; toremifene; quinine; tamoxifen); competition for binding sites (verapamil; reserpine; quinine; elacridar; cyclosporine A); alteration in P-gp expression (verapamil; cyclosporine A; valspodar)	Toxicities; nephrotoxicity, myelosuppression, and neurotoxicity	Varma et al., 2003
Second	Dexverapamil; valspodar; biricodar		More affinity, efficacy; low toxicity and interaction with cytochrome P450 enzymes	Kathawala et al., 2015
Third	Elacridar; laniquidar; zosuquidar; dofequidar; mitotane; annamycin; tariquidar		More specific and potent with diminutive pharmacokinetic interactions	Pusztai et al., 2005

2018), and the existing inhibitors are nonspecific for P-gp. Besides, some inhibitors may interact with chemotherapeutic drugs when coadministered and may influence the integrity and functions of the brain. Furthermore, there lacks enough preclinical data and appropriate animal models to evaluate the efficiency of P-gp inhibitors.

Traditional Chinese Medicine Modulation of P-gp

At present, researchers have performed intensive efforts in the area of identifying MDR modulators from TCM. Various naturally originating compounds and plant extracts were reported for their modulation effect of MDR (Li et al., 2014; Abdallah et al., 2015). This review focuses on major classes of TCM, including flavonoids, alkaloids, coumarins, terpenoids, and quinonoids, and other TCM extracts. In this section, TCMs that have an MDR modulation effect are discussed. These compounds could inhibit or decrease the activity of P-gp (Table 2).

Flavonoids. Flavonoids are ubiquitous in many vegetables and herbs and are closely related to a number of human life benefits. Current research has demonstrated that flavonoids have various pharmacological properties, including antioxidant, anti-inflammatory, anticancer, antifungal, and antiviral activity (Karabin et al., 2015). Flavonoids have also been reported to inhibit ABC transporters that contribute to the development of MDR.

A few flavonoids, including naringin (Zhu et al., 2018) and taxifolin (Chen et al., 2018), have been identified as substrates of P-gp. The cellular uptake of 40 flavonoids was measured in parental human mouth epidermal carcinoma (KB) cells and KB/MDR cells with or without elacridar. Molecular docking was also performed to investigate the structure-affinity relationship between P-gp and the flavonoids. The results indicated that 3-OH, 5-OH, 3′-OCH3, and 4′-OCH3 are crucial for the interplay between flavonoids and P-gp (Fang et al., 2019). Epigallocatechin-3-gallate could downregulate the expression level of P-gp and BCRP but could not affect the expression of MDR-associated protein 1 in Adriamycin (ADR)-resistant human leukemia (CEM/ADR) cells (Li et al., 2018). 7-O-geranylquercetin (GQ), a derivative of quercetin, could reverse drug resistance of ADR-resistant human breast adenocarcinoma (MCF-7/ADR) cells. GQ inhibited the efflux of ADR by downregulating the expression of P-gp protein and its encoding *MDR1* gene in MCF-7/ADR cells (Zhang et al., 2019). Silychristin A modulates MDR by the direct inhibition of P-gp, whereas anhydrosilychristin and isosilychristin modulate MDR by downregulating the expression of P-gp (Viktorova et al., 2019). Chen et al. (2018) evaluated the effects of some natural flavonoids on P-gp activity, including taxifolin, luteolin, (−)-gallocatechin, and (−)-catechin. They found that taxifolin could significantly resensitize MDR cancer cells in combination with chemotherapeutic agents and enhance the efficacy. This result suggested that taxifolin could be considered as a potential P-gp modulator for the synergistic treatment of MDR cancers. Saeed et al. (2015) found that apigenin could not only inhibit the activity of P-gp but also inhibit the activity of BCRP by increasing cellular uptake of ADR and synergistic inhibition of cell viability in combination with ADR in MDR cells. The molecular docking experiment indicated that apigenin could bind to the NDBs of P-gp, which suggested that apigenin may compete with ATP on NDBs and lead to energy depletion to fuel the transport of P-gp substrates. Curcumin has been described to inhibit both the function of P-gp and the expression of P-gp (Lopes-Rodrigues et al., 2016). Glabridin can increase the accumulation of ADR in drug-induced resistant human breast cancer (MDA-MB-231/MDR1) cells by suppressing the expression of P-gp and competitively inhibiting the P-gp efflux pump and enhance the apoptosis of MDA-MB-231/MDR1 cells induced by ADR, thus realizing reversal effects on MDR. Therefore, the combination therapy of anticancer drugs and glabridin is a promising strategy to overcome P-gp–mediated MDR (Qian et al., 2019).

Some flavonoids have been reported to possess significant P-gp inhibitory activity via diverse mechanisms. Mohana et al. (2018) studied the interplay between flavonoids (theaflavin, quercetin, rutin, epicatechin 3 gallate, and tamarixetin) and glycogen synthase kinase 3β by performing molecular docking. Curcumin could enhance anticancer efficacy through the ablation of nuclear factor-κB, the wingless/integrated beta-catenin pathway, as well as mammalian target of rapamycin signaling (Mohana et al., 2018). Dihydromyricetin, a dihydroflavonol compound with anti-inflammatory, antioxidant, antibacterial, and antitumor actions, could reverse MDR in MCF-7/ADR and K562/ADR cell lines. It enhanced the cytotoxicity of ADR by downregulating *MDR1* mRNA and P-gp expression through a mitogen-activated protein kinase/extracellular signal–regulated kinase pathway and inhibiting the function of P-gp significantly (Sun et al., 2018).

Alkaloids. Alkaloids are a group of naturally originating chemicals containing one or more basic nitrogen atoms and are classified into different groups based on the amino acid they are derived from. Many reports have revealed the ability of alkaloids to inhibit P-gp. The structural analysis of alkaloids suggested that the P-gp inhibitory function is due to the presence of 1) a basic nitrogen atom and 2) two planar aromatic rings. Alkaloids have been reported to inhibit the function of P-gp via diverse mechanisms.

Among various alkaloids investigated in in vitro experiments as P-gp inhibitors or MDR reversal agents, a natural alkaloid called CBT-01 (tetrandrine) has already been tested on clinical trials. Tetrandrine can antagonize MDR in both drug-induced and *MDR1* gene–transfected cancer cells by downregulating the expression of P-gp, followed by increasing the intracellular concentration of chemotherapeutic agents. The combination therapy using tetrandrine and other anticancer drugs could promote the treatment efficiency of drugs that are substrates of P-gp (Liao et al., 2019). Tetrandrine and fangchinoline, isolated from *Stephania tetrandra*, could significantly reduce the expression level of P-gp expression in a concentration-dependent manner. Tetrandrine and fangchinoline showed a significant synergistic, cytotoxic effect in MDR human colon cancer cells (Caco-2) and CEM/ADR cancer cells in combination with ADR (Sun and Wink, 2014).

Besides, there exists a large number of natural alkaloids possessing potent inhibition of the P-gp responsible for the development of

TABLE 2

Inhibition of P-gp function and/or expression by TCM

Compound	Test Model	Pharmacological Results	References
Flavonoids			
EGCG	CEM/ADR	Downregulation of P-gp	Li et al., 2018
GQ	MCF-7/ADR	Downregulation of P-gp and *MDR1* gene	Zhang et al., 2019
Silymarin A	A2780/ADR	Inhibition of P-gp efflux; downregulation of P-gp	Viktorova et al., 2019
Dihydromyricetin	MCF7/ADR; K562/ADR	Inhibition of P-gp efflux; downregulation of P-gp	Sun et al., 2018
Curcumin	KB-C2	Inhibition of P-gp	Lopes-Rodrigues et al., 2016
Apigenin	CEM/ADR	Inhibition of P-gp	Saeed et al., 2015
Taxifolin	HeLaS3; KB/VCR	Inhibition of P-gp	Chen et al., 2018
Alkaloids			
Chelidonine	Caco-2; CEM/ADR	Inhibition of P-gp	Herrmann et al., 2018
Tetrandrine	MCF-7/ADR	Downregulation of P-gp	Liao et al., 2019
Matrine	MCF-7/ADR	Inhibition of P-gp	Zhou et al., 2018
Terpenoids			
Ursolic acid	MCF-7/ADR	Inhibition of P-gp	Zong et al., 2019
Parthenolide	A549/ADR	Downregulation of P-gp	Carlisi et al., 2017
Costunolide	K562/ADR	Downregulation of P-gp	Cai et al., 2019
Cryptotanshinone Dihydrotanshinone	CEM/ADR	Downregulation of P-gp and *MDR1* gene	Hu et al., 2014
Coumarins			
Galbanic acid	HL60	Inhibition of P-gp	Maruszewska and Tarasiuk, 2019
Decursinol	NCI/ADR-RES	Inhibition of P-gp	Choi et al., 2016
Osthole	K562/ADR	Downregulation of *MDR1* gene	Wang et al., 2016
Quinonoids			
Miltirone	HepG2	Inhibition of P-gp	Zhou et al., 2015
Emodin	K562/ADR; Caco-2	Downregulation of P-gp	Min et al., 2017
Shikonin	A2780/PTX	Inhibition of P-gp	Wang et al., 2019
TCM extracts			
Asiatic toad	CRC	Inhibition of P-gp	Yuan et al., 2017
S. miltiorrhiza	Everted rat gut sacs	Inhibition of P-gp	Dai et al., 2012
S. chinensis	K562/A02	Downregulation of P-gp; inhibition of P-gp	Wang et al., 2017
CR	LS180	Activation of P-gp transportation	Yu et al., 2018
Liquorice	Caco-2	Inhibition of P-gp	He et al., 2020

CRC, colorectal cancer cell; EGCG, epigallocatechin-3-gallate; VCR, vincristine.

resistance (Joshi et al., 2017). Chelidonine, isolated from *Chelidonium majus*, could also inhibit the function of P-gp and consequently upregulate the xenobiotic metabolism genes *CYP1A1* and *MDR1* (Herrmann et al., 2018). Matrine, isolated from *Sophora flavescens*, is another quinolizidine alkaloid that has an inhibition effect on P-gp. Zhou et al. (2018) reported that matrine could inhibit MCF-7/ADR cell growth, induce cell apoptosis, and reverse MDR for breast cancer cells through the mediation of downstream apoptosis factors of phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) signaling pathway by decreasing the cell phosphorylation level of AKT.

Terpenoids. Terpenoids are one of the most extensively studied and structurally diverse classes of TCM. Based on the number of isoprene units in the parent structure, terpenoids can be classified as monoterpenoids, sesquiterpenoids, diterpenoids, sesterterpenoids, triterpenoids, tetraterpenes, and polyterpenes. Terpenoids show promising inhibitory effects on primary ABC transporters. The rosemary-originated terpenoid ursolic acid (Zong et al., 2019) was found to inhibit P-gp and increase the cellular accumulation of ADR and rhodamine 123 (Rh123). The chemosensitization effect of ursolic acid was tested; the result indicated that these compounds could significantly reduce the growth of cells in the presence of ADR.

Terpenoids have been reported to possess significant P-gp inhibitory activity via several mechanisms. Parthenolide inhibited P-gp upregulation and promoted the intracellular accumulation of ADR in ADR-resistant human non–small-cell lung cancer (A549/ADR) cells. Parthenolide inhibited the development of the resistance toward ADR, which exhibited an inhibitory effect on the overexpression of nuclear factor E2-related factor 2 and P-gp (Carlisi et al.,2017). Costunolide dramatically enhanced ADR-induced antiproliferative activity against ADR-resistant human erythroleukemia (K562/ADR) cells through inhibition of the PI3K/Akt pathway and downregulation of P-gp expression (Cai et al., 2019). Hu et al. (2014) found that cryptotanshinone and dihydrotanshinone could increase the intracellular accumulation of anticancer drugs by downregulating P-gp mRNA and protein levels and inhibiting P-gp ATPase activity. Tenulin and isotenulin significantly inhibited P-gp efflux by stimulating P-gp ATPase activity. The combinations of tenulin and isotenulin with chemotherapeutic drugs significantly resensitized MDR cancer cells (Chang et al., 2019).

Coumarins. Coumarins, belonging to the benzopyrone family of TCM, are mostly found in plants rich in oils. They can be classified as simple coumarins, furanocoumarins, pyranocoumarins, and pyrone-substituted coumarins based on the position of the substituents. The P-gp inhibitory activity of galbanic acid was evaluated using Rh123 efflux assay in multidrug-resistant leukemia HL60 cells. It was found to be more potent than verapamil in terms of P-gp inhibition (Maruszewska and Tarasiuk, 2019). Decursinol inhibited ADR-resistant ovarian cancer cell proliferation and induced apoptosis via P-gp expression inhibition (Choi et al., 2016).

Coumarins have been reported to inhibit P-gp through multiple mechanisms. Osthole decreased the expression of P-gp at both mRNA and protein levels. Further experiments demonstrated that osthole could suppress P-gp expression by inhibiting the PI3K/Akt signaling pathway, which is possibly the main mechanism accounting for the reversal potential of osthole in K562/ADR cells (Wang et al., 2016).

Quinonoids. Quinone compounds are a class of chemical components with the quinoid structure in TCM and are mainly divided into four types: benzoquinone, naphthoquinone, phenanthrenequinone, and anthraquinone. Miltirone, an abietane-type diterpene quinone isolated from *Salvia miltiorrhiza*, demonstrates anticancer activities in P-gp–overexpressing human cancer cells. Current studies have suggested that miltirone can inhibit the activity of P-gp and apoptotic induction in a human hepatoma (HepG2) cell line and its P-gp–overexpressing HepG2/ADR cell line (Zhou et al., 2015). The inhibition activities of six anthraquinones

(alizarin, purpurin, chrysophanol, emodin, aloe-emodin, and 1,3,8-trihydroxyanthraquinone) toward P-gp were estimated using docking analysis. The results indicated that all the investigated anthraquinones were potential inhibitors of P-gp under physiological conditions, indicating their roles as potential protectors against patient resistance toward various anticancer drugs (Jeremić et al., 2018). Emodin reversed ADR resistance in K562/ADR cells by decreasing the expression of P-gp. It can increase the accumulation of Rh123 in both K562/ADR and Caco-2 cells and, hence, inhibit P-gp efflux (Min et al., 2017). Shikonin/paclitaxel (PTX) cotreatment led to synergistically enhanced cytotoxicity and apoptosis in PTX-resistant ovarian cancer cells, reflecting the reverse of MDR. Further studies indicated that the MDR reversal effect of shikonin was independent of inhibiting the activity of P-gp (Wang et al., 2019). Moreover, the shikonin derivatives (acetylshikonin and acetoxyisovalerylshikonin) showed inhibition of P-gp and increased uptake and reduced efflux of anticancer drugs in the malignant cancer cells, suggesting that chemotherapy in combination with shikonin compounds may be beneficial to cancer cells (Wang et al., 2019).

Traditional Chinese Medicine Extracts. *Bufo gargarizans* (Asiatic toad), a popular Chinese herb, showed a reversal effect on P-gp–mediated MDR in colorectal cancer cells. More studies showed that the representative ingredient, cinobufagin, significantly enhanced the sensitivity of P-gp–overexpressing cells to ADR without affecting the corresponding parental cells. Studies further revealed that the mechanism of action involved noncompetitive inhibition of P-gp (Yuan et al., 2017). *Salvia miltiorrhiza (S. miltiorrhiza)* is a Chinese herb with significant antifungal, antioxidant, anti-inflammatory, and anticancer activities. The diterpenoid tanshinone, which coexisted in the *S. miltiorrhiza* extract, could significantly enhance the absorption of cryptotanshinone. Danxiongfang is a useful preparation composed of the *S. miltiorrhiza* extract, which mainly includes lipophilic diterpenoid tanshinones, water-soluble salvianolic acids, and ferulic acid. It could be used to treat coronary heart diseases and cerebrovascular disease. Danxiongfang could influence the absorption of cryptotanshinone. It has been proposed that the oral bioutilization of cryptotanshinone can be enhanced by reducing the efflux and transport of P-gp in combination with diterpenoid tanshinones and danxiongfang (Dai et al., 2012). *Schisandra Chinensis(S. chinensis)*, known as wu wei zi in Chinese, could inhibit the efflux of P-gp. Its active components include schizandrol A, γ-schisandrin, schizandrin B, and schizandrin C. Some experiments showed that schizandrin B increased the intracellular accumulation of ADR through inhibiting expression and activity of P-gp (Wang et al., 2017). The MDR reverse effect of schizandrol A was demonstrated with P-gp overexpressed drug-resistant K562 cells (Arken, 2019). Coptidis rhizoma (CR), the rhizome of *Coptis chinensis*, is a well known Chinese herb. CR contains abundant isoquinoline alkaloids, such as berberine, coptisine, and palmatine. Cell studies have shown that CR decoction, berberine, coptisine, and palmatine can activate the efflux of P-gp (Yu et al., 2018). The wutou-gancao herb pair is extensively used to attenuate the toxicity and enhance the efficacy of aconite. The active components of liquorice (gancao) could inhibit the efflux of P-gp through Caco-2 cells. Since aconitine from *Aconitum* (wutou) has been shown to be a P-gp substrate; the synergic effect is attributed to the inhibition of P-gp by liquorice. The results showed the potential synergic mechanism of the wutou-gancao herb pair, which could help to elucidate the compatibility principle of the two herbs by inhibiting P-gp function and enhancing the systemic circulation exposure of aconitine and further anti-inflammatory effects (He et al., 2020).

Approaches for Studying the Interaction of P-gp and TCM

In Vitro Approaches for TCM Interactions with P-gp. Several in vitro screening assays can be used to characterize the interplay between test compounds and P-gp to identify drugs as P-gp substrates or inhibitors (Polli et al., 2001). The basic principle of the classification method is determining the effect on P-gp function and expression. Functional assays used to screen P-gp inhibitors include the detection of the changes in P-gp content and the expression level of the *MDR1* gene, the evaluation of the P-gp efflux by colorimetric chemosensitivity assay, and bidirectional transcellular transport assays. A colorimetric chemosensitivity assay was used to determine the effect of reversal agent on P-gp via the calculation of IC_{50} and the degree of drug resistance. Concentration changes in P-gp content and the levels of *MDR1* genes were typically measured in cells by reverse-transcription polymerase chain reaction, immunoblotting, and/or flow cytometry (Xia et al., 2017). Li et al. (2018) used 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay to determine the cytotoxicity of doxorubicin, polyphenols, and digitonin and the cytotoxicity of their combinations. Rhodamine 123 and Calcein-Acetoxymethylester were further used to detect the effects of polyphenols on the activity of P-gp. It has been validated that the combination of nontoxic concentrations of each polyphenol with ADR could synergistically improve the drug efficacy toward Caco-2 and CEM/ADR cells. Polyphenols could modulate the activity of P-gp and may be used as chemosensitizers.

Bidirectional transcellular transport assay is the gold standard for identifying P-gp substrates in vitro and represents the most accurate predictive model for the identification of P-gp inhibitors in vivo (Feng et al., 2008). Drug-resistant cell lines with overexpression of P-gp are widely used to investigate the effect of test compounds as potential inhibitors of P-gp in this method. There are many cell lines suitable for use in bidirectional transcellular transport assays, including the naturally P-gp–expressing Caco-2 cells and polarized *MDR1*-transfected Madin-Darby canine kidney cells, with distinct apical and basolateral membrane domains. The apparent permeability coefficient (apical to basolateral/basolateral to apical) was used to judge whether the intestinal absorption of a drug would be affected by P-gp. It was considered that the transport of a drug was directional when the apparent permeability coefficient (apical to basolateral/basolateral to apical) was more than 1.5 (Liang et al., 2012). When the efflux rate of a probe substrate decreased, it was considered that the agent may inhibit the activity of P-gp; the schematic diagram of the method is shown in Fig. 3A. The effect of different tanshinones on the P-gp efflux function was first investigated by using a digoxin bidirectional transport assay. According to the results, among the five tanshinones tested, only cryptotanshinone and dihydrotanshinone could decrease the efflux ratio of digoxin bidirectional transport across a Caco-2 cell monolayer, indicating their inhibition on P-gp function (Hu et al., 2014). Meanwhile, these in vitro methods have their limitations and should be fully considered in practice. Different studies have used the same assay for the same compound but reported contrasting results. In addition to the discordance in the results obtained from different in vitro studies, there have also been some disagreements between in vitro and in vivo findings regarding the P-gp substrates or inhibitors (Lund et al., 2017).

In Vivo Approaches for TCM Interactions with P-gp. The generation of *MDR1*-knockout mice has boosted the study of the pharmacological influence on the function of P-gp (Li et al., 2020). By comparing brain/plasma ratios between these P-gp–deficient mice to those of the wild-type controls, it has been possible to screen drugs as potential P-gp substrates in vivo (Mittapalli et al., 2012). Alternatively, P-gp inhibitors can be evaluated by investigating the influence of P-gp inhibition on drug pharmacokinetics in wild-type animals. Coadministration of digoxin with verapamil or emodin can increase the area under the curve of digoxin by 55% and 51%, respectively. Emodin demonstrated inhibition of P-gp to specific extents in vivo (Li et al., 2014).

In vivo studies were also conducted on tumor-bearing mice. The chemosensitization effects of TCM have been investigated through

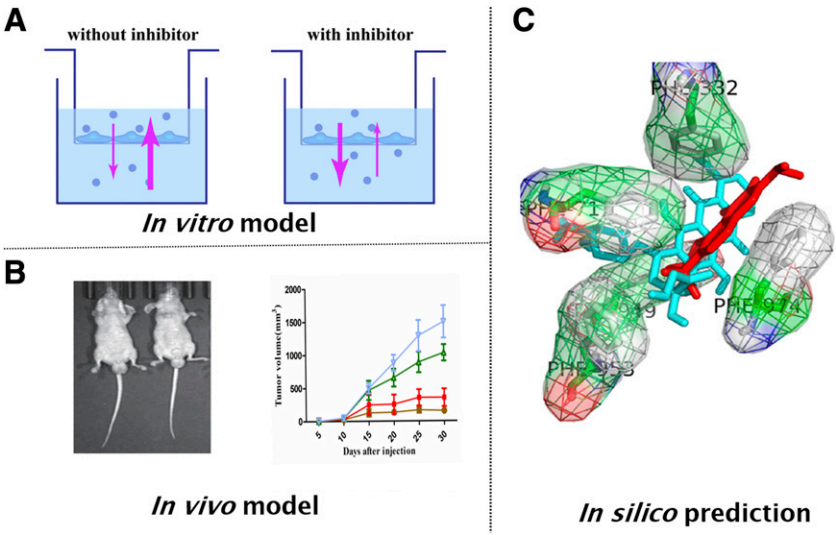

Fig. 3. Approaches to studying TCM interactions with P-gp. (A) In vitro method using bidirectional transcellular transport assay; (B) in vivo method conducted on tumor-bearing rats; (C) in silico prediction method by molecular docking (Zhou et al., 2015).

changes in tumor growth or survival rate of tumor-bearing mice (Fig. 3B) (Tiwari et al., 2013). If a TCM has MDR-reversing activity, tumor growth should be inhibited, and the survival period of tumor-bearing rats should be prolonged. An in vivo antitumor study showed that coencapsulated PTX and baicalein in nanoemulsions demonstrated higher antitumor efficacy than other PTX formulations. The antitumor effect of various PTX formulations in vivo was evaluated in models using mice bearing MCF-7/Tax xenograft tumors (Meng et al., 2016). Combining PTX with vitamin D3 and curcumin could potentially synergize their ability to decrease drug resistance by decreasing P-gp. In vivo, the triple therapy group (PTX + curcumin + D3) resulted in the smallest tumor size, indicating that the addition of curcumin and D3 could enhance the therapeutic effect on the tumor (Attia et al., 2020).

In Silico Prediction Method. With the rapid development of machine learning and artificial intelligence, in silico methods have acquired an increasing interest in P-gp inhibitor discovery. Compared with the classic methods, these in silico methods have an enormous potential to speed up the preclinical development processes at minimal costs. Using in silico virtual screening to detect novel P-gp inhibitors is an important application of molecular simulations.

At present, no human crystallized P-gp has been published. Since the sequence identity between the human protein and the murine protein is 87%, the available structures of the murine protein are important for molecular docking from different perspectives (Pajeva et al., 2009; Jabeen et al., 2011). Shityakov and Forster (2014) ran molecular docking in the crystallized murine structure with polynomial empirical scores using a P-gp inhibitor library of 1300 molecules. To understand the interaction of ADR and miltirone with P-gp, molecular docking analysis was conducted. The results indicated the most energetically optimal binding modes of ADR and miltirone at the drug-binding cavities of mouse P-gp (Protein Data Bank Identification: 3G61), which is shown in Fig. 3C (Zhou et al., 2015). However, the human P-gp homology model showed more limitations to predict the experimental data. In addition, no human crystallized P-gp has been made available, and extrapolation of the results from animals to humans is not always satisfactory (Montanari and Ecker, 2015).

Conclusions and Outlook

Despite the promising preclinical results of P-gp inhibitors, no compound has yet been approved for clinical use as an MDR reversal agent. Studies were terminated because of unexpected toxicities in clinical trials or insufficient efficacy in vivo. Consequently, identifying MDR reversal agents with high efficacy and limited toxicity remains a big challenge. Because of the characteristic of low toxicity, an increasing number of TCMs, including flavonoids, alkaloids, terpenoids, coumarins, quinonoids, and other TCM extracts, have been investigated for their potential as MDR reversal agents and have shown good efficacy for reversing MDR in experimental model systems. TCM has been reported to reverse P-gp–mediated MDR with diverse mechanisms, such as inhibiting P-gp–mediated drug efflux (Abdallah et al., 2015), hindering the activity of P-gp ATPase, and reducing P-gp levels by downregulating the expression of the *MDR1* gene.

Currently, studies on identifying TCM as P-gp reversal agents have mainly been conducted in vitro using cell models, rat models, and in silico methods. However, the in vitro approaches for screening are labor-intensive and time-consuming at present. The molecular docking results from the human P-gp homology model is not always satisfactory. Technologies such as proteoliposome, which can predict the interaction of the active compound with the target protein, should be explored to establish a new high-throughput screening method.

On the other hand, the influence of P-gp inhibition on tissue pharmacokinetics and intratumoral distribution of anticancer drugs is very important when coadministered with a P-gp inhibitor. More efforts should be made to explore the effects of TCM on the pharmacokinetic processes of anticancer drugs in vivo to choose the optimal therapeutic protocol for cancer treatment. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) is commonly used to determine the drug concentration in homogenized tissues, whereas it is not accurate for tumors because of heterogeneous characteristics. Since the heterogeneous tissue and the tumor microenvironment markedly affect drug penetration, it is necessary to detect not only the level of exposure but also the spatial distribution of the drug within the tumor tissue. Thus, the use of only LC-MS/MS analysis is insufficient for clarifying the behavior of antitumor drugs. Mass spectrometry imaging (MSI), which could offer spatial information for the drug in the tumor tissue, has recently been used as an innovative tool for detecting the molecular distribution of pharmacological agents in heterogeneous targets. Compared with LC-MS/MS, MSI does not require homogenization of tumor tissue, so it can provide information on the spatial distribution of the detected drugs in the tissue. This technique would certainly improve our understanding of the heterogeneous and irregular tumor drug distribution.

In summary, TCMs will play a crucial role in the development of effective MDR modulators in the future. The in vitro techniques, such as

bidirectional transcellular transport assays and in silico prediction method, will greatly influence drug development. These approaches can help predict the effect of TCM on P-gp and provide an impetus to the discovery of more MDR reversal agent candidates for clinical trials. However, precise techniques, such as MSI, discussed in this review were needed for in vivo validation. In brief, the current challenge is how to identify suitable P-gp inhibitors with significant MDR reversal and acceptable toxicity from TCM and thus improve the treatment effect of chemotherapy drugs in clinical cancer therapy.

Authorship Contributions

Participated in research design: Cao, Hong, Chai.
Conducted experiments: Cao, Shi, Hong, Cai.
Contributed new reagents or analytic tools: Cao, Shi, Cai.
Performed data analysis: Cao, Hong, Chai.
Wrote or contributed to the writing of the manuscript: Cao, Shi, Hong.

References

Abdallah HM, Al-Abd AM, El-Dine RS, and El-Halawany AM (2015) P-glycoprotein inhibitors of natural origin as potential tumor chemo-sensitizers: a review. *J Adv Res* **6**:45–62.

Arken N (2019) Schizandrol A reverses multidrug resistance in resistant chronic myeloid leukemia cells K562/A02. *Cell Mol Biol* **65**:78–83.

Attia YM, El-Kersh DM, Ammar RA, Adel A, Khalil A, Walid H, Eskander K, Hamdy M, Reda N, Mohsen NE, et al. (2020) Inhibition of aldehyde dehydrogenase-1 and p-glycoprotein-mediated multidrug resistance by curcumin and vitamin D3 increases sensitivity to paclitaxel in breast cancer. *Chem Biol Interact* **315**:108865.

Binkhathlan Z and Lavasanifar A (2013) P-glycoprotein inhibition as a therapeutic approach for overcoming multidrug resistance in cancer: current status and future perspectives. *Curr Cancer Drug Targets* **13**:326–346.

Cai H, Li L, Jiang J, Zhao C, and Yang C (2019) Costunolide enhances sensitivity of K562/ADR chronic myeloid leukemia cells to doxorubicin through PI3K/Akt pathway. *Phytother Res* **33**: 1683–1688.

Carlisi D, De Blasio A, Drago-Ferrante R, Di Fiore R, Buttitta G, Morreale M, Scerri C, Vento R, and Tesoriere G (2017) Parthenolide prevents resistance of MDA-MB231 cells to doxorubicin and mitoxantrone: the role of Nrf2. *Cell Death Discov* **3**:17078.

Chang Y-T, Wang CCN, Wang J-Y, Lee T-E, Cheng Y-Y, Morris-Natschke SL, Lee K-H, and Hung C-C (2019) Tenulin and isotenulin inhibit P-glycoprotein function and overcome multidrug resistance in cancer cells. *Phytomedicine* **53**:252–262.

Chen CJ, Chin JE, Ueda K, Clark DP, Pastan I, Gottesman MM, and Roninson IB (1986) Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. *Cell* **47**:381–389.

Chen HJ, Chung YL, Li CY, Chang YT, Wang CCN, Lee HY, Lin HY, and Hung CC (2018) Taxifolin resensitizes multidrug resistance cancer cells via uncompetitive inhibition of P-glycoprotein function. *Molecules* **23**:3055.

Choi HS, Cho SG, Kim MK, Kim MS, Moon SH, Kim IH, and Ko SG (2016) Decursin in Angelica gigas Nakai (AGN) enhances doxorubicin chemosensitivity in NCI/ADR-RES ovarian cancer cells via inhibition of P-glycoprotein expression. *Phytother Res* **30**:2020–2026.

Dai H, Li X, Li X, Bai L, Li Y, and Xue M (2012) Coexisted components of Salvia miltiorrhiza enhance intestinal absorption of cryptotanshinone via inhibition of the intestinal P-gp. *Phytomedicine* **19**:1256–1262.

Dong J, Qin Z, Zhang WD, Cheng G, Yehuda AG, Ashby CR Jr., Chen ZS, Cheng XD, and Qin JJ (2020) Medicinal chemistry strategies to discover P-glycoprotein inhibitors: an update. *Drug Resist Updat* **49**:100681.

Fang Y, Xia M, Liang F, Cao W, Pan S, and Xu X (2019) Establishment and use of human mouth epidermal carcinoma (KB) cells overexpressing P-glycoprotein to characterize structure requirements for flavonoids transported by the efflux transporter. *J Agric Food Chem* **67**: 2350–2360.

Feng B, Mills JB, Davidson RE, Mireles RJ, Janiszewski JS, Troutman MD, and de Morais SM (2008) In vitro P-glycoprotein assays to predict the in vivo interactions of P-glycoprotein with drugs in the central nervous system. *Drug Metab Dispos* **36**:268–275.

Gherbovet O, García Alvarez MC, Bignon J, and Roussi F (2016) Original vinca derivatives: from P-glycoprotein substrates to P-glycoprotein inhibitors. *J Med Chem* **59**:10774–10780.

Gottesman MM and Ling V (2006) The molecular basis of multidrug resistance in cancer: the early years of P-glycoprotein research. *FEBS Lett* **580**:998–1009.

Gottesman MM and Pastan I (1993) Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* **62**:385–427.

He Y, Wei Z, Xie Y, Yi X, Zeng Y, Li Y, and Liu C (2020) Potential synergic mechanism of Wutou-Gancao herb-pair by inhibiting efflux transporter P-glycoprotein. *J Pharm Anal* **10**: 178–186.

Herrmann R, Roller J, Polednik C, and Schmidt M (2018) Effect of chelidonine on growth, invasion, angiogenesis and gene expression in head and neck cancer cell lines. *Oncol Lett* **16**: 3108–3116.

Hrycyna CA, Ramachandra M, Ambudkar SV, Ko YH, Pedersen PL, Pastan I, and Gottesman MM (1998) Mechanism of action of human P-glycoprotein ATPase activity. Photochemical cleavage during a catalytic transition state using orthovanadate reveals cross-talk between the two ATP sites. *J Biol Chem* **273**:16631–16634.

Hu T, To KK, Wang L, Zhang L, Lu L, Shen J, Chan RL, Li M, Yeung JH, and Cho CH (2014) Reversal of P-glycoprotein (P-gp) mediated multidrug resistance in colon cancer cells by cryptotanshinone and dihydrotanshinone of Salvia miltiorrhiza. *Phytomedicine* **21**:1264–1272.

Hussaarts KGAM, Veerman GDM, Jansman FGA, van Gelder T, Mathijssen RHJ, and van Leeuwen RWF (2019) Clinically relevant drug interactions with multikinase inhibitors: a review. *Ther Adv Med Oncol* **11**:1758835918818347.

Jabeen I, Wetwitayaklung P, Klepsch F, Parveen Z, Chiba P, and Ecker GF (2011) Probing the stereoselectivity of P-glycoprotein-synthesis, biological activity and ligand docking studies of a set of enantiopure benzopyrano[3,4-b][1,4]oxazines. *Chem Commun (Camb)* **47**:2586–2588.

Jeremić S, Amić A, Stanojević-Pirković M, and Marković Z (2018) Selected anthraquinones as potential free radical scavengers and P-glycoprotein inhibitors. *Org Biomol Chem* **16**: 1890–1902.

Joshi P, Vishwakarma RA, and Bharate SB (2017) Natural alkaloids as P-gp inhibitors for multidrug resistance reversal in cancer. *Eur J Med Chem* **138**:273–292.

Karabin M, Hudcova T, Jelinek L, and Dostalek P (2015) Biotransformations and biological activities of hop flavonoids. *Biotechnol Adv* **33**:1063–1090.

Kathawala RJ, Gupta P, Ashby CR Jr., and Chen ZS (2015) The modulation of ABC transporter-mediated multidrug resistance in cancer: a review of the past decade. *Drug Resist Updat* **18**: 1–17.

Kumar A and Jaitak V (2019) Natural products as multidrug resistance modulators in cancer. *Eur J Med Chem* **176**:268–291.

Leopoldo M, Nardulli P, Contino M, Leonetti F, Luurtsema G, and Colabufo NA (2019) An updated patent review on P-glycoprotein inhibitors (2011-2018). *Expert Opin Ther Pat* **29**: 455–461.

Li H, Krstin S, and Wink M (2018) Modulation of multidrug resistant in cancer cells by EGCG, tannic acid and curcumin. *Phytomedicine* **50**:213–222.

Li W, Sparidans R, El-Lari M, Wang Y, Lebre MC, Beijnen JH, and Schinkel AH (2020) P-glycoprotein (ABCB1/MDR1) limits brain accumulation and cytochrome P450-3A (CYP3A) restricts oral availability of the novel FGFR4 inhibitor fisogatinib (BLU-554). *Int J Pharm* **573**: 118842.

Li X, Hu J, Wang B, Sheng L, Liu Z, Yang S, and Li Y (2014) Inhibitory effects of herbal constituents on P-glycoprotein in vitro and in vivo: herb-drug interactions mediated via P-gp. *Toxicol Appl Pharmacol* **275**:163–175.

Li Y, Chen M, Yao B, Lu X, Zhang X, He P, Vasilatos SN, Ren X, Bian W, and Yao C (2019) Transferrin receptor-targeted redox/pH-sensitive podophyllotoxin prodrug micelles for multidrug-resistant breast cancer therapy. *J Mater Chem B Mater Biol Med* **7**:5814–5824.

Liang XL, Zhao LJ, Liao ZG, Zhao GW, Zhang J, Chao YC, Yang M, and Yin RL (2012) Transport properties of puerarin and effect of Radix Angelicae Dahuricae extract on the transport of puerarin in Caco-2 cell model. *J Ethnopharmacol* **144**:677–682.

Liao D, Zhang W, Gupta P, Lei ZN, Wang JQ, Cai CY, Vera AA, Zhang L, Chen ZS, and Yang DH (2019) Tetrandrine interaction with ABCB1 reverses multidrug resistance in cancer cells through competition with anti-cancer drugs followed by downregulation of ABCB1 expression. *Molecules* **24**:4383.

Lopes-Rodrigues V, Sousa E, and Vasconcelos MH (2016) Curcumin as a modulator of P-glycoprotein in cancer: challenges and perspectives. *Pharmaceuticals (Basel)* **9**:71.

Lund M, Petersen TS, and Dalhoff KP (2017) Clinical implications of P-glycoprotein modulation in drug-drug interactions. *Drugs* **77**:859–883.

Ma X, Hu M, Wang H, and Li J (2018) Discovery of traditional Chinese medicine monomers and their synthetic intermediates, analogs or derivatives for battling P-gp-mediated multi-drug resistance. *Eur J Med Chem* **159**:381–392.

Maruszewska A and Tarasiuk J (2019) Antitumour effects of selected plant polyphenols, gallic acid and ellagic acid, on sensitive and multidrug-resistant leukaemia HL60 cells. *Phytother Res* **33**: 1208–1221.

Meng L, Xia X, Yang Y, Ye J, Dong W, Ma P, Jin Y, and Liu Y (2016) Co-encapsulation of paclitaxel and baicalein in nanoemulsions to overcome multidrug resistance via oxidative stress augmentation and P-glycoprotein inhibition. *Int J Pharm* **513**:8–16.

Min H, Niu M, Zhang W, Yan J, Li J, Tan X, Li B, Su M, Di B, and Yan F (2017) Emodin reverses leukemia multidrug resistance by competitive inhibition and downregulation of P-glycoprotein. *PLoS One* **12**:e0187971.

Mistry P, Stewart AJ, Dangerfield W, Okiji S, Liddle C, Bootle D, Plumb JA, Templeton D, and Charlton P (2001) In vitro and in vivo reversal of P-glycoprotein-mediated multidrug resistance by a novel potent modulator, XR9576. *Cancer Res* **61**:749–758.

Mittapalli RK, Vaidhyanathan S, Sane R, and Elmquist WF (2012) Impact of P-glycoprotein (ABCB1) and breast cancer resistance protein (ABCG2) on the brain distribution of a novel BRAF inhibitor: vemurafenib (PLX4032). *J Pharmacol Exp Ther* **342**:33–40.

Mohana S, Ganesan M, Rajendra Prasad N, Ananthakrishnan D, and Velmurugan D (2018) Flavonoids modulate multidrug resistance through wnt signaling in P-glycoprotein overexpressing cell lines. *BMC Cancer* **18**:1168.

Mollazadeh S, Sahebkar A, Hadizadeh F, Behravan J, and Arabzadeh S (2018) Structural and functional aspects of P-glycoprotein and its inhibitors. *Life Sci* **214**:118–123.

Montanari F and Ecker GF (2015) Prediction of drug-ABC-transporter interaction--recent advances and future challenges. *Adv Drug Deliv Rev* **86**:17–26.

Nguyen PH, Sigdel KP, Schaefer KG, Mensah GAK, King GM, and Roberts AG (2020) The effects of anthracycline drugs on the conformational distribution of mouse P-glycoprotein explains their transport rate differences. *Biochem Pharmacol* **174**:113813.

O'Brien FE, Dinan TG, Griffin BT, and Cryan JF (2012) Interactions between antidepressants and P-glycoprotein at the blood-brain barrier: clinical significance of in vitro and in vivo findings. *Br J Pharmacol* **165**:289–312.

Pajeva IK, Globisch C, and Wiese M (2009) Combined pharmacophore modeling, docking, and 3D QSAR studies of ABCB1 and ABCC1 transporter inhibitors. *ChemMedChem* **4**:1883–1896.

Polli JW, Wring SA, Humphreys JE, Huang L, Morgan JB, Webster LO, and Serabjit-Singh CS (2001) Rational use of in vitro P-glycoprotein assays in drug discovery. *J Pharmacol Exp Ther* **299**:620–628.

Pusztai L, Wagner P, Ibrahim N, Rivera E, Theriault R, Booser D, Symmans FW, Wong F, Blumenschein G, Fleming DR, et al. (2005) Phase II study of tariquidar, a selective P-glycoprotein inhibitor, in patients with chemotherapy-resistant, advanced breast carcinoma. *Cancer* **104**:682–691.

Qian J, Xia M, Liu W, Li L, Yang J, Mei Y, Meng Q, and Xie Y (2019) Glabridin resensitizes p-glycoprotein-overexpressing multidrug-resistant cancer cells to conventional chemotherapeutic agents. *Eur J Pharmacol* **852**:231–243.

Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, and Gottesman MM (2018) Revisiting the role of ABC transporters in multidrug-resistant cancer. *Nat Rev Cancer* **18**:452–464.

Saeed M, Kadioglu O, Khalid H, Sugimoto Y, and Efferth T (2015) Activity of the dietary flavonoid, apigenin, against multidrug-resistant tumor cells as determined by pharmacogenomics and molecular docking. *J Nutr Biochem* **26**:44–56.

Schinkel AH (1997) The physiological function of drug-transporting P-glycoproteins. *Semin Cancer Biol* **8**:161–170.
Sharom FJ (2011) The P-glycoprotein multidrug transporter. *Essays Biochem* **50**:161–178.
Shityakov S and Förster C (2014) In silico structure-based screening of versatile P-glycoprotein inhibitors using polynomial empirical scoring functions. *Adv Appl Bioinform Chem* **7**:1–9.
Shustik C, Dalton W, and Gros P (1995) P-glycoprotein-mediated multidrug resistance in tumor cells: biochemistry, clinical relevance and modulation. *Mol Aspects Med* **16**:1–78.
Sun Y, Wang C, Meng Q, Liu Z, Huo X, Sun P, Sun H, Ma X, Peng J, and Liu K (2018) Targeting P-glycoprotein and SORCIN: dihydromyricetin strengthens anti-proliferative efficiency of adriamycin via MAPK/ERK and Ca^{2+} -mediated apoptosis pathways in MCF-7/ADR and K562/ADR. *J Cell Physiol* **233**:3066–3079.
Tiwari AK, Sodani K, Dai CL, Abuznait AH, Singh S, Xiao ZJ, Patel A, Talele TT, Fu L, Kaddoumi A, et al. (2013) Nilotinib potentiates anticancer drug sensitivity in murine ABCB1-, ABCG2-, and ABCC10-multidrug resistance xenograft models. *Cancer Lett* **328**:307–317.
Varma MV, Ashokraj Y, Dey CS, and Panchagnula R (2003) P-glycoprotein inhibitors and their screening: a perspective from bioavailability enhancement. *Pharmacol Res* **48**:347–359.
Viktorová J, Dobiasová S, Řehořová K, Biedermann D, Káňová K, Šeborová K, Václavíková R, Valentová K, Ruml T, Křen V, et al. (2019) Antioxidant, anti-inflammatory, and multidrug resistance modulation activity of silychristin derivatives. *Antioxidants* **8**:303.
Wang H, Jia XH, Chen JR, Wang JY, and Li YJ (2016) Osthole shows the potential to overcome P-glycoprotein-mediated multidrug resistance in human myelogenous leukemia K562/ADM cells by inhibiting the PI3K/Akt signaling pathway. *Oncol Rep* **35**:3659–3668.
Wang S, Wang A, Shao M, Lin L, Li P, and Wang Y (2017a) Schisandrin B reverses doxorubicin resistance through inhibiting P-glycoprotein and promoting proteasome-mediated degradation of survivin. *Sci Rep* **7**:8419.
Wang Z, Yin J, Li M, Shen J, Xiao Z, Zhao Y, Huang C, Zhang H, Zhang Z, Cho CH, et al. (2019) Combination of shikonin with paclitaxel overcomes multidrug resistance in human ovarian carcinoma cells in a P-gp-independent manner through enhanced ROS generation. *Chin Med* **14**:7.
Xia X, Cole SPC, Cai T, and Cai Y (2017) Effect of traditional Chinese medicine components on multidrug resistance in tumors mediated by P-glycoprotein. *Oncol Lett* **13**:3989–3996.
Xie B, Wan J, Chen X, Han W, and Wang H (2020) Preclinical evaluation of a cabazitaxel prodrug using nanoparticle delivery for the treatment of taxane-resistant malignancies. *Mol Cancer Ther* **19**:822–834.
Yu C-P, Huang C-Y, Lin S-P, and Hou Y-C (2018) Activation of P-glycoprotein and CYP 3A by Coptidis Rhizoma in vivo: using cyclosporine as a probe substrate in rats. *Yao Wu Shi Pin Fen Xi* **26** (Suppl):S125–S132.
Yuan Z, Shi X, Qiu Y, Jia T, Yuan X, Zou Y, Liu C, Yu H, Yuan Y, He X, et al. (2017) Reversal of P-gp-mediated multidrug resistance in colon cancer by cinobufagin. *Oncol Rep* **37**:1815–1825.
Zhang E, Liu J, Shi L, Guo X, Liang Z, Zuo J, Xu H, Wang H, Shu X, Huang S, et al. (2019) 7-O-geranylquercetin contributes to reverse P-gp-mediated adriamycin resistance in breast cancer. *Life Sci* **238**:116938.
Zhou BG, Wei CS, Zhang S, Zhang Z, and Gao HM (2018) Matrine reversed multidrug resistance of breast cancer MCF-7/ADR cells through PI3K/AKT signaling pathway. *J Cell Biochem* **119**:3885–3891.
Zhou X, Wang Y, Lee WYW, Or PMY, Wan DCC, Kwan YW, and Yeung JHK (2015) Miltirone is a dual inhibitor of P-glycoprotein and cell growth in doxorubicin-resistant HepG2 cells. *J Nat Prod* **78**:2266–2275.
Zhu H, Gao J, Wang L, Qian K-J, and Cai L-P (2018) *In vitro* study on reversal of ovarian cancer cell resistance to cisplatin by naringin via the nuclear factor-κB signaling pathway. *Exp Ther Med* **15**:2643–2648.
Zong L, Cheng G, Liu S, Pi Z, Liu Z, and Song F (2019) Reversal of multidrug resistance in breast cancer cells by a combination of ursolic acid with doxorubicin. *J Pharm Biomed Anal* **165**:268–275.

Address correspondence to: Zhanying Hong, School of Pharmacy, Second Military Medical University, Shanghai Key Laboratory for Pharmaceutical (Chinese Materia Medica) Metabolites Research, Shanghai 200433, China. E-mail: hongzhy001@163.com