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Effect of traditional Chinese medicine components on multidrug resistance in tumors mediated by P-glycoprotein (Review)

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Abstract. Multidrug resistance (MDR) is a major cause of chemotherapy failure. It occurs when an organism is resistant to one type of drug, but also develops resistance to other drugs with different structures and targets. There is a high incidence of MDR in cancer chemotherapy, therefore, finding an effective and non-toxic MDR reversal agent is an important goal, particularly for P-glycoprotein-mediated MDR in cancer. Improvements continue to be made to the status and understanding of traditional Chinese medicine (TCM), due to the advantages of low toxicity and relatively minor side effects. Therefore TCM is currently being used in the treatment of various types of diseases. In recent years, numerous components of TCM have been identified to be effective in reversing MDR by downregulating expression of the drug transporter membrane protein, recovering changes in enzymes involved in detoxification and metabolism and repairing the cell apoptosis pathway. The present study summarizes the anticancerous properties and MDR reversing components of traditional medicinal plants commonly used in the treatment of cancer.

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Abbreviations: MDR, multidrug resistance; TCM, traditional Chinese medicine; P-gp, P-glycoprotein; MRP1, multidrug resistance protein 1; TMD, transmembrane domain; TM, transmembrane segment; NBD, nucleotide binding domain

Key words: MDR, reversal agent, TCM components, P-pg, drug efflux pump, doxorubicin

Contents

1. Introduction

Multidrug resistance (MDR) in cancer refers to tumor cells that not only exhibit resistance to a single drug to which they have been exposed, but they also develop cross-resistance to multiple drugs with different structures, cellular targets and mechanisms of action (1,2). MDR thus reduces the sensitivity of tumor cells to cytotoxic and targeted chemotherapeutic agents, and is one reason why individuals with cancer may not respond to other effective chemotherapeutic regimens. Traditional Chinese medicine (TCM) is recognized by an increasing number of individuals for its potential applications in tumor therapy due to the advantages of its relatively low toxicity, its reported efficacy and its ability to target multiple cellular pathways. Following a comprehensive number of studies, it has been demonstrated that various components of TCM may have the potential to reverse MDR in tumors. TCM may be particularly effective in tumors in which resistance is mediated through the elevated expression of the drug transporter membrane protein, P-glycoprotein (P-gp). The present study provides a brief review of the field of TCM and P-gp.

2. Mechanisms of MDR

The mechanisms of MDR are multifaceted and complex, and include several factors that are summarized in Fig. 1. Among these factors are: An adenosine 5'-triphosphate (ATP) dependent decrease in cellular drug accumulation in tumor cell lines associated with elevated levels of the 170 kDa drug transporter P-gp, the 190 kDa multidrug resistance protein 1 (MRP1), or other ATP-binding cassette (ABC) drug efflux pump (3-5); changes in enzymes involved in detoxification

and metabolism (e.g. increased levels of glutathione S-transferases and decreased topoisomerase II levels) (6); changes in DNA damage repair capacity of the tumor cells; and the dysregulation of apoptosis-related genes (7) (e.g. increased B-cell lymphoma 2, mutation of tumor protein p53 and activation of RAS). Other factors involved include changes in the tumor microenvironment *in vivo*, decreases in cytokine secretion and changes in hormone levels (8-10). Of all these factors, drug efflux mediated by P-gp is perhaps the most studied.

As mentioned, P-gp is a member of the ABC superfamily of membrane proteins, and was first identified in the plasma membrane of mammalian cells that had been selected for resistance to drugs (1,11). It is present in a number of normal tissues (12), and uses the energy from ATP binding and hydrolysis to effect the conformational changes in the protein necessary to pump xenobiotics (including anticancer drugs) across the cell membrane (13,14). Human P-gp is encoded by the ATP-binding cassette sub family B member 1 (ABCB1) gene (formerly termed MDR1), and has 1,280 amino acids that are organized in 2 homologous halves, each containing a hydrophobic transmembrane domain (TMD) with 6 transmembrane segments (TM), and a cytoplasmic nucleotide binding domain (NBD) (1,15). Substrate binding and translocation occurs largely through the TMDs, while ATP binding and hydrolysis requires the cooperation of the two NBDs (Fig. 2) (1,13,16).

3. P-gp as a target for reversing MDR in cancer

P-gp mediated drug efflux in tumor cells is an important resistance mechanism, and studies into MDR reversal agents almost always include this transporter protein as a target. Numerous small molecule inhibitors of P-gp have been developed during the last 4 decades (17). Verapamil was the first small molecule reported to reverse MDR mediated by P-gp in 1981 (18). The multiple P-gp MDR reversal agents described since then have been assigned to one of three generations, according to the timing of their discovery and development, and their individual features, selectivity and effectiveness. Although all MDR reversal agents possess a certain degree of effectiveness, each individual agent has shortcomings (see Table I for details).

The majority of small molecule reversal agents interact with P-gp at its drug binding sites through a competitive or non-competitive mechanism to inhibit the transport of anticancer drugs. Despite promising pre-clinical results in experimental systems, none have yet been approved for clinical use as reversal agents (19). Studies of certain reversal agents were terminated due to unacceptable patient toxicities in clinical trials or apparent lack of efficacy (20-22). Consequently, the search for effective and less toxic tumor MDR reversal agents continues to be a target pursued by numerous studies. An increasing number of non-toxic natural plant medicines are now being studied for their potential as MDR reversal agents. A variety of TCM components have been shown to have good activity with respect to reversing tumor MDR in experimental model systems. Studies on TCM components as P-gp reversal agents have mainly been performed *in vitro* using cultured cells and in rat models bearing MDR tumors.

4. *In vitro* experimental studies on TCM components as P-gp reversal agents

MDR tumor cell lines with elevated P-gp levels have been used *in vitro* to study the effect and mechanism of TCM components on the reversal of MDR. Frequently, a colorimetric MTT chemosensitivity assay is used to determine the effect of the P-gp reversal agent on the IC_{50} (concentration which inhibits cell viability by 50%) of the cytotoxic drug as well as the degree of drug resistance (fold change in resistance). Changes of P-gp content and the levels of related genes are typically measured in the cells by reverse transcription-polymerase chain reaction (RT-PCR), immunoblotting, and/or flow cytometry.

Rh2 ginsenosides. Rh2 ginsenosides are mainly derived from the dry roots and leaves of *Panax ginseng* C.A. Meyer (Araliaceae), and are given to patients with cancer to promote immunity against cancer through enhancing immune cell activity. Different concentrations of Rh2 ginsenosides were added to cultured MDR breast cancer MCF7/ADM cells and then resistance to doxorubicin (DOX; also termed adriamycin) and 5-fluorouracil (5FU), two agents commonly used to treat breast cancer clinically, were examined (23). The Rh2 ginsenosides were also tested for their ability to influence the fluorescence intensity of MDR cells incubated in rhodamine 123 as a measure of their effect on P-gp efflux activity. The Rh2 ginsenosides increased the sensitivity of MCF7/ADM cells to DOX and 5FU. In addition, the Rh2 ginsenosides significantly inhibited the cellular efflux of rhodamine 123 from the MDR cells (23). This indicates that Rh2 ginsenosides can effectively reduce P-gp activity to reverse tumor cell MDR. Rh2 ginsenosides perform an additional important role in leukemia and breast cancer cells. In addition to reducing P-gp activity, they have been demonstrated to decrease the levels of phospho-protein kinase B (p-AKT) and matrix metalloproteinase-2, and reduce the invasion and metastasis of MCF7/ADM cells through the suppression of the phosphoinositide 3-kinase/AKT signaling pathway (24). Rh2 ginsenosides could be excellent anti-leukemic agents due to their ability to inhibit growth, induce apoptosis and reverse the MDR of human leukemia K562/VCR cell lines (25).

Matrine. Matrine is a tetracycline quinolizidine alkaloid found mainly in members of the legume genus *Sophora flavescens*. Matrine has anti-inflammatory (26,27), antiviral (28), and anti-tumor effects (29-32), and can also reverse MDR (33-35). Thus, the effect of celecoxib, alone and combined with matrine, on the resistance of MDR erythroleukemia K562/AO2 cell lines was examined. Gui *et al* (33) revealed that in the presence of matrine, the DOX IC_{50} in erythroleukemia K562/AO2 cells was reduced almost 4-fold (from 33.31 to 9.44 μg/ml). The extent of apoptosis also increased from 4.81 to 15.31%. RT-PCR analyses demonstrated that ABCB1 and cyclooxygenase-2 (COX-2) mRNA levels were downregulated, as were the levels of the corresponding P-gp and COX-2 proteins. These data indicate that matrine likely reverses MDR (and enhances chemosensitivity) by reducing P-gp levels through the downregulation of ABCB1. In similarly designed studies using MDR breast cancer MCF-7/ADR (34) and hepatoma CRBH-7919/MDR1 cell lines (35), similar conclusions were reached that matrine reverses P-gp mediated MDR.

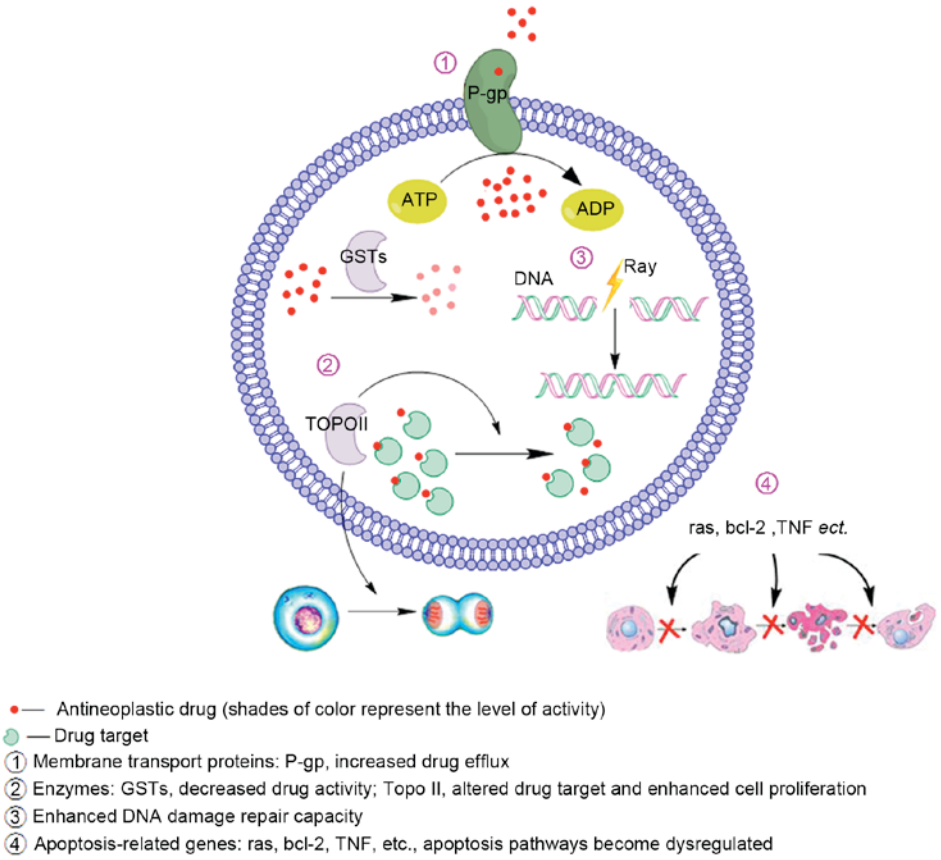


Figure 1. Main mechanisms by which multi drug resistance is thought to occur in cells. ATP, adenosine 5'-triphosphate; ADP, adenosine 5'-diphosphate; P-gp, P-glycoprotein; TOPOII, topoisomerase II; bcl-2, B-cell lymphoma 2; TNF, tumor necrosis family.

Quercetin. Quercetin is a natural flavonoid compound that exists widely in TCM, and in certain vegetables, fruits and grains. Quercetin appears to act as an MDR reversal agent through a variety of different mechanisms. For example, Wang *et al* (36) established a drug resistant glioma cell line (U87/TR) by exposing drug sensitive cells to temozolomide (TMZ). The authors determined that, compared with TMZ alone, the cell survival rate with a combination of quercetin with TMZ was significantly decreased ($P<0.01$), and cell toxicity was enhanced in a dose-dependent manner. Wang *et al* (37) also revealed that quercetin inhibited proliferation and enhanced apoptosis of a tamoxifen resistant breast cancer MCF-7Ca/TAM-R cell line, in a dose-dependent manner. In addition, Wang *et al* (38) reported that quercetin could significantly diminish P-gp activity in drug resistant lung adenocarcinoma A549/DDP cell lines and enhance the accumulation of chemotherapeutic agents. Wei *et al* (39) and He *et al* (40) also reported that quercetin resulted in a decrease in ABCB1 gene expression.

Emodin. Emodin (EM) is a type of anthraquinone extracted from rhubarb that possesses a variety of physiological activities, including inhibiting tumor cell proliferation, promoting apoptosis, and reversing MDR. EM can augment cisplatin cytotoxicity in platinum-resistant ovarian cancer COC1/DDP cells via reactive oxygen species-dependent downregulation of MRP1 (41). Li *et al* (42) reported that EM (10 μM) could markedly promote apoptosis in MDR human ovarian A2780/taxol tumor cells. It could also enhance the sensitivity of these cells to paclitaxel, and downregulate the intracellular levels of P-gp and inhibitor of apoptosis protein. Consequently, the authors concluded that the mechanism of EM-mediated reversal of MDR is associated with P-gp.

Tetramethylpyrazine. Tetramethylpyrazine (TMP; also termed ligustrazine) is a type of pyrazine alkaloid that occurs in the chuanqiong TCM. TMP has calcium channel blocking activity, and thus may be considered as an MDR reversal agent in tumors. The effect of TMP on P-gp activity was examined by Yu *et al* (43) using hepatoma BEL-7402/ADM cells. In one study, the authors examined 4 groups: BEL-7402 (drug sensitive) cells; BEL-7402/ADM (drug resistant) cells; BEL-7402/ADM cells exposed to verapamil (positive control group); and BEL-7402/ADM exposed to TMP (experimental group). Levels of P-gp were then measured by flow cytometry and immunohistochemistry. The results indicated that P-gp expression in BEL-7402/ADM cells was significantly increased compared with BEL-7402 cells, as expected. However, the authors also revealed that P-gp expression in BEL-7402/ADM cells was significantly lower following the treatment of cells with TMP ($P<0.01$).

Zhang *et al* (44) examined the effects of TMP on MDR breast cancer cells and demonstrated that TMP increased the intracellular concentration of DOX and inhibited P-gp mediated efflux of DOX in a dose-dependent manner. Additionally, TMP inhibited the ATPase activity of P-gp and suppressed the expression of P-gp in MCF-7/DOX cells.

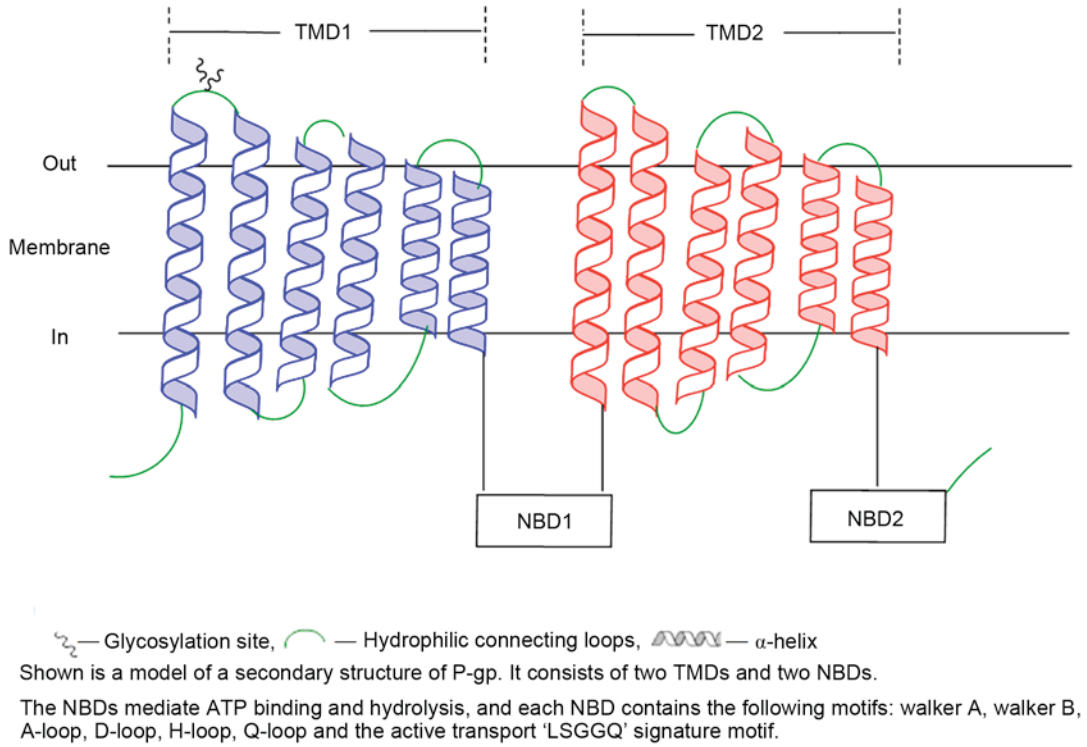


Figure 2. Structure of human P-gp in a cellular membrane. P-glycoprotein uses the energy from ATP binding and hydrolysis to pump xenobiotics (including anticancer drugs) across the cell membrane. It contains 2 TMDS and 2 NBDs. Substrate binding and translocation occurs largely through the TMDs, while ATP binding and hydrolysis requires the cooperation of the two NBDs. P-gp, P-glycoprotein; TMD, transmembrane domain; NBD, nucleotide binding domain.

Baicalin. Baicalin is a flavonoid compound isolated from the *Scutellaria baicalensis* root and is reported to demonstrate antibacterial, anti-inflammatory, anti-allergy and anticancer activity. Yang *et al* (45) observed that baicalin could reverse the resistance of leukemia K562/ADR cells. Thus the DOX resistance of these cells was reversed 5.2 and 19.3 fold with baicalin at 10 and 20 mg/l, respectively. Intracellular DOX accumulation was also increased significantly. The authors concluded that resistance reversal by baicalin may be associated with its ability to inhibit expression of the ABCB1 gene.

Schizandrin B. It has been reported that 5 schizandrins isolated from the Chinese herb *Fructus schizandrae* (FS) could reverse P-gp mediated MDR (46). Schizandrin B is the biphenyl cyclooctene lignans present at the highest levels in FS. It demonstrated effective reversal of drug resistance in bladder tumor (47), human osteosarcoma (48) and human colon cancer cells (49). Pan *et al* (50) has reported that Schizandrin B demonstrated MDR reversal activity in 4 MDR human tumor cell lines, which express elevated P-gp levels. These cell lines are K562/Adr, MCF-7/Adr, KBv200 and Bcap37/Adr. Through direct interaction with P-gp, Schizandrin B reduces drug efflux activity and thus completely restores the ability of the tumor cells to accumulate drugs.

5. Studies of TCM components as P-gp reversal agents in tumor-bearing rat models

TCM components can also reverse P-gp mediated MDR *in vivo* as demonstrated in studies conducted on tumor bearing rats (45,51). The chemosensitizing abilities of TCM components have been analyzed by studying changes in cell protein levels (51-54), tumor growth (55) or survival rate of tumor-bearing rats (56). A TCM component with MDR reversing activity should show an ability to decrease resistance related protein expression levels, to inhibit tumor growth or prolong the survival rate of tumor-bearing rats.

Matrine. In addition to having MDR reversal activity *in vitro* as described above, matrine also has activity in intact mice. Li *et al* (52) emulated the clinical chemotherapy of Cisplatin/5-FU/Cytoxan (PFC) to induce resistance in S_{180} tumors in mice. Following 10 days of continuous lavage with matrine solution, the study obtained S_{180} cells from mouse ascites. The S_{180} cells were then analyzed by flow cytometry, and it was determined that P-gp levels and the drug target topoisomerase II were both reduced. The authors concluded that the MDR reversing activity of matrine was associated with its ability to regulate a variety of drug resistance-related macromolecules.

Cepharanthine. Cepharanthine is a type of bisbenzylisoquinoline alkaloid extracted from the radix, stem or leaves of *Menispermaceae stephania*. It has been reported that cepharanthine hydrochloride can downregulate the ABCB1 gene, and may activate c-Jun/c-Jun N-terminal kinases in K562/ADR cells (53). Due to the difficulty of directly analyzing intratumoral drug concentrations or P-gp changes *in vivo*, Han (54) established an alternative surrogate method that took advantage of the presence of P-gp in peripheral $CD8^+$ lymphocytes. Thus, P-gp activity was measured in $CD8^+$ peripheral lymphocytes rather than in whole tumor bearing

Table I. Selected examples of P-gp multi drug resistance reversal agents.

Generation	Representative agent	Characteristics	Shortcomings
1st	Verapamil, cyclosporin A, auinidine	Competitive inhibitors of P-gp	Weakly competitive, no target specificity, adverse side effects
2nd	R-verapamil, cyclosporin A derivatives, quinidine analogues	Often structural analogs of first generation of reversal agents	Alters metabolism/pharmacokinetics of chemotherapeutic agent
3rd	XR9576, LY335979, R101933, Tariquidar	Designed according to structure or activity predictions; more specific, non-competitive P-gp inhibitors	May inhibit certain P-gp pump functions in normal cells; may alter pharmacokinetics

P-gp, P-glycoprotein.

rats. The mean fluorescence intensity (MFI) of $CD8^+$ cells incubated with the fluorescent P-gp substrate Rhodamine123 was used as a measure of P-gp activity, and used to study the MDR reversing effect of cepharanthine hydrochloride *in vivo*. Different doses of cepharanthine (2.5, 5.0 and 10.0 mg/kg), verapamil (5.0 mg/kg; positive control) and saline (vehicle control) were injected in hepatoma tumor-bearing mice (Hca/Fap), which had received tail-vein injections of Rhodamine 123. The MFI was 8.6±0.4 in the absence of cepharanthine or verapamil. Following cepharanthine treatment, the MFI in $CD8^+$ peripheral lymphocytes increased in a dose-dependent manner to 18.9±0.8, 13.1±0.8 and 11.9±0.4, respectively; following treatment with verapamil, the MFI was 10.2±0.2. The differences among the groups were statistically significant ($P<0.05$), indicating that cepharanthine can reverse MDR by inhibiting the P-gp activity.

Curcumin. In a study by Lu *et al* (55), human colon tumor HCT-8/VCR cells were implanted in nude mice to establish MDR tumor-bearing mice. The mice were then divided into 4 groups, and administered saline (vehicle control), vincristine (VCR) alone, curcumin alone, and VCR + curcumin together, respectively. The tumors were excised and weighed following 2 weeks of treatment, and RT-PCR and immunoblotting were used to detect levels of ABC1 and survivin mRNAs as well as P-gp and survivin proteins in tumor tissue, respectively. The tumor mass and levels of ABCB1 mRNA, survivin mRNA and P-gp and survivin proteins in the VCR/curcumin combination group and the curcumin alone group were significantly lower than the control (saline) group and VCR alone group, indicating that curcumin is more effective than VCR in MDR. Other studies have also shown that curcumin could inhibit the migration and invasion of Hca-F cells (57), and induce apoptosis in gallbladder carcinoma GBC-SD cells (58).

Other TCM components. Diallyltrisulfide could overcome P-gp-mediated MDR in K562/A02 cells by the downregulation of nuclear factor-κB (NF-κB)/p65 (59). In addition, grape seed procyanidin (GSP) belongs to a class of polyphenol flavonoid compounds that significantly increase the efficacy of paclitaxel and adriamycin in A2780/T cells by blocking the function of P-gp and inhibiting the transcription of ABCB1. Additionally, GSP suppressed the NF-kB activity and mitogen activated protein kinase (MAPK)/extracellular signal-related kinases (ERK) pathway mediated YB-1 nuclear translocation, which may be associated with the downregulation of P-gp (60).

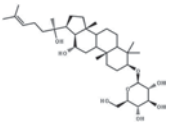
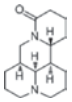
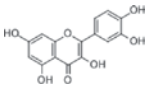
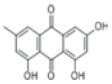
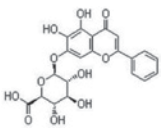
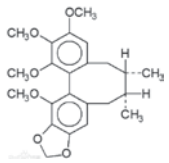
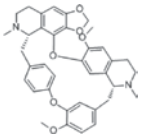
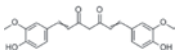
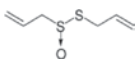
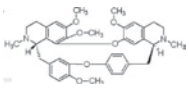
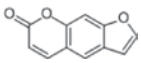
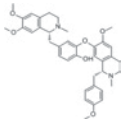
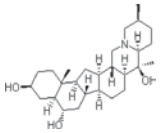
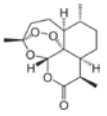
Annonaceous acetogenins can also reverse MDR by reducing P-gp pump function and increasing the intracellular concentration of chemotherapeutic drugs. Thus, 15 annonaceous acetogenins demonstrated significant inhibitory activities against MCF-7/ADR cells; among them, annotemoyin-1 was 190X more active than verapamil (61).

Tetrandrine also significantly reduced P-gp expression in a concentration-dependent manner, and thus can reverse MDR by increasing the intracellular concentration of anticancer drugs (62,63). In addition, the study demonstrated that H1 (a novel derivative of tetrandrine) inhibited P-gp expression in a dose-dependent manner by promoting P-gp degradation apparently through decreasing its half-life, which may be associated with a downregulated MAPK-ERK signaling pathway. H1 also inhibited the ATPase activity of P-gp in a dose-dependent manner (64). Psoralen (65), neferine (66,67), peimine (68,69), guggulsterone (69,70) and artemisinin (71) are also reported to reverse MDR by reducing P-gp expression or promoting the ATPase activity of P-gp in drug-resistant tumor cells (for details of TCM components, see Table II).

6. Conclusions

Reversing MDR in cancer is a challenging task, and over the past several decades (72,73), a number of studies have proposed numerous strategies to combat it and 4 generations of small molecule MDR P-gp inhibitors have been reported (17). Among these are a variety of TCM components that have been shown to reverse P-gp mediated MDR by several mechanisms including inhibiting P-gp drug efflux activity, hindering P-gp ATPase activity and reducing P-gp levels by downregulating the expression levels of the ABCB1 gene. Despite these interesting observations, there are still a number of challenges that need to be addressed. Firstly, MDR is the result of numerous biochemical and cellular factors. Therefore, if studies remain narrowly focused on the classical resistance mechanism mediated by P-gp (i.e. a single target), the contributions of other resistance mechanisms will remain unexplored. Secondly, studies to date have mostly used cultured MDR tumor cell model systems. By contrast, studies utilizing tumor-bearing

Table II. Physicochemical characteristics of selected TCM components.

TCM component	Main source	Formula	MWt	Structure
Rh_2 ginsenosides	*Panax ginseng*	$C_{36}H_{62}O_8$	622	
Matrine	*Sophora flavescens*	$C_{15}H_{24}N_2O$	248	
Quercetin	Numerous vegetables, fruits and grain	$C_{15}H_{10}O_7$	302	
Emodin	*Rheum rhabarbarum*	$C_{15}H_{10}O_5$	270	
Tetramethylpyrazine HCl	*Ligusticum wallichii*	$C_8H_{12}N_2 \cdot HCl$	172.5	
Baicalin	*Scutellaria baicalensis*	$C_{21}H_{18}O_{11}$	446	
Schizandrin B	*Schisandra chinensis*	$C_{23}H_{28}O_6$	400	
Cepharanthine	*Rheum rhabarbarum*	$C_{37}H_{38}N_2O_6$	606	
Curcumin	*Polygonum cuspidatum*, and *Rheum rhabarbarum*	$C_{21}H_{20}O_6$	368	
Diallyltrisulfide	*Allium sativum*	$C_6H_{10}S_2O$	162	
Tetrandrine	*Stephania tetrandra*	$C_{38}H_{42}N_2O_6$	622	
Psoralen	*Psoralea corylifolia*	$C_{11}H_6O_3$	186	
Neferine	*Nelumbo nucifera*	$C_{38}H_{44}N_2O_6$	624	
Peimine	*Fritillaria thunbergii*	$C_{27}H_{45}NO_3$	431	
Artemisinin	*Artemisia annua*	$C_{15}H_{22}O_5$	282	

TCM, traditional Chinese medicine; MWt, molecular weight.

animals are relatively rare, and clinical studies with human patients even rarer. In addition, the majority of studies use TCM components in their purified forms, and studies of TCM components in relevant and appropriate formulations should also be examined.

To date, no single compound (or combination of compounds) has been used as a MDR reversal agent successfully in the clinic. However, as TCM components with higher efficacy and lower toxicity, and with confirmed MDR reversing mechanisms are identified, it remains a promising prospect. Additional studies are also required to explore the influence of TCM components on pharmacokinetic processes *in vitro* and *in vivo* in order to choose the optimal formulation and dosage for clinical cancer treatment.

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