Application of alkaloids in reversing multidrug resistance in human cancers

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[ABSTRACT] Multidrug resistance (MDR) in human cancer is one of the greatest challenges in cancer therapy. Natural products, especially the alkaloids, exert reversed effects on MDR with low toxicity, by interacting with various targets. In this review article, we summarize the recent progress made in the research of the main alkaloids, including classification, function, mechanism, research status, and application in reversing MDR.

[KEY WORDS] Multidrug resistance; Alkaloids; Cancer

[Introduction] Nowadays, cancer is one of the leading causes of human death, and chemotherapy-based comprehensive treatment is the main measure in clinical management of cancer. Along with the sustainable development of science and medical technology, many modified and new methods have been used to treat these patients; some of them have a relatively good therapeutic effect and prognosis, but there are still more than 50% of the tumor rapidly becomes multidrug-resistant to chemotherapeutic agents, unable to obtain satisfying results [1]. Domestic and foreign researchers have been committed to investigating the mechanism for multidrug resistance (MDR) and developing reversal agents; several drugs, such as verapamil and cyclosporine have been shown to have certain effects in reversing MDR. Unfortunately, these drugs are impotent for clinical using due to their required high concentration, high toxicity, and simplistic target. Natural products have garnered increased attention in cancer drug discovery field because of the superiorities of rich resources, fewer side effects, diversity of chemical elements. They have great potential in finding more applicable drugs with high efficiency and low toxicity, providing a promising and broaden prospect in searching novel cancer chemotherapeutics [2].

Many natural products have exerted vital effects on reversing MDR, such as alkaloids, terpenoids, flavonoids, coumarins, and lignin [2], and alkaloids are the most concerned with apparent functions by targeting the physiological capabilities of cancer cells, which accounts for the largest part in the research field.

Mechanism of MDR

Multidrug resistance (MDR) refers to the cross-resistance of cancer cells to various anticancer drugs with different structures and mechanisms after they are exposed to anticancer drugs and develop resistance. Additionally, the mechanism of MDR in the cancer is complex and diverse, which mainly relates to the following aspects [3-4].

Decrease in intracellular accumulation of chemotherapeutics

High expression of transmembrane transporter ATP-binding cassette transporters super family (ABC) directly delivers the drug to extracellular compartment by using energy of ATP hydrolysis and resistance-related proteins, such as lung resistance-related protein (LRP), locks the nuclear pole and prevents the entry of anticancer drugs into nucleus, while transporting drugs outside the nucleus [3].

Changed volume and activity of enzymes

GST-π in Glutathione-S-transferase (GST) exerts an op-
positive effect on anti-cancer drugs and inhibits mitogen protein kinase (MAPK). Moreover, the drug-resistant mechanism of topoisomerase II relates to the mutation of ATP/DNA-binding site, intracellular reduction of concentration and activity, and increased levels of phosphorylation. Additionally, glucosylceramide synthetase and PKC may link to the cell differentiation and apoptosis.

**Abnormal control of apoptosis-related genes**

Most chemotherapeutic drugs exert anti-cancer effect by inducing apoptosis, and both overexpression and lack of anti-apoptosis gene can lead to MDR, including p53 (divided into wild and mutant types; wild one as cancer suppressor gene mainly regulates cell proliferation and apoptosis, inhibits malignant proliferation and maintains the growth of normal cells. Mutant type of p53 as an oncogene, loses regulation of cell growth, apoptosis, and DNA repair, which inhibits chemotherapy-induced apoptosis, resulting in MDR), Bcl-2 (an anti-apoptotic gene in the BCL family and its overexpression could promote cell living and inhibit apoptosis induced by chemotherapeutics) and so on [6].

**Multidrug-resistant signaling pathways of cancer**

P13K/Akt (phosphatidylinositol-3-kinases/Akt) is one of canonical signaling pathways in inhibiting apoptosis, and MAPK transport signal from the surface into the nucleus of the cell, including ERK1/2, JNK/SPAK, P38MAPK, and ERK5/BMK1 [7].

### Table 1 Main classification of natural products and their mechanism of reversing MDR

<table>
<thead>
<tr>
<th>Classification</th>
<th>Compounds</th>
<th>Resource</th>
<th>Function</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoquinoline</td>
<td>Berberine</td>
<td>Coptis japonica</td>
<td>Anti-inflammatory Analgesia</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp)] decreasing Enzyme activity (PKC)</td>
<td>[13, 97]</td>
</tr>
<tr>
<td></td>
<td>Tetrandrine</td>
<td>Stephenie tetrandra</td>
<td>Anti-inflammatory Analgesia</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp)]</td>
<td>[28, 96]</td>
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<td></td>
<td></td>
<td></td>
<td>Decreasing Enzyme activity (PKC)</td>
<td>Regulation of signaling pathway [nuclear factor (NFκB)] apoptosis induction</td>
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<td></td>
<td></td>
<td></td>
<td>miRNA regulation</td>
<td></td>
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<tr>
<td></td>
<td>Camptothecin</td>
<td>Camptotheca acuminata</td>
<td>Anti-cancer</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp)]</td>
<td>[39-40]</td>
</tr>
<tr>
<td>Indole</td>
<td>Evodiamine</td>
<td>Evodia rutaecarpa</td>
<td>Anti-cancer Low pressure; Bringing down the fever Analgesia Anti-inflammatory Anti-allergy</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp)] Regulation of apoptosis–related genes (Bcl-2) Regulation of Enzyme activity (Raf-1 kinase) Signaling pathway (P13K/AKT/mTOR)</td>
<td>[41-42, 44]</td>
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<td></td>
<td>Antofine</td>
<td>Cynanchum paniculatum</td>
<td>Analgesia Low blood lipids Reducing the heart rate Anti-inflammatory Anti-allergy</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp)] decreasing the expression of the MDR-1 gene</td>
<td>[47]</td>
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<tr>
<td>Piperidine</td>
<td>Piperine</td>
<td>Piper nigrum</td>
<td>Sedative hypnosis Anti-bacterial Analgesia Bringing down the fever Anti-oxidation</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp), MDR-related protein (BCRP, MRP1)] Decreasing the expression of gene (ABCB1, ABCG1, ABCG2)</td>
<td>[49-50, 98-99]</td>
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<tr>
<td></td>
<td>Sophora flavescens</td>
<td>Sophora flavescens</td>
<td>Anti-inflammatory Anti-allergy</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp), MDR-related protein (LRP, MRP, MDR1)] Increasing the expression of apoptosis-related genes (Fas/Fas L, Bax/Bcl-2, Caspase, p53, Survivin) Decreasing the expression of apoptosis-related proteins (P170) Regulation of Enzyme activity (TOPO II)</td>
<td>[55, 100]</td>
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<tr>
<td></td>
<td>Sophora flavescens and S. subprostrata</td>
<td></td>
<td>Anti-inflammatory Anti-allergy Bringing down the fever Diuresis Insecticide</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp), MDR protein (LRP)]</td>
<td>[54, 67]</td>
</tr>
</tbody>
</table>
### Classification

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Resource</th>
<th>Function</th>
<th>Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harmine</td>
<td>Peganum harmala</td>
<td>Anti-cancer</td>
<td>Regulating the expression of apoptosis-related genes (COX-2, PCNA, Bcl2, MPP-2, Bax)</td>
<td>[71, 101]</td>
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<td></td>
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<td>Lower the blood glucose</td>
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<td>Anti-leukemia</td>
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<td>Insecticide</td>
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<td>Antibacterial</td>
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<td>Lobeline</td>
<td>Obelia</td>
<td>Exciting the central nervous system</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp)]</td>
<td>[72]</td>
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<tr>
<td>Organic amines</td>
<td>Tetramethylpyrazine</td>
<td>Improving blood circulation</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp), MDR-related protein (MRP1, MDR1, MRP2, MRP3, MRP5)]</td>
<td>[66-75, 77]</td>
</tr>
<tr>
<td></td>
<td>Szechwan lovage</td>
<td>Bringing down the fever</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp), MDR-related protein (MRP, LRP)]</td>
<td>[36, 82]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Clearing up the phlegm to stop coughing</td>
<td>Inhibiting the expression of ERCC1m RNA Apoptosis promotion</td>
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<td></td>
<td></td>
<td>Anti-cancer</td>
<td>Regulating the expression of apoptosis-related genes (Bax, Bcl-2)</td>
<td>[85]</td>
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<td>Insecticide</td>
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<td>Antibacterial</td>
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<td></td>
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<td>Bringing down the fever Analgesia</td>
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<tr>
<td>Purine</td>
<td>Theophylline</td>
<td>Tea</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp)]</td>
<td>[86]</td>
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<td></td>
<td></td>
<td>Cardiotonic</td>
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<td>Diuresis</td>
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<td>Vasodilation</td>
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<tr>
<td>Pyrrole</td>
<td>Lamellarin</td>
<td>Ianthella sp.</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp), MDR-related protein (MRP1, BCRP)]</td>
<td>[88]</td>
</tr>
<tr>
<td>Tropolium</td>
<td>Pervilleine</td>
<td>Erythroxylum pervill</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp)]</td>
<td>[91-92]</td>
</tr>
<tr>
<td>Acridone</td>
<td>Arborinina</td>
<td>Opiliaceae</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp)]</td>
<td>[93]</td>
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<td></td>
<td></td>
<td>Anti-cancer</td>
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<tr>
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<td></td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp), MDR-related protein (MDR1)]</td>
<td>[95-96]</td>
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<td></td>
<td>Anti-cancer</td>
<td>Increasing intracellular accumulation of chemotherapeutics [Transmembrane transporter (p-gp), MDR-related protein (MDR1)]</td>
<td>[102]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-cancer</td>
<td>Decreasing the expression of apoptosis-related proteins (NF-kB p65)</td>
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<td>Regulating the expression of apoptosis-related genes (Bax, Bcl-2, Bak, Bim)</td>
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### Application of Alkaloids in Reversing MDR

#### Isoquinolines

**Berberine (BBR)**

Berberine, a benzylisoquinoline alkaloid, has been reported as a promising drug to reverse MDR in many studies. For instance, the IC_{50} values of adriamycin alone and BBR in combination in K562/A02 cells were 25.70 and 0.72 μg·mL⁻¹, respectively. In the presence of BBR at 20 and 40 μg·mL⁻¹, the sensitivity of K562/AO2 cells was improved by 20.56 and 35.69 times and the accumulation of adriamycin in cells was risen by 1.5 and 2.5 times, assuming that BBR could remarkably reverse the multidrug-resistant activity of K562/A02 cells [8].

Similar results were subsequently observed in K562/DOX under the nontoxic dose (< 1 μmol·L⁻¹), which had a 1.5-fold reduction in IC_{50} and increased the intracellular accumulation of DOX in K562/DOX [9], as well as in breast cancer resistance protein BCRP/ABCG2 and ABCB1 [10]. Moreover, P-glycoprotein (P-gp)/MDR1-expressing CEM (human CCRF-CEM leukemia cell line)/ADR5000 and CEM/ VLB100 were selected by doxorubicin and vinblastine.
respectively, multidrug resistance-related protein 1 (MRP1)-expressing CEM/E1000 was selected by etoposide, which was highly resistant to the corresponding selecting agents. To the MDR was reversed by berberine at 6 folds with minimal degrees in both growth inhibition assay and MTT assay [11].

In addition, the combination of BBR and anti-cancer drugs showed a synergetic effect on MDR. In HCT-8 (Colon cancer cell line)/VCR, the reverse rate of 3.57 was observed when combined with VCR [12]. BBR at different concentrations (0.5, 1, 2, 11, and 22 mol·L⁻¹) significantly increased uptake rate of ADM in HL-60/ADR (human multi-drug leukemia cell line) when combined with DAC [13]. There were similar trends in the combination of BBR and other chemotherapeutic drugs (5-FU, CPT, and TAX), which was more effective in inhibiting the growth of A549 cells [14]. YU Ai-Rong et al. found the combination of BBR and CSA could significantly inhibit the expression of MDR1a and MDR1b genes in CYP3A1 in mice and rats, which decreased the metabolism and elimination of CSA in liver [15]. WU Xiao-Xiang et al. discovered that berberine could increase the sensitivity of lung cancer cells to CDDP by reversing MDR [16]. LIN H-L et al. found not only pgp-170 expression could be induced but also its function could be elevated by berberine in two oral cancer cell lines (OC2, KB), two gastric cancer cell lines (SC-M1, NUGC-3), and two colon cancer cell lines (CT26, COLO 205) [17]. Moreover, cytotoxicity in OC2, SCM1, and COLO 205 cells was induced by the treatment of paclitaxel for 24 h in a concentration-dependent manner. Compared with paclitaxel-treated cells, pretreatment of cells with BBR (100 mg·kg⁻¹) for 24 h before led to increased viability. Berberine hydrochloride was discovered to decrease the expression of lung multidrug-resistant protein (LRP), which interfered the production of MDR by transportation and exocytosis of the vesicle [18].

**Tetrandrine (TET)**

It was reported that more than 1 μmol·L⁻¹ of TET could reverse the MDR at least 10 times in vitro with a plasma peak concentration of about 2 μmol·L⁻¹ and after TET administration (i.p.) at 30 mg·kg⁻¹ until 18 h at a concentration of not less than 1 μmol·L⁻¹ [19]. Much of researches has confirmed the reversal effect on MDR of cancer as follows: Tet was discovered to reverse the expression of MDR-related protein P170 in S180 sarcoma cells, which was related to the reduction of the expression of MDR cell membrane mucin P170, inhibition of chemotherapy induced S180 cells after TOPO II activity and significantly increased chemotherapy-induced multidrug-resistant S180 apoptosis [20].

The similar results were also observed in following studies. In HNE-1/ADM cells, TET at a low toxic concentration showed a 3.5 fold reverse rate to ADM [21]. The inhibitory rates of anticancer drugs such as DDP, 5-Fu, VCR, and ADM were increased when combined with TET, the reverse rate was up to 3.19 times in HNE-1 (200) cell line [22]. The 5% inhibitory concentration of Lo Vo (Colon cancer resistant cell line)/L-OHP cells was 0.45 μg·mL⁻¹ after 48 h of TET as a non-toxic dose. The IC₅₀ of L-OHP to Lo Vo/L-OHP cells was 112.5 ± 23.6 μg·mL⁻¹, and the IC₅₀ values were significantly decreased to 58.9 ± 26.3 μg·mL⁻¹ when TET (0.45 μg·mL⁻¹) was added [23].

The IC₅₀ of DNR on K562/A02 cells was 6.52 ± 0.43 mg·L⁻¹ and the reversal fold was 2.19 ± 0.09. After combined with nilotinib, the IC₅₀ of DNR in K562/A02 cells decreased to 3.12 ± 0.13 mg·L⁻¹, and the reversal rate was 4.58 ± 0.17 [24]. In addition, the effect of reversing the MDR was also confirmed in several cancer resistant cell lines, such as KB-MRP1 (Human epidermal carcinoma cell lines) [25], Hep-2/ADM (Laryngeal cancer resistant cell line) [26], Hep-2/2 (2.22 fold to TET and 1.88 fold to TMP) [27], KBV200 (the concentration of VRP was 25 fold to TET, reverse rate was 45% of that) [28-29], MCF-7/TAM (reverse multiple was 2.0) (human breast cancer resistant cell line) [30] and combined with MAT, which exerted a synergetic effect [31], MCF-7/ADR [32], MCF-7/DOX [33], YES-1-DDP (Human esophageal cancer multidrug resistant cell line) [34], and K562/A02 when combined with DNR (which linked to regulation of glucosylceramide synthase (GCS) and MDR1 genes except p-gp inhibition) [35]. Moreover, it was shown that TET exerted a remarkable reversal effect in P-gp overexpressing Caco-2 (human epithelial colon adenocarcinoma cells) and CEM/ADR5000 (human leukemia lymphoblast cell line) cells. It significantly and synergistically enhanced the cytotoxicity of doxorubicin at the same time [36]. Additionally, some derivatives of TET were discovered as well. H1, a derivative of TET, led to apoptosis of both sensitive and resistant cancer cells and displayed an anti-MDR activity on KB and KBx200 cells in vitro and in vivo, which was linked to the mechanism of morphologic change. Fragmentation of DNA and Erk1/2 and Akt1/2 activation resulted in increased ROS production, rising Bax/ Bcl-2 ratio, loss of mitochondrial transmembrane potential (Δψm), release of cytochrome and AIF from mitochondria into cytosol, and activation of caspase 3 and 9 [37]. Furthermore, BrTET exerted the reversing effect on resistance of K562/ A02 cells to DNR, which increased the accumulation of that in K562/A02 cells by 271% with 2 μmol·L⁻¹ of BrTET. In addition, the protein level of MRP7 dropped by 83.7%, assuming that the activity of reversing pgp-mediated MDR may relate to protein MRP7 [38].

**Quinolones**

**Camptothecin**

A series of novel 7-(N-substituted-methyl)-camptothecin derivatives were observed to be more potent than paclitaxel against the multidrug-resistant KB vin subline, and compounds 9d, 9e, and 9r were more efficient and cytotoxic to cell lines A-549, MDA-MB-231, KB, especially the KB vin [39-40].

**Indole**

**Evodiamine (EV)**

Evodiamine could effectively reverse the MDR of lung
cancer resistant strain A549/DDP to cisplatin, and the resistant-fold was 8.075 times. The reversed capability of drug resistance of A549/DDP cells was 3.668 and 11.48 respectively after adding 0.125 and 0.25 mg·L⁻¹ EV, which significantly increased the sensitivity of A549/DDP to chemotherapeutic drugs [41]. LI Xiao-Peng et al. [42] reported that EV could reverse MDR of human leukemia cell line K562/ADR to DNR, which was also proven in human breast cancer cells [43]. LIAO Cho-Hwa et al. discovered that EV exhibited the activity of anti-cancer and reversing MDR in adriamycin-resistant human breast cancer NCI/ADR-RES cells in a time- and concentration-dependent manner [44].

Moreover, EV displayed cytotoxicity in HCT 116 (p53+) colon cancer cells and its knock out clone HCT 116 (p53−/−), with the IC50 values ranging from 6.11 μmol·L⁻¹ [towards HCT116 (p53+/+) cells] to 80.99 μmol·L⁻¹ [towards HepG2 cells] [45]. Wei et al. also evaluated the effects of EV on multidrug-resistant pancreatic cancer cell and L5178 mouse lymphoma cells, which could inhibit MDR [46].

**Antofine**

Eun-Hye Kim et al. established the paclitaxel-resistant cell line A549-PA, which was created by the parental A549 cells. The concentration of paclitaxel was gradually increased for about 5 months, and the drug resistance was 200 times that of the parental cells. It was shown that antofine could inhibit the growth of A549-PA cells with similar IC₅₀ to that of parental A549 cells and inhibit the growth of drug-resistant cancer cells alone or combined with conventional cancer chemotherapeutics [47].

**Piperidine**

Piperine

It was shown that piperine could enhance the activity of the anticancer drug in various drug resistant cancer cells [48]. ZHOU Yi et al. found that piperine augmented the cytotoxicity of DOX in a concentration-dependent manner after a 72-h incubation in MCF-7/DOX cells [49]. The inhibition also was shown in the efflux of doxorubicin and mitoxantrone in MCF-7 and A549 cancer cells, which reduced the IC₅₀ values of doxorubicin against MCF-7 and A549 cells from 40.5 and 4.43 to 1.26 and 0.31 mol·L⁻¹, respectively, and the IC₅₀ values of mitoxantrone against MCF-7 cells from 37.72 to 5.41 μmol·L⁻¹ with 50 μmol·L⁻¹ of piperine [50]. OKURA Takashi et al. reported the effect of inhibition of P-glycoprotein-mediated efflux in Caco-2 and KB-C2 without increasing P-glycoprotein-expressing cells, but significantly increased mRNA expression of MRP3 by using LS-180V cells [51].

Other studies have shown that piperine had certain degrees of effects on resistance to MCF-7/ADM cells at different concentrations, and the nontoxic dose ranged from 60 to 100 μmol·L⁻¹, which was in dose-dependent manner [52].

**Matrine (MAT)**

It was reported that the combination of matrine and paclitaxel was more sensitive than paclitaxel alone in HS20/TAX25, the reverse-fold was 17.4 [53]. LI Gui-Hai et al. found that matrine could significantly inhibit the expression of P170 (cell membrane mucin) in S180 cells after chemotherapy induction (PFC: DDP + 5-FU + CTX) [54]. The combination of ATRA and MAT could significantly decrease the NBT-positive reduction rate of multidrug-resistant acute promyelocytic leukemia (APL) cell line, indicating that MAT could promote the differentiation of NB4-R1 cells (MAT-resistant APL cell line) in vitro and reverse MDR to some extent [55].

Many studies also confirmed the effect of MAT on MDR in the cancer. One of them was to inhibit the expression of p-gp in multidrug-resistant cancer cell lines, such as human bladder cancer [56], T24/ADM [57], BIU-87/ADM [58], Sensitive rat hepatoma cell CRBH-7919/MDR1 [59], lung cancer cell QGY/CDDP (reversal multiple to CDDP, 5-FU, VCR, MMC, MTX were 1.900, 1.108, 1.500, 1.40, and 1.020, respectively) [60], and human colon cancer cell HT-29/OXA [61]. The other reversed mechanisms were via inhibition of proteins and signaling pathways, such as human nasopharyngeal carcinoma HONE1/DDP (reversal multiple 1.45), breast cancer MCF-7/ADR [62], MCF-7/DOX [63], and had a synergetic effect when combined with other anti-cancer drugs.

The inhibitory rate of stomach cancer SGC7901/DDP-resistant cells was increased after the intervention of VCR, ADM, 5-FU with different concentrations (0.01, 0.1, 1, 10, and 100 times PPC) when combined with MAT (5 mg·L⁻¹). The expressions of mir-7, mir-125b, mir-200a, mir-200c, and mir-146a were significantly higher than that of VCR, ADM, and 5-FU alone, which was a dose-dependent regulation [64]. SUN Ting-Li et al. showed that matrine (0.5, 1.0, and 2.0 mg·mL⁻¹) was used as a reversal agent to reverse the MDR human gastric cancer cell line SGC7901/VCR with a reversed rate of 9.4, 13.4, and 24.5, respectively [65].

Additionally, there is a great number of studies in reversing the effect of the marine on human leukemia cell lines. Wang S et al. found that marine could partly reverse the MDR in K562/A02, increasing the reversing effect and enhancing toxicity when combined with a progesterone [66].

**Oxymatrine (Oxy)**

Oxymatrine inhibited the expression of P-gp in nasopharyngeal carcinoma (HNE-1) cell line and HNE-1 (200) which was induced by highly energetic radiation. The cytotoxicity was similar to that of VRP, and the inhibitory rate of HNE-1 (200) cells was increased by 287.13%, 293.02%, and 144.69%, respectively when combined with VCR [67]. Moreover, The IC₅₀ of ADM alone to multidrug-resistant MCF-7/ADM was 38.6 μg·mL⁻¹, and the reversal multiple was about 45.4, which was dropped to 11.8 μg·mL⁻¹ after adding Oxy (0.8 mg·mL⁻¹) with reversal multiple at 3.27 [68]. PENG Xiang-qian et al. found Oxy exerted a toxic effect on K562/A02, and the reversal multiple was 2.62 [69].

**Carbazole**

Pinnacles, one of β-carboline alkaloids, can re-sensitize
mitoxantrone and camptothecin resistance with non-toxic concentrations (1 or 5 μmol·L⁻¹, respectively) interacting with Breast Cancer Resistance Protein (BCRP) [70].

**Harmane (HM)**

HM was reported to inhibit the growth of human stomach cancer cells in vitro and in vivo [71]. Recent research found that the IC₅₀ was significantly lower than that of the control group with the combination of HM, 5-FU, and DDP in vitro and the sensitization ratio was 1.47 and 5.78 in human hepatocellular carcinoma cell line HepG2 [72].

**Lobeline**

WU Min et al. and MA Yong-gang et al. [70, 72] confirmed that lobeline could reverse the MDR of human colon cancer cell HCT-8 to VCR and 5-FU. Ma et al. also indicated that lobeline could increase the concentrations of rhodamine 123 in Caco-2 cells and p-gp overexpressing cell lines CEM ADR5000, which significantly reduced the IC₅₀ values of ADR by 3.5 and 2.5 times, respectively. Meantime, the same concentration of VCR and 5-FU showed a stronger toxic effect on colon cancer cell Hct-8/VCR under the nontoxic concentration of lobeline, and more shrinkage cells and cell debris were observed. Compared with the untreated cells, the resistance of HCT-8/VCR cells was partially reversed, the reversal factor of lobeline to VCR and 5-FU were 3.65 and 1.35, respectively. CHEN Jia et al. [73] found that lobeline could reverse human breast cancer cells MCF-7/ADM to ADM and 5-FU, and the reversal multiples were 2.68 and 1.37 respectively, which was also discovered in stomach cancer cells SGC7901/VCR.

**Organic amines**

**Tetramethylpyrazine (TMP)**

Teng Zuo et al. [74] found that TMP could inhibit the expression of P-glycoprotein in human breast cancer cell line MCF-7/DOX. CUI Xiao-Bo et al. [75] discovered that TMP could increase the sensitivity of multidrug-resistant rectal cancer cells to chemotherapeutics when combined with 5-FU. TANG Xiao-Yong et al. [76] found that the inhibitory rate of A549/DDP cells increased with the increase of DDP concentration, and the reversal ratio was 3.62. Moreover, TMP was capable of reversing the MDR of human hepatocellular carcinoma cells BEL-7402/ADM in response to ADM [77], and TMP reversed the MDR of BEL 7402/ADM cells by 9.23-fold at the concentration of 600 μmol·L⁻¹ in vitro [71]. TMP was shown to enhance the cytotoxicity of anticancer drugs by reversing MDR of bladder cancer cell lines Pumc-91/ADM and T24/DDP cells in response to ADM, with a dose-dependency [78]. ZHANG Yin-Xu et al. [79] found that the resistance of MCF-7/dox cells to VCR, DOX, and TAX could be reversed in the presence of 300 mg·L⁻¹ of TMP with the reverse folds of 16.7, 30.6, and 4.2, respectively.

**Steroids**

**Peimine**

HU Kai-wen et al. [80] found that PM1 and PM2 had a significant reversal effect of MDR in malignant cells with non-cytotoxic dose. Peimine could play a significant role in the sensitivity of peimine to MDR cells in vitro, which could improve the reversal effect of ADR on the MDR cell lines K562/A02 and HL-60/ADR with different resistant mechanisms. LI D-Y et al. [81] also found that the reversal multiples of PM1 and PM2 to drug-resistant cell line KBV200 were 2.6 and 3.2. After that, much of corresponding research was also reported. LI Wei et al. [82] selected 30 cases of AL with increased P170, which were randomly divided into treatment group (reversal group) and control group. The control group was treated with conventional chemotherapy regimen (ALL using VDCP or VAMP or the two regimens plus L-asparaginase, and AML using DA or HA or the two regimens plus VP-16). The treatment group was supplemented with peimine. The results confirmed that the CR rate was 55% in the treatment group, while the control group had only 20%. Further, TANG Xiao-Yong et al. [76] also proved that peimine could reverse the MDR of A549/DDP, and the reversed effect of drug resistance in human lung adenocarcinoma cell line A549/DDP was 3.73 via MTT assay, which was positively correlated with the concentration of DDP.

Many reversal effects were discovered in fritillaria. TONG Xiao-Lin et al. [83] showed that peimine (BMJS) and peiminine (BMY5) could reverse the MDR of human breast cancer cell lines MCF to ADM and TAX in vitro. ZHANG Pei et al. [84] discovered that BMSY could significantly increase the sensitivity of multidrug-resistant cells to chemotherapeutics, and the reversed multiples were 16.61, 6.27, 6.47, and 7.18 to VCR, ADR, CDDP and 5-FU, respectively. The combination of BMSY and VCR was observed to inhibit nude mice transplanted tumor SGC7901/VCR and improve the sensitivity to VCR.

**Solanine (SM)**

WAN Pei-Hong et al. [85] found that SM had a wide range of cytotoxic effects on various MDR cell lines and induced K562/A02 cell apoptosis, which not only caused the imbalance between Bax and Bel-2, but also reduced p-gp expression and destroyed microfilm network over the nontoxic concentrations.

**Purine**

**Theophylline**

Theophylline, one of the xanthines, could inhibit the downregulation of MDR membrane protein ABCG2 by inducing its translocation and subsequent lysosomal degradation, inhibiting ABCG2 substrate and sensitizing resistant breast cancer cells with mitoxantrone, commonly used to treat acute myeloid leukemia, metastatic breast cancer and non-Hodgkin's lymphoma [86].

**Pyrryl**

**Lamellarin**

Plisson F et al. reported that several amellarins from two southern Australian didemnum sp. were P-gp substrates
and P-gp inhibitors capable of reversing MDR \[87\]. Moreover, some researches had specifically pointed out lamellarin compounds and derivatives exerted a reversing effect on MDR in cancer. For instance, it exhibited proliferative inhibitory activity against human colon cancer SW260 cell lines \[88\]. Marie Vanhuyse et al. \[89\] also indicated that MDR was not an obstacle to the anticancer activity of the lamellarins, such as lamellarin D, which was toxic to P388 and P388CPT5 cells, compared to CPT, therelative resistance index (RRI) was considerably reduced with LAM-D, which mediated by topoisomerase I, and several DNA-manipulating enzymes are interfered as well. Lamellarin O was reported to reverse the MDR of cancer cell lines as well. Lamellarin O exhibited modest cytotoxicity towards SW620 and SW620 Ad300 cell lines (IC\(_{50} > 22 \mu\text{mol·L}^{-1}\)), which could inhibit the efflux of pump P-glycoprotein \[90\]. Moreover, the cytotoxicity was also detected, measured and quantified in human colon cancer (SW620 and SW620 Ad300), ovarian carcinoma (2008 and 2008/MRP1) and non-small cell lung (NCI-H460 and NCI-460/MX20) cells, with further combination of Hoechst 33342, doxorubicin and mitoxantrone, respectively \[88\].

### Tropinium

**Pervilleine**

It was reported that pervilleine A was effective as an inhibitor of P-gp and exhibited higher activity compared to verapamil \[91\]. Moreover, KB-V1 cells (a model for the discovery of various agents of reversing MDR) was more resistant in inhibiting growth with vinblastine than that of parental KB-3 cells. It also effectively reversed the MDR phenotype with KB-8-5 and CEM/VLB100 cells \[91\]. It also was demonstrated that pervilleines B and C (PB and PC), which were obtained from a chloroform extract of the roots of *Erythroxylum pervillei* as two new tropane alkaloid aromatic esters, could restore the vinblastine (VLB) sensitivity of cultured multidrug-resistant KB-V1 cells with IC\(_{50}\) values being 0.17 \(\mu\text{mol·L}^{-1}\) in each case. Moreover, the rate of inhibition was up to 77.7% when combined with vinblastine \[92\].

### Acridone

**Arborinine**

Rethy B et al. \[93\] evaluated the acridone alkaloids for reversing activity of MDR (MDR) in human P-gp-transfected L5178 mouse lymphoma cells and found that arborinine could increase the intracellular anticancer drug levels by modulating the P-gp activity at the mRNA level. Kuete V also reported that arborinin displayed cytotoxic effects towards a panel of MDR cancer cell lines \[94\].

### Others

**Ellipticine**

Ellipticine is one of the alkaloids fused by isoquinoline and indole. HUANG Y et al. \[95\] investigated five ellipticines for their effects on MDR1. Two of them met the criteria as the substrate, and three ellipticines met the criteria of inhibitor. Among the three inhibitors, MDR1 efflux was blocked by NSC 86715 in a different mechanism compared to the other two based on compound–compound and compound–gene correlations. S16020-2 was one of the most potent olivacine and ellipticine derivative yet characterized, which was a potent cytotoxic compound, globally as cytotoxic as ADR, and 46 folds more than elliptinium acetate (ELP). P388/ADR-1, P388/ADR, P388/VCR-20, KB-A1, DC-3F/AD, S1/TMDR and COL0320DM cells were more sensitive to S16020-2 than to ADR or ELP, which was linked to p-gp overexpression \[96\].

### Clitocine

Both R-HEPG 2 and MES-SA/Dx5 cells showed the excessive expression of p-gp, and P-gp expression in the two cell lines could be down-regulated by clitocine \[93\]. These two cells are much less sensitive to doxorubicin than the parent cells \[97\]. However, P-gp level in R-HepG2 cells appeared to be much higher than that in MES-SA/Dx5 cells and clitocine exerted more effective regulatory activity in the former \[97\].

### Conclusion

Traditional Chinese medicine and natural medicine have their unique advantages. The compounds with MDR reversal activity are also found to cover almost every type of natural products and have their own structural characteristics and alkaloids account for a large part of these compounds. The current studies focus on the monomer and its derivatives or extracts in cells and animal experiments. In comparison, the clinical trials are far less with insufficient scientific basis. Therefore, it is crucial to find more effective and less toxic compounds and explore their pharmacological mechanisms through using modern technologies to provide more helpful therapeutics reversing MDR to the clinic.

### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>ADM</td>
<td>Doxorubicin</td>
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<tr>
<td>ADR</td>
<td>Adriamycin</td>
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<td>ATRA</td>
<td>All-trans retinoic acid</td>
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<td>BBR</td>
<td>Berberine</td>
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<tr>
<td>BrTet</td>
<td>5-Bromotetrandrine</td>
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<td>CDDP/DDP</td>
<td>Cisplatin</td>
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<td>CPT</td>
<td>Camptothecin</td>
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<td>CSA</td>
<td>Cyclosporin</td>
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<tr>
<td>CTX</td>
<td>Cyclophosphamide</td>
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<td>DEC</td>
<td>Decitabine</td>
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<td>DNR</td>
<td>Daunorubicin</td>
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<td>DOX</td>
<td>Doxorubicin</td>
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<td>EV</td>
<td>Evodiamine</td>
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<td>5-FU</td>
<td>Fluorouracil</td>
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<td>HM</td>
<td>Harmine</td>
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<td>L-OHP</td>
<td>Oraliplatin</td>
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<td>MAT</td>
<td>Matrine</td>
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<td>NF-Kb</td>
<td>Nuclear transcription factor</td>
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<td>Oxy</td>
<td>Oxymatrine</td>
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<td>P-gp</td>
<td>P-glycoprotein</td>
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<td>SM</td>
<td>Solanine</td>
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<td>TAM</td>
<td>Tamoxifen</td>
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<td>TET</td>
<td>Paclitaxel</td>
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<td>TBP</td>
<td>Tetramethylpyrazine</td>
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<td>VCR</td>
<td>Vincristine</td>
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<td>VRP</td>
<td>Verapamil</td>
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