



Multidrug-resistant cancer cells and cancer stem cells hijack cellular systems to circumvent systemic therapies, can natural products reverse this?

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Abstract Chemotherapy is one of the most effective and broadly used approaches for cancer management and many modern regimes can eliminate the bulk of the cancer cells. However, recurrence and metastasis still remain a major obstacle leading to the failure of systemic cancer treatments. Therefore, to improve the long-term eradication of cancer, the cellular and molecular pathways that provide targets which play crucial roles in drug resistance should be identified and characterised. Multidrug resistance (MDR) and the existence of tumor-initiating cells, also referred to as cancer stem cells (CSCs), are two major contributors to the failure of chemotherapy. MDR describes cancer cells that become resistant to structurally and functionally unrelated anti-cancer agents. CSCs are a small population of cells within cancer cells with the capacity of self-renewal, tumor metastasis, and cell differentiation. CSCs are also believed to be associated with chemoresistance. Thus, MDR and CSCs are the greatest challenges for cancer chemotherapy. A significant effort has been made to identify agents that specifically target MDR cells and CSCs. Consequently, some agents derived from nature have been developed with a view that they may overcome MDR and/or target CSCs. In this review, natural products-targeting MDR cancer cells and CSCs are summarized and clustered by their targets in different signaling pathways.

Keywords Cancer therapy · Drug treatment · Drug efflux pumps · Cancer-initiating cells · Multidrug resistance · Cancer stem cells · Natural products

Introduction

Cancer is a complex array of diseases with a morbidity of around 7.5 million deaths per year worldwide, and these numbers are rising, owing partly to aging populations and environment pollution [1]. Chemotherapy using anti-cancer agents with structural and functional diversity has been developed to treat cancers. These drugs have been applied alone or in combination to prolong life or to alleviate the symptoms of cancer for decades. However, chemotherapy has failed to completely eradicate cancers for several reasons. Multidrug resistance (MDR) is one of the major obstacles for chemotherapy. MDR is a phenomenon, in which cancer cells become resistant to mechanistically and structurally unrelated anti-cancer drugs [2]. Various factors can contribute to MDR, including inappropriate drug delivery and genetic alterations, that prolong cell surviving [3]. The overexpression of drug efflux pumps, such as the ATP-binding cassette (ABC) transporter family of proteins, is one of the major factors that confer drug resistance [2]. It is the largest transporter superfamily which exports specific molecules through cell membranes. For example, the activation of one of the ABC transporter P-glycoproteins (P-gp), the product of the *MDR1* (also referred to as *ABCB1*) gene, can cause active drug release from cells [4]. The overexpression of ABC transporters can reduce the cellular concentration of the drugs, such as the well-known anti-cancer compounds vinblastine, vincristine, and doxorubicin (DOX) [5–7]. Therefore, ABC transporters play an important role in the development of drug resistance in cancers.

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Cancer stem cells (CSC) are another obstacle for chemotherapy. A large body of evidence indicates that there exists a small subset of tumor cells within a cancer population which are heterogeneous in proliferation rates and clonogenic potency (reviewed in [8]). This subset of tumor cells was termed cancer stem cells. It is believed that this subset of cells has the capacity of self-renewal, differentiation, cancer relapse, and tumor metastasis [9]. Subsequently, more and more CSCs were identified in various tumors, including colorectal cancer [10], breast cancer [11], prostate cancer [12], colon cancer [13], and brain cancer [14]. How do CSCs drive the failure of cancer therapy? CSCs share the capacity of self-renewal and differentiation with normal stem cells and can be characterised by the expression of specific stem-cell markers. Some cell-surface markers used to identify and enrich the CSCs are also markers for normal stem cells. For example, CD133 was characterised as a marker for the identification of brain CSCs [15] and colorectal carcinoma [16], but it is also a marker of normal neural stem cells [17] and probably pancreatic stem cells [18]. Therefore, CSCs might share properties with normal stem cells, including the expression of some specific ABC drug transporters, prosurvival and anti-apoptotic molecules, and other factors. This review discusses how CSC and chemoresistance may be correlated. The following sections give a brief outline of some of the mechanisms utilized by CSC and MDR cells with a view to highlighting cellular pathways that can be targeted by natural products and their derivatives in the fight to halt cancer progression (Fig. 1).

The ABC transporter family

The ABC transporter family acts by pumping drugs to either the external leaflet of the membrane or the extracellular domain and requires ATP hydrolysis to do so [4]. To date, 49 human ABC genes have been identified and were clustered in seven subfamilies (ABCA–ABCG) according to the homology of amino-acid sequences, the constitution of transmembrane (TM) domains, and ATP-binding sites [19]. There are three major transporters correlated with MDR, including P-glycoprotein (MDR1/ABCB1), MDR-associated protein (MRP/ABCC1), and breast cancer resistance protein (BCRP/ABCG2) [3], and these are discussed in the following sections.

P-glycoprotein in cancer (ABCB1)

P-glycoprotein (P-gp), a 170 kDa TM phosphoglycoprotein, is one of the most important ABC transporters and its overexpression is directly associated with MDR in humans. Similar to several other ABC transporter members, P-gp

consists of two TM domains, each of which comprises six helices and two ATP-binding sites. Two paralogs, expressed by the *MDR1/ABCB1* and *MDR2/3/ABCB4* genes, have been identified so far. The P-gp expressed from *MDR1/ABCB1* was initially demonstrated to have a protective role from toxins in susceptible tissues, such as the brain, inner ear, testis, and mammary tissue [20–22]. When substrates or drugs enter the binding site, ATP will be hydrolyzed by ATPase to supply the energy to release the substrates/drugs to the outer leaflet or the extracellular space. There are three patterns that modulate the ATPase activity of P-gp by drug substrates and modulators; many drugs stimulate the activity of ATPase at low concentrations while inhibiting activity at higher concentrations, other drugs can only inhibit the activity of ATPase, whereas some of the drugs stimulate the ATPase activity. P-gp can transport a large range of compounds from chemotherapeutics to peptides; however, priority is given to amphipathic and relatively hydrophobic drugs, including the large complex drugs, such as paclitaxel, vinblastine and small molecules, such as daunorubicin and DOX. Most of the commonly recognized constitutions of the substrates of P-gp contained planar aromatic rings and positively charged tertiary N atoms. Therefore, a therapeutic approach exists which targets P-gp, so that efflux-driven drug resistance of cancer cells could be reversed. In a cellular environment, ABCB1 expression can be regulated by both transcriptional regulation and the translation or stability of the mRNA. For example, P-gp has been shown to bypass translational silencing [23] in specialized Ribonucleoprotein particles RNP called stress granules (reviewed in [24]). ABCB1 can also be regulated via its upstream signaling pathways, such as protein kinase C and mitogen-activated protein kinase cascades, fluxion of Ca^{2+} , and NF- κB [25], and some drugs were developed to regulate these pathways. For instance, curcumin and capsaicin were used to block NF- κB activation. With a greater understanding of the mechanisms of ABCB1 activation, new therapeutics can be developed to target these pathways.

MDR-associated proteins (ABCC1)

The existence of P-gp by itself cannot explain all the occurrences of MDR, since the overexpression of P-gp was not found in all types of MDR cells. Further studies discovered other efflux pumps, such as multidrug-resistance-associated proteins (MRPs) and breast cancer resistant protein (BCRP/ABCG2). The MRP family is comprised of 13 members, 9 of which were associated with drug resistance. MRP1 and MRP9 played the most important roles in drug resistance among the MRPs [26]. MRP1 shares 15 % identity, at the protein level, with P-gp, and consists of one membrane-spanning domain

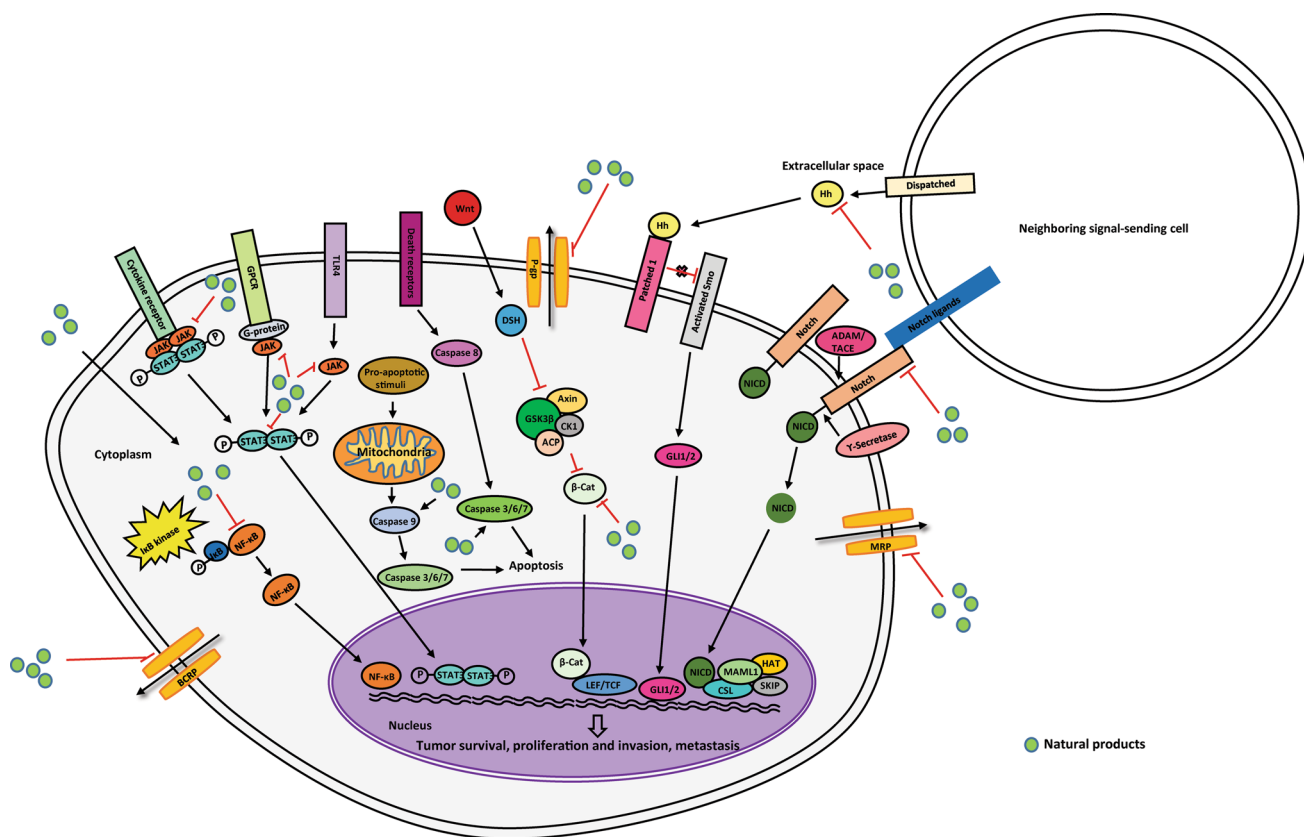


Fig. 1 Depicts the major pathways that are targeted by natural products in multidrug-resistant cancer cells and cancer stem cells. The figure depicts natural products (green circles) interacting with their

target cell-surface receptor (labelled rectangles) or protein (labelled circles) within signaling pathways

(MSD) with five TM helices, and two other MSDs with six TM helices. Subsequently, MRP1 has an overlapping resistance with P-gp. MRP1 is a 190 kDa protein encoded by the *ABCC1* gene, which is expressed in normal tissues, such as lung [27], testis [22], kidney [28], placenta [29], macrophages [30], and skeletal and cardiac muscles [31]. Primarily, the expression of MRP1 can make a contribution to some drug protection in these tissues of humans. For example, the defective expression of MRP1 can enhance the damage caused by etoposide in the mucosa of the oropharyngeal cavity and the seminiferous tubules of the testis [32]. In parallel, the diversity of substrates of MRP1 resulted in decreasing accumulation of drugs, including certain anti-cancer drugs, such as anthracyclines, vinca alkaloids, cisplatin, epipodophyllotoxins, camptothecins, saquinavir, methotrexate, and mitoxantrone [26]. MRP9 is another member within the family which is related to drug resistance and is comprised of two MSD and 12 helices. The high expression of an unusual truncated *MRP9* mRNA was discovered in breast cancer, and MRP9 could be a significant target for breast cancer treatment, because of the relatively low-expression level in normal breast tissue [26].

Breast cancer resistance protein (ABCG2)

Interestingly, the overexpression of P-gp or MRP cannot be detected in the drug-resistant cell line, MCF7/AdrVp. However, a related 2.4 kb mRNA which encodes a 663 amino-acid protein was identified in the cell line and was termed as the breast cancer resistance protein (BCRP/ABCG2) [33]. The G subfamily of ABC transporters is comprised of six half-transporters with a nucleotide-binding domain (NBD) at the N-terminus and a TM domain at the C-terminus [34]. The expression level of ABCG2 mRNA in some tissues may shed some light on its potential function. There were no *ABCG2* transcripts detected in heart, lung, skeletal muscle, kidney, pancreas, spleen, thymus, and peripheral blood leukocytes, while approximately 100 times more expression was detected in placental tissue than that in the brain, prostate, small intestine, testis, ovary, colon, or liver [35]. It appears to play a protective role in human stem cells, because ABCG2 has been reported as a potential phenotypic marker for stem cells in normal lung [36] and breast tissue [37]. Some anti-cancer therapeutics were identified as the substrates of ABCG2, including mitoxantrone, topotecan, irinotecan,

flavopiridol, and methotrexate [38]. In addition, bioflavonoid kaempferol is a substrate of ABCG2 and inhibits ABCG2-mediated quercetin efflux [39]. To overcome the resistance caused by ABCG2, some inhibitors were developed. The first reported inhibitor of ABCG2 was fumitremorgin C (FTC), 5 μ M of which reversed cellular resistance to mitoxantrone, DOX, and topotecan in drug-selected colon carcinoma cells [40]. However, FTC can cause severe side effects, such as neurotoxicity, which precluded its clinical application. Ko143, an analog of FTC, caused an increase in intracellular drug accumulation and reversed mouse BCRP1- and human BCRP-mediated MDR without neurotoxic effects [41]. In recent years, more and more inhibitors have been discovered to reverse the effects of BCRP and these will be discussed below.

The Hedgehog receptor, patched, functions in multidrug resistance

The Hedgehog (Hh) gene was discovered in 1980 in fruit fly [42], and since then, three Hh genes have been identified in mammals, including Desert hedgehog (Dhh), Indian hedgehog (Ihh), and Sonic hedgehog (Shh) [43]. Hedgehog-activated signal transduction occurs via its receptor, patched (Ptch), which is a 12-transmembrane transporter-like protein [44] that has been implicated in multidrug transport in some cancer cell lines [45]. The Hh/patched pathway plays a crucial role in the early embryonic development as well as the tumor development, progression, and metastasis. In particular, the dysregulation of the hedgehog pathway has been observed in a number of cancers, especially in cells showing drug resistance [46]. The analysis of Ptch revealed sequence and topology conservation with the resistance-nodulation-division (RND) family of prokaryotic permeases. Furthermore, the GXXXD motif in the RND bacterial drug efflux pumps was highly conserved in the fourth putative transmembrane segment of human and drosophila Ptch. The RND family transports a broad range of agents, and therefore, Ptch has also been proposed to be an efflux transporter. Not surprisingly, yeast-expressing Ptch showed resistance to certain chemotherapeutics, such as DOX, methotrexate, temozolomide, and 5-FU [47]. Therefore, the development of new agents to target Ptch has been considered as a strategy for the treatment of cancer.

The ABC family in CSCs

The current research trends suggest that several characteristics of CSCs are shared with common features identified in normal stem cells, including the

overexpression of ABC transporter family members, protecting the cells from drugs and the toxins. Glioblastoma CSCs were characterised by the expression level of the MDR gene *BCRP1* [48]. Normal stem cells were believed to possess drug resistance by the expression of ATP-binding cassette transporters, DNA-repair systems, and anti-apoptosis systems to provide protection to these cells. For example, a high level of *ABCG2* expression was detected in hematopoietic stem cells (HSC) as compared with most committed progenitor cells and mature blood cells [49]. *RBM3* is a proto-oncogene that encodes for an RNA-binding protein that promoted a stem-like phenotype and spheroid formation in the colorectal cancer cell line HCT116. The overexpression of *RBM3* also enhanced drug resistance to DOX and paclitaxel by upregulating the gene expression of MRP2 and P-gp [50]. Another ABC transporter member *ABCB5*, identified in oral squamous carcinoma cells (OSCC), was associated with tumor formation, metastasis, and a putative CSC compartment through gene expression studies [51]. In a study of leukemia, leukemia stem cells exhibited MDR by the expression of ABC transporters, such as P-gp, BCRP, and MRP8. This resistance was reversed by the agent salinomycin [52]. The occurrence of ABC expression in CSC and the evidence which suggests that they can be blocked with drugs make this family of genes an interesting target for future lead therapeutics.

Anti-apoptotic pathways in CSCs

Apoptosis can be triggered by the extrinsic and intrinsic pathways. The extrinsic pathway requires extracellular ligands, such as TNF- α , FasL, and TNF-related apoptosis-inducing ligand (TRAIL) binding to the cell-surface receptor, which is termed the death receptor. The intrinsic pathway is activated by the stimuli, such as cytotoxic signals, resulting in cell death. Bcl2 family members function as important regulators of programmed cell death pathways by blocking the extrinsic and intrinsic pathways and inhibiting downstream caspase activity. Bcl2 was a predicted target of miR-1915 in human colorectal carcinoma cells. In accordance with this, it was observed that the increased expression of miR-1915 reduced Bcl2 protein levels and increased cell sensitivity to some anti-cancer drugs [53]. The cellular FLICE-like inhibitory protein (cFLIP) is another regulator of apoptosis and works by blocking the activated death receptor signals, consequently inhibiting the activation of caspase 8. Importantly, cFLIP is not only expressed in many cancer cells but also in CSCs [54], such as glioblastoma stem cells which overexpress cFLIP. A study on glioblastoma CSCs demonstrated that the CD133⁺ cells not only overexpress BCRP1, but also

the anti-apoptosis genes *Bcl2*, *Bcl-X_L*, *XIAP*, and *FLIP*, which have been correlated to their chemoresistance [55]. NF- κ B has been the most extensively examined transcription factors. It controls the expression of more than 500 different products that are relevant to inflammation, cellular transformation, tumor cell survival, proliferation, apoptosis suppression, invasion, and metastasis [56]. NF- κ B signaling is multifunctional in many cells depending on its signaling context. In the immune system, NF- κ B-mediated activation has been potent to inhibit tumor growth, in part through the production of growth inhibitory cytokines [57]. NF- κ B is constitutively active in a wide range of cancers, including acute lymphocyte leukemia [58], chronic myelogenous leukemia [59], prostate [60], and breast cancers [61]. The previous study suggested that the activation of NF- κ B could potentiate the expression level of anti-apoptosis genes, including *Bcl2*, *Bcl-X_L*, *XIAP*, *cFLIP*, *survivin*, *cIAP-1*, and *cIAP-2* [62]. A study on the breast CSCs indicated that blocking NF- κ B signaling by parthenolide (PTL), pyrrolidinedithiocarbamate (PDTC), and its analog diethylthiocarbamate (DETC) resulted in the inhibition of the proliferation of sphere cells [63]. The examples described above are only a snapshot of the roles that anti-apoptotic systems play in cancer CSC survival but provide us with some of the scope of survival mechanisms available to CSC.

Prosurvival signaling in CSCs

In addition to the expression of efflux pumps and anti-apoptotic genes, drug resistance in cancer is also mediated by signaling cascades that control self-renewal, differentiation, and survival, such as the Notch, Wnt, and Hedgehog pathways. Notch induces the high expression of the anti-apoptotic gene *BIRC5* (*survivin*) as well as the upregulation of the cyclin D1 protein [64]. It has been shown that Notch might be involved in the resistance to antitumor agents, such as trastuzumab, a HER2 inhibitor, that was used for the treatment of ErbB-2-positive breast cancer. The resistance to trastuzumab could be prevented and reversed by the inhibition of the Notch pathway [65]. The blockage of Notch by γ -secretase inhibitors (GSIs) in glioblastoma-derived neurospheres reduced tumor growth and expression of CSC markers, including CD133, NES-TIN, BMI1, and OLIG2. Therefore, a high level of Notch expression maintained the CSC properties in CSCs and promoted proliferation, decreased apoptosis, and caused chemoresistance.

Wnt/ β -catenin proteins play an important role in embryonic development and the maintenance of stem-cell properties. Not surprisingly, the β -catenin signaling cascade was also described as an essential pathway for

sustaining the CSC phenotype in cutaneous cancer. In contrast, the depletion of the β -catenin gene led to the loss of CSCs and tumor regression [66]. Furthermore, it was reported that Wnt signaling played an important role in spheroidal CSC cultures that contained heterogeneity [67]. In haematopoietic stem cells, proliferation and self-renewal can be promoted by the overexpression of the β -catenin or stimulation with Wnt protein in vitro [68]. In addition, the expression of Wnt in CSCs derived from the intestine and mammary gland was also essential for their maintenance, which subsequently gave rise to the drug resistance [69]. Thus, the Wnt signaling pathway could be an important target to overcoming drug resistance.

The Hedgehog (Hh) signaling pathway is also critical for the embryonic development, regulating cell proliferation, metastasis, and differentiation in a tightly controlled mechanism. Emerging evidence based on the study of human cancers, such as glioblastoma, breast cancer, pancreatic adenocarcinoma, multiple myeloma, and chronic myeloid leukemia indicated that Hh signaling pathway was involved in the regulation of CSCs undergoing self-renewal and differentiation. Hh-Gli signaling affected stem-like gene expression and self-renewal in glioma CSCs, sustaining glioma proliferation, and survival [70]. The inhibition of the Hh signaling pathway by cyclopamine resulted in the ablation of stem-like cancer cells in glioblastoma [71]. A recent study demonstrated that Hh-Gli1 drove the UDP glucuronosyltransferase (GUT1A)-dependent glucuronidation of ribavirin and Ara-C, leading to the drug resistance [72]. On the other hand, the stimulation of the Hh pathway produced chemoresistance partly due to the upregulation of drug efflux by ABC transporters, including P-gp and BCRP [73]. In addition, the expression of the transcription factor Oct4 in CSCs played an important role in the maintenance of the survival of CSCs, and in drug resistance in prostate and liver cancer through the Oct4-AKT-ABCG2 signaling pathway [74, 75].

Aldehyde dehydrogenases in CSCs

Aldehyde dehydrogenases (ALDHs) are a family of intracellular enzymes, which can be used as molecular markers to identify normal stem cells and CSCs, such as the ovarian CSCs [76]. This superfamily consists of 19 genes in humans, including 11 families and four subfamilies. The most common studied members in normal stem cells and CSCs include ALDH1, ALDH2, and ALDH3A1. ALDH1 is commonly used to identify and enrich CSCs from many cancers, including liver cancer [77], head and neck cancer [78], breast cancer [79], colon cancer [80], and bladder cancer [81].

In CSCs, ALDHs are crucial for the maintenance of stemness. Treating lung adenoma stem cells with

ALDH1A1 siRNA resulted in a lower capacity of clonogenicity, which suggested that ALDH1A1 was involved in the maintenance of stem-like properties by inhibiting the Notch/CDK2/CCNE pathway [82]. Blockage of ALDH in breast CSCs inhibited cell growth. Furthermore, the ALDH inhibitor, diethylaminobenzaldehyde (DEAB) obstructs tumor metastasis to the lung. In conclusion, a high level of ALDH expression promotes stemness features in breast cancer [83]. In addition, ALDHs play a detoxifying role, functioned in self-protection and conferred drug resistance to alkylating agents by metabolic inactivation [84]. Malignant pleural mesothelioma (MPM) cells with resistance to cisplatin and DEAB exhibited the overexpression of ALDH1A2, ALDH1A3 isozymes, and CD44, which suggest that ALDH^{high}CD44⁺ cells were implicated in conferring drug resistance [85]. ALDH inhibition and CD44 knockdown resulted in decreased stem-like gene expression and the enhancement of sensitivity to chemotherapeutics in lung cancer [86]. In accordance, stem-like ALDH^{high}CD44⁺ human breast cancer cells exhibited chemotherapy and radiation resistance, which was reversed by the inhibition of ALDH [87]. In these cases, ALDHs can be used as therapeutic targets, and subsequently, some ALDH inhibitors were developed to kill CSCs. For example, one of the inhibitors, copper (Cu)-dependent disulfiram (DS), was toxic to glioblastoma multiforme (GBM) stem-like cells by inhibiting the ALDH and NF- κ B pathways, and enhanced the antitumor activity of gemcitabine (dFdC) in a synergistic manner [88]. Another CSC killer, salinomycin, also used for the reversal of MDR in cancer, was identified and found to be specifically toxic on the ALDH^{high} population of stem-like cells in gastric cancer [89].

DNA repair and quiescence in CSCs

Genotoxic damage in mammalian cells, caused by endogenous and exogenous chemical, physical, and biological mutagens, results in DNA degradation and cell apoptosis. Under the normal conditions, the cellular integrity of genomic material is repaired to maintain the normal functions of the cells. This is especially true in the stem cells, as they have the capacity of self-renewal and give rise to a daughter cell that must retain an identical genome, and the potential to differentiate into tissue-specific cells. Thus, to ensure the original stemness properties, stem cells are endowed with multiple DNA-repair mechanisms, such as nucleotide excision repair, base excision repair, mismatch repair, direct repair, and the double-strand break recombinational repair [90].

The malignant counterpart of stem cells, CSCs, exhibits similar protective mechanisms. Accumulating evidence has demonstrated that CSCs show radioresistance and chemoresistance which were mediated by the DNA-repair mechanisms. Chronic myelogenous leukemia is mainly caused by the acquisition of BCR/ABL in HSCs. BCR/ABL expression increased reactive oxygen species (ROS) expression, which subsequently promoted oxidative stress and DNA damage. When DNA damage occurs, the inhibition of apoptosis can also be mediated by BCR/ABL, which subsequently induces the acquisition of radioresistance and chemoresistance [91]. In the breast CSCs, high levels of gene expression are observed for genes involved in DNA damage response and repair, such as *Nek1*, *Brac1*, *Chek1*, *Hus1*, *Ung*, *Xrcc5*, *Sfpq*, and *Uhrf1*, suggesting that breast CSCs are also resistant to chemotherapy and radiotherapy [92]. Compared with normal glioblastoma multiform cells, glioblastoma multiform CSCs were more radioresistant to cell death by the down-regulation of DNA damage checkpoint proteins, including ATM, Chk1, and Chk2 [93]. In recent research, prostate CSCs (PCSCs) exhibited chemoresistance in response to etoposide and docetaxel, the most commonly used chemotherapeutic drugs. This was achieved through the elevated expression of γ H2AX (a marker for DNA double-strand breaks and genomic instability) and G₂/M arrest. This study demonstrated that the upregulation of DNA damage responses made a contribution to chemoresistance in PCSCs [94].

Another mechanism to prevent apoptosis is for a cell to remain quiescent. Leukemia CSCs, transplanted into immunodeficient mice, conferred chemoresistance by quiescence as a defensive mechanism [95]. Esophageal CSCs (ECSCs) also remain in quiescence and, as a result, are more resistant to DNA damage agents. ECSCs demonstrated a low-expression level of EGFR, phosphorylated STAT3, and c-Myc, but elevated expression of p27. These factors appear to maintain quiescence and attenuate DNA damage responses and contributed to cell survival [96].

The studies described above highlighted a few of the mechanisms, such as the expression of DNA damage response and repair genes, as well as quiescence, that are utilized by cells to achieve chemoresistance which helped cells avoid apoptosis.

In conclusion, CSCs acts as one of the major obstacles to chemotherapy mainly due to their highly tumorigenic and chemoresistance properties. Understanding the mechanisms to achieve chemoresistance in CSCs has been exploited for the development of novel anti-cancer drugs. Therefore, the development of the new ABC inhibitors, anti-apoptotic antagonists, pro-survival pathway inhibitors, ALDH inhibitors, and the agents that target DNA-repair pathways holds promise for effective treatment of CSCs.

Natural products as MDR-reversing agents

MDR in cancer is a major obstacle for cancer therapy. To reverse MDR in cancer cells, researchers have developed antisense therapies, such as MDR1-antisense RNA [97], TAT-conjugated mesoporous silica nanoparticle drug delivery systems [98], and adjuvant therapy (i.e., fluoxetine synergies). Chemotherapeutics have also been developed to directly inhibit the activity of P-gp, MRPs, and BCRPs. For example, vinblastine and azidopine bound to the transport sites of P-gp, leading to the intracellular accumulation of the substrates [99–101], while amoxapene and Ioxapine non-competitively bound at the allosteric modulatory sites of P-gp, resulting in a 3.5-fold reduction of the DOX GI₅₀ in K562Dox cells [102]. To date, a number of natural products have been identified to reverse drug resistance by modulating ABC transporters, including P-gp, MRP1, and BCRP.

P-gp inhibitors

A number of flavonoids have shown to be MDR reversal agents by inhibiting P-gp. Baicalein (Table 1, 1), a flavonoid from *Scutellariae radix*, was shown to exert strong anti-cancer activity against ovarian cancer with LD₅₀ values ranging from 25 to 40 μM [103]. It induced G₀/G₁ phase arrest in hepatocellular carcinoma cells and obstructed H22 xenograft tumor growth. It has been shown that baicalein can inhibit tumor growth and apoptosis by affecting the phosphatidylinositol 3-kinase-AKT, Bcl-2, Bax, NF-κB, and p53 pathways [104]. Baicalein can also enhance the cytotoxicity of other anti-cancer chemotherapeutics, such as cisplatin, by increasing gap junction intercellular communication [105]. Hypoxia-induced 5-fluorouracil resistance in gastric cancer AGS cells can be reversed by baicalein via the suppression of glycolysis through the regulation of the PTEN/Akt/HIF-1α signaling pathway [106]. Baicalein increased nimodipine bioavailability by inhibiting cytochrome P450 3A4 (CYP3A4)-mediated metabolism of nimodipine in the small intestine and/or in the liver. Baicalein also elevated intracellular rhodamine-123 (the substrate of P-gp, RH123) concentration in P-gp overexpressed MCF7/ADR cells in a dose-dependent manner [107]. Icaritin (Table 1, 2), a flavonoid isolated from *Herba epimedii*, exhibited a broad range of pharmacological and biological activities, including anti-cancer activity in hepatoma cells SMMC-7721 with an IC₅₀ of 9.6 μM [108]. Icaritin can reduce renal cell carcinoma (RCC) by reducing the activation of the protein, signal transducer, and transcription-3 (STAT3), which is critical for tumor survival, proliferation, and angiogenesis [109].

Further studies indicated that icaritin can also reverse MDR in HepG2/adriamycin (HepG2/ADR) human hepatoma cells by down-regulating the expression of *MDR1*. Icaritin reversed the resistance to ADR by more than 7-folds at the concentration of 30 μM [110]. Icarin (Table 1, 3), a flavonoid glycoside, isolated from the same plant, is commonly prescribed for the treatment of cardiovascular diseases, osteoporosis, and cancer in China [111, 112]. The MDR reversal activity study revealed that icaritin enhanced the sensitivity of MCF7/ADR cells to ADR by about twofold at the concentration of 25 μM [113]. Recent study suggested that icaritin inhibited P-gp-mediated efflux pump by competitively binding at the P-gp drug-binding site [114]. Other flavonoids, including quercetin (Table 1, 4), biochanin A (Table 1, 5), phloretin (Table 1, 6), silymarin (Table 1, 7), and morin (Table 1, 8), also showed activity in reversing the drug resistance by inhibiting P-gp-mediated drug efflux [115, 116].

Sesquiterpenes from the plant family *Celastraceae* have also been used for the treatment of MDR cells in cancers. Sesquiterpene ester 1 (Table 1, 9), celafolin A-1 (Table 1, 10), and celorbicol ester (Table 1, 11) all showed drug-resistance reversal activities with an IC₅₀ value of 61.91, 14.00, and 14.36 μM against MCF7/ADR, respectively [117]. Munoz-Martinez et al. investigated the MDR reversal potency of 28 dihydro-β-agarofuran sesquiterpenes in human MDR1-transfected NIH-3T3 cells in vitro. The results suggested that the agarofuran sesquiterpenes reversed the MDR phenotype by interacting with the TM domain of P-gp and modulating P-gp ATPase activity [118].

Curcumin (diferuloylmethane, Table 1, 12) is a polyphenol natural product isolated from the rhizomes of *Curcuma longa*. The compound is known to regulate signaling pathways related to cell growth, differentiation, and apoptosis [119, 120], and has widely been used as antioxidant, anti-inflammatory, anti-cancer, and antimetastatic agent. It was reported that curcumin inhibited NF-κB signaling pathway activated by TNF, TPA, and hydrogen peroxide in human myelomonoblastic leukemia cell line ML-1a [121]. In a different study, treatment of human cervical carcinoma cells, KB-V1, with low concentrations (1, 5, and 10 μM) of curcumin reduced the expression of *MDR1* gene. Curcumin also increased the accumulation of RH123 by inhibiting P-gp [122]. Demethoxycurcumin (Table 1, 13) and bisdemethoxycurcumin (Table 1, 14) from *C. longa* also modulated the *MDR1* gene expression in human cervical carcinoma cells. Bisdemethoxycurcumin reduced P-gp expression by 88 % at 5 μM for three days [123].

Ecteinascidin 743 (Et-743, Table 1, 15), a marine natural product from Caribbean sea squirt *Ecteinascidia turbinata*, was used for the treatment of several cancers,

Table 1 Natural products that have been implicated in the reversal of multidrug resistance in cancers

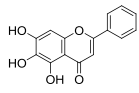
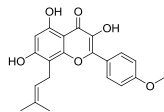
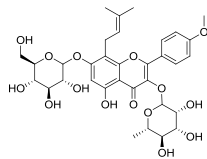
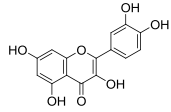
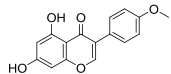
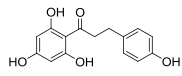
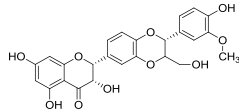
No.	Compound	Structure	Source	Biological mechanisms	Cancer type
1	Baicalein		<i>S. radix</i>	phosphatidylinositol 3-kinase-Akt Bcl-2 Bax NF-κB p53 PTEN/Akt/HIF-1α P-gp	Ovarian cancer [103] Hepatocellular carcinoma [104] Gastric cancer [106] Breast cancer [107]
2	Icaritin		<i>H. epimedii</i>	STAT3 P-gp	Hepatocellular carcinoma [108] Renal cell carcinoma [109]
3	Icariin		<i>H. epimedii</i>	P-gp	Breast cancer [114]
4	Quercetin		<i>A. aequalis</i>	P-gp	Leukemic cells [200] Prostate cancer [201] Gastric carcinoma cells [202] Breast cancer [203] Pancreatic tumor [204]
5	Biochanin A		<i>C. fistula</i>	P-gp	Breast cancer [116]
6	Phloretin		<i>P. mandshurica</i>	P-gp	
7	Silymarin		<i>S. marianum</i>	P-gp	

Table 1 continued

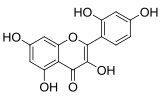
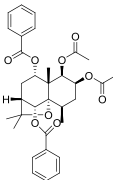
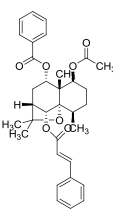
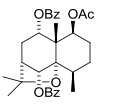
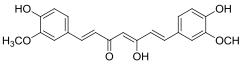
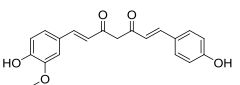
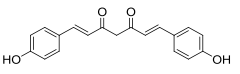
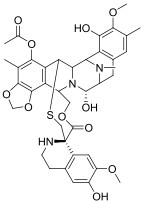
8	Morin		<i>M. alba</i>	P-gp	
9	Sesquiterpene ester 1		<i>C. orbiculatus</i>	P-gp	
10	Celafofin A-1		<i>C. orbiculatus</i>	P-gp	Cervical carcinoma and breast cancer [117]
11	Celorbicol ester		<i>C. orbiculatus</i>	P-gp	
12	Curcumin		<i>C. longa</i>	NF-κB P-gp	Cervical carcinoma [122]
13	Demethoxycurcumin		<i>C. longa</i>	P-gp	Cervical carcinoma [123]
14	Bisdemethoxycurcumin		<i>C. longa</i>	P-gp	
15	Ecteinasidin 743		<i>E. turbinata</i>	P-gp	Cervix carcinoma [127] Colon cancer, ovarian cancer, breast cancer, renal cancer and lung cancer [124]

Table 1 continued

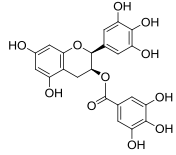
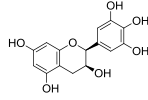
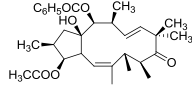
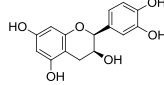
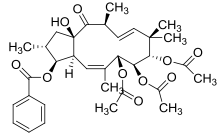
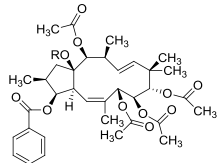
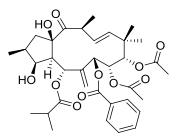
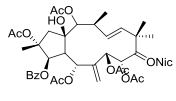
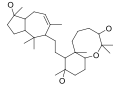
16	(-)-Epigallocatechin-3-gallate		<i>C. sinensis</i>	NF-κB P-gp	Cervix carcinoma [124, 125]
17	(-)-epigallocatechin		<i>C. sinensis</i>	P-gp	
18	(-)-epicatechin-3-gallate		<i>C. sinensis</i>	P-gp	
19	(-)-epicatechin		<i>C. sinensis</i>	P-gp	
20	Euphomelliferine		<i>E. mellifera</i>	P-gp	Mouse lymphoma and colon adenocarcinoma [134]
21	Euphomelliferine A		<i>E. mellifera</i>	P-gp	
22	Euphodendroidin D		<i>E. dendroides</i>	P-gp	Leukemic cells [135]
23	Pepluanin A		<i>E. peplus</i>	P-gp	
24	Siphonolol A		<i>C. siphonella</i>	P-gp	Cervix carcinoma [136]

Table 1 continued

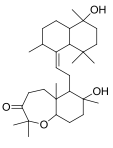
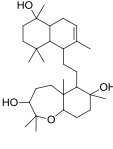
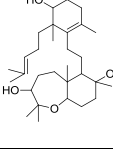
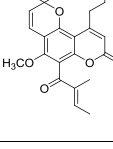
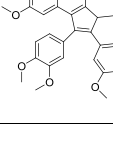
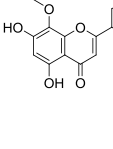
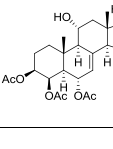
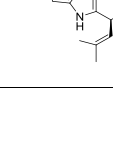
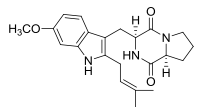
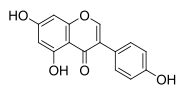
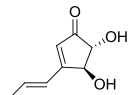
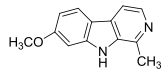
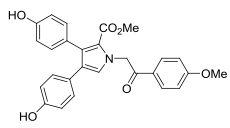
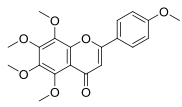
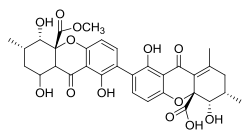
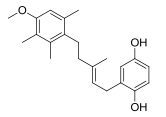
25	Sipholenone E		<i>C. siphonella</i>	P-gp	
26	Sipholenol L		<i>C. siphonella</i>	P-gp	Cervix carcinoma [137]
27	Siphonellinol D		<i>C. siphonella</i>	P-gp	
28	GUT-70		<i>C. brasiliense</i>	P-gp Apoptosis	Leukemic cells [138]
29	Lamellarin I		<i>D. cactos</i>	P-gp	Colon adenocarcinoma and murine leukaemia [139]
30	Wogonin		<i>S. baicalensis</i>	Cycle-cycle associated proteins Wnt/ β -catenin Nrf2 P-gp MRP1	Breast cancer [140] Myelogenous leukemia [141]
31	Aposterol A		<i>Spongia</i> sp.	P-gp MRP1	Cervix carcinoma [143]
32	Fumitremorgin C		<i>A. fumigatus</i>	BCRP	Colon cancer [40] Breast cancer [144]

Table 1 continued

33	Tryprostatin A		<i>A. fumigatus</i>	BCPR	Gastric carcinoma and breast cancer [145]
34	Genistein		<i>H. sibirhorioides</i>	BCRP NF-κB Akt	Breast cancer [147]
35	Terrein		<i>A. terreus</i>	G ₂ /M phase arrest ABCG2 Caspase-7 pathway Akt pathway	Breast cancer [149] Ovarian cancer [150]
36	Harmine		<i>P. harmala</i>	BCRP Cyclooxygenase-2	Breast cancer and gastric cancer [152]
37	Lamellarin O		<i>Ianthella</i> sp.	BCRP P-gp	Colon cancer and non-small lung cancer [154]
38	Tangeretin		<i>Citrus</i>	P-gp BCRP	Myelogenous leukemia [156] Colon cancer [157]
39	Secalonic acid D		<i>P. oxalicum</i>	ABCG2 P-gp MRP1	Oral epidermoid carcinoma, breast cancer [160]
40	Panicin A hydroquinone		<i>H. mucosa</i>	Ptch	Melanoma [45]

The table lists the compound, its structure, the natural source of the compound, the biological mechanism or signaling pathway targeted by the compound, and the types of cancers that have been targeted by the compound

including ovarian, breast, non-small lung, and renal cancers [124–126]. It has been shown that Et-734 can down-regulate P-gp expression at a concentration of 0.1 nM. In addition, Et-743 increased the cellular accumulation of DOX/VCR in P-gp-overexpressed cervix cells [127].

Polyphenolic catechins, such as (–)-epigallo catechin-3-gallate (EGCG, Table 1, 16), (–)-epigallocatechin (EGC, Table 1, 17), (–)-epicatechin-3-gallate (ECG, Table 1, 18), and (–)-epicatechin (EC, Table 1, 19), from tea, can be used for the treatment of cancers, including skin, lung, oral cavity, esophagus, breast, stomach, small intestine, colon, liver, pancreas, and mammary glands [128]. EGCG, one of the major water-soluble compounds in green tea (*Camellia sinensis*), has been shown to have the chemopreventive, anticarcinogenic, anti-atherogenic, and antioxidant effects [129]. EGCG was used as the chemopreventive agent by inducing apoptosis and increasing cell growth arrest via regulating the expression of cell-cycle regulation proteins. The previous studies have shown that EGCG inhibited carcinogenesis by regulating a wide range of signaling pathways. It can activate NF- κ B, and then induce the expression of more than 200 genes that inhibited apoptosis, and promoted cell proliferation, invasion, metastasis, and chemoresistance [130]. It was also reported that EGCG inhibited NF- κ B signaling pathway by blocking TPA- and UV-induced phosphorylation of I κ B [131, 132]. In a study, catechins, including EGC, ECG, and EGCG, enhanced the cellular accumulation of P-gp substrates, RH123, and daunorubicin, in P-gp overexpressed KB-C2 cells. Therefore, the catechins played an important role in reversing drug resistance by inhibiting the activity of P-gp.

Three macrocyclic jatrophone diterpenes and one tetracyclic triterpene were isolated from *Euphorbia mellifera*. Their ability to reverse drug resistance was evaluated through the RH123 accumulation test on human *MDR1* gene-transfected mouse lymphoma cells (L5178Y MDR) and multidrug-resistant human colon adenocarcinoma cells (COLO 320). Macrocyclic jatrophone diterpenes, euphomelliferine (Table 1, 20), and euphomelliferine A (Table 1, 21) exhibited high MDR reversal activity with the fluorescence activity ratio (FAR) values of 72.9 and 82.2 at 60 μ M, respectively [133]. Two other compounds, euphodendroidin D (Table 1, 22) and pepluanin A (Table 1, 23), isolated from genus *Euphorbia* were also studied. At the concentration of 5 μ M, euphodendroidin D inhibited daunomycin-efflux activity twofold ($183 \pm 17\%$) more than cyclosporine A, a conventional modulator, by specifically modulating P-gp activity. Pepluanin A also showed higher inhibition ($207 \pm 17\%$) [134].

Sipholenol A (Table 1, 24), one of the sipholane triterpenes isolated from the Red Sea sponge *Callyspongia siphonella*, reversed MDR in P-gp overexpressed cervix carcinoma cells. At the concentration of 2.5, 5, and 10 μ M, sipholenol A potentiated the toxicity of P-gp substrates,

colchicine, vinblastine, and paclitaxel in a dose-dependent manner [135]. Three other triterpenoids isolated from the same sponge, sipholenone E (Table 1, 25), sipholenol L (Table 1, 26), and siphonellinol D (Table 1, 27) also enhanced the inhibitory effect of colchicine, vinblastine, and paclitaxel, and reversed the MDR in P-gp-overexpressing MDR cancer cell line KB-C2 at 1, 3 and 10 μ M [136].

A tricyclic coumarin, GUT-70 (Table 1, 28), derived from the stem bark of Brazilian plant *Calophyllum brasiliense*, exhibited the inhibitory effect on six human leukemic cell lines, including P-gp overexpressed cells in a concentration- and time-dependent manner. GUT-70 induced caspase-mediated and p53-independent apoptosis to overcome MDR [137]. Anti-cancer effects of several lamellarins isolated from genus *Didemnum* were also studied on P-gp-mediated MDR cancer cell lines. Lamellarin I (Table 1, 29) reversed MDR by inhibiting P-gp efflux. This compound completely reversed resistance of DOX, vinblastine, and daunorubicin at 2 μ M in multidrug-resistant P388/Shabel cells [138].

P-gp and MRP1 inhibitors

Wogonin (Table 1, 30), a flavonoid isolated from *Scutellaria baicalensis*, reversed DOX resistance in MCF7/DOX cells by inhibiting nuclear factor erythroid 2-related factor 2 (Nrf2), which played a vital role in cell survival and MDR. Wogonin increased the sensitivity of MCF7/DOX cells to DOX by 1.24-, 1.93-, and 3.24-fold at 20, 40, and 60 μ M, respectively [139]. A recent study implicated that wogonin can reverse MDR in human myelogenous leukemia K562/A02 cells. Wogonin promoted the sensitivity of K562/A02 cells to ADR by 1.22-, 2.31-, and 3.85-fold at the concentrations of 10, 20, and 40 μ M, respectively. It also repressed the Nrf2 signaling pathway, resulting in the down-regulation of MRP1 expression [140]. In addition, wogonin showed P-gp inhibitory activity and suppressed excretion of calcein-AM, a substrate of P-gp, in Jurkat cells, and A549 cells at a concentration of 10 μ M [141]. Aposterol A (Table 1, 31), a polyhydroxylated sterol acetate, was isolated from a marine sponge *Spongia* sp. [142]. Aposterol A increased the vincristine accumulation in both P-gp-mediated MDR cells (KB-C2) and MRP1-mediated MDR cells (KB-CV60) at 3 μ M and showed reversal effect on P-gp and MRP1 [142].

BCRP inhibitors

Fumitremorgin C (FTC, Table 1, 32), a prenylated indole alkaloid isolated from *Aspergillus fumigatus*, was the first reported BCRP inhibitor. FTC significantly potentiated the

sensitivity of S1-M1-3.2, a BCRP-overexpressed colon carcinoma cell line, to mitoxantrone (93-fold), DOX (26-fold), and topotecan (24-fold) at the concentration of 5 μM [40]. At the same concentration, FTC sensitized BCRP-overexpressed MCF7 cells to DOX (6.6-fold), mitoxantrone (29.4-fold), and topotecan (6.5-fold) [143]. However, FTC can cause severe side effects, such as neurotoxicity, which limited its clinical application. In recent years, FTC analogs have been developed and showed inhibitory activity against BCRP. One of the natural analogs, tryprostatin A (Table 1, 33), reversed mitoxantrone-resistant gastric carcinoma cell line EPG85-257RNOV, breast cancer cell line MCF7/AdrVp, and BCRP cDNA-transfected breast cancer cell line MCF7/BCRP at the concentrations of 10–50 μM [144]. An isoflavone, genistein (Table 1, 34) isolated from *Hydrocotyle sibthorpioides* [145], competitively inhibited BCRP-mediated drug efflux. Genistein potentiated cytotoxicity of SN-38 (7.23-fold) and mitoxantrone (6.28-fold) at the concentration of 3 μM [146]. The marine sponge-derived fungal metabolite, terrein (Table 1, 35), was isolated from *Aspergillus terreus* [147]. Terrein significantly decreased ABCG2-expressed MCF7 cells at the concentrations of 1 or 10 nM. It induced apoptosis by potentiating the caspase-7 signaling pathway and repressing the Akt signaling pathway. Terrein showed 100-fold more toxicity against MCF7 cells than paclitaxel. The IC_{50} values of terrein against breast cancer MCF7 cells, pancreatic cancer PANC-1 cells, and liver cancer HepG2 cells were 1.1 nM, 9.8 μM , and 66.8 nM, respectively [148]. A later study suggested that terrein can also be used in the treatment of ovarian cancer, since the compound induced G₂/M phase cell cycle arrest in the ovarian CSCs [149]. The β -carboline alkaloid, harmine (Table 1, 36), was isolated from *Peganum harmala* [150], and was identified as a BCRP inhibitor in a BCRP-overexpressed breast cancer cell line MDA-MB-231. The anti-cancer activity of harmine was evaluated in gastric cancer and it induced apoptosis and inhibited cell proliferation, migration, and invasion [151]. Harmine showed some side effects, such as neurotoxicity and cytotoxicity; however, it could be used as a lead compound for the development of BCRP inhibitors [152].

P-gp and BCRP inhibitors

Lamellarin O (Table 1, 37), isolated from an Australian marine sponge *Ianthella* sp., showed growth inhibition against P-gp overexpressed colon cancer cell line SW620 Ad300 with an IC_{50} of 22.3 μM . Treatment with lamellarin O increased sensitivity towards DOX, a P-gp substrate by 4.8-fold. Calcein-AM accumulation- and cell flow cytometry-based assays indicated that lamellarin O acted as a potent and selective BCRP inhibitor in non-small lung cancer with an IC_{50} of 4.7 μM [153].

Tangeretin (Table 1, 38) is a natural *Citrus* flavonoid known with antiproliferative activity [154]. Tangeretin was previously identified as MDR reversal agent by inhibiting P-gp in K562/ADM human myelogenous leukemia with an EC_{25} of 12.84 μM [155]. Tangeretin induced apoptosis by caspase-3 activation, and reversed multidrug resistance in colon cancer by inhibiting P-gp [156]. A recent study suggested that tangeretin showed potent inhibitory effect on BCRP with an EC_{50} of 1.19 μM against human BCRP transfected MDCK-II cells. Tangeretin also significantly enhanced dasatinib intracellular accumulation (341 % in mean value) by inhibiting P-gp at 50 μM [157].

P-gp, MRP1, and BCRP inhibitors

Secalonic acid D (SAD, Table 1, 39) was isolated from *Penicillium oxalicum* [158]. It showed potent inhibitory effect against P-gp-, MRP1-, and BCRP-overexpressed MDR cells and their parental cells, with an IC_{50} value of 0.27, 1.20, 0.13, and 1.04 μM against ABCB1-overexpressed oral epidermoid carcinoma cell line KBv200, ABCB1-overexpressed breast cancer cell line MCF7/Adr, ABCC1-overexpressed epidermoid carcinoma cell line CA120, and ABCG2-overexpressed colon carcinoma cell line S1-M1-80, respectively. Particularly, SAD suppressed the expression of ABCG2 and shortened the half-life of ABCG2 protein via the activation of calpain 1. Therefore, SAD was implicated in the treatment of cancer as a MDR inhibitor [159].

Ptch inhibitors

Four natural products, panicein A hydroquinone, panicein B2, panicein B3, and panicein C, were isolated from *Haliclona (Soestella) mucosa*. These four compounds inhibited the growth of yeast-expressing Ptch in the presence of DOX with IC_{50} values of 1, 2, 0.8, and 4.9 μM , respectively. Treatment with Panicein A hydroquinone significantly increased cell death 5–8-fold after treatment by DOX in melanoma MEWO cells and caused a 2–3-fold increase in A375 cells. Further studies indicated that Panicein A hydroquinone inhibited 40 % of the DOX efflux activity of Ptch [45].

Natural products-targeting CSCs

The past few decades have seen many achievements in cancer prevention and therapy. Our better understanding of the CSCs and the mechanisms of chemoresistance have

helped researchers to explore new strategies for cancer treatment. Many natural products have shown biological activities against CSCs by interacting with apoptotic genes, survival genes, and cell cycles.

Berberine from *Berberis aristata* or *Coptis chinensis*

Berberine (Table 2, 1) from the roots and bark of *Berberis aristata* or *Coptis chinensis* [160] has been shown to have antitumor activity. In an early study, berberine was shown to inhibit cell proliferation of human tumor U937 and murine melanoma B16 cell lines, inducing apoptosis in the U937 cells with an IC_{100} of 100 $\mu\text{g}/\text{mL}$ and causing cytoplasmic membrane damage on the B16 cells with an IC_{100} of 1 $\mu\text{g}/\text{mL}$ [161]. Recent studies on CSCs suggested that berberine was an effective inhibitor of CSCs; it suppressed cancer invasion and metastasis in A549 lung cancer with an IC_{50} of 56.15 μM , up-regulated epithelial phenotype marker E-cadherin, down-regulated the expression of mesenchymal phenotype marker Vimentin, and suppressed TGF- β 1-induced epithelial-to-mesenchymal (EMT) cell transition [162]. Berberine decreased the proportion of pancreatic CSCs from 9.7 to 5.7 % at the concentration of 15 μM . Stem-cell-associated genes (SOX2, POU5F1, and NANOG) were down-regulated by the treatment of berberine [163]. In contrast, berberine increased the side-population fraction, referred to as CSCs, by 7.6 % in H460 lung cancer cells [164].

Curcumin from *Curcuma longa*

More and more evidence suggested that curcumin (Table 2, 2) can target CSCs by regulating self-renewal pathways, such as Wnt/ β -catenin, Notch and sonic hedgehog, and specific microRNAs [165]. Curcumin inhibited the mammosphere formation in breast cancer cells by 50 % at the concentration of 5 μM and reduced the proportion of ALDH1A1-expressing cells by 5.8 % at the concentration of 10 μM [166]. Another study suggested that curcumin dramatically decreased the percentage of $CD44^+/CD24^-$ expressing cells after 6-h treatment at the concentration of 50 μM [167]. Studies of curcumin on breast CSCs suggested that curcumin caused the apoptotic effects on mammospheres of MCF7 and T47D cells at the concentration of 15 μM . Curcumin suppressed β -catenin nuclear translocation, subsequently increased E-cadherin/ β -catenin complex formation, down-regulated EMT-promoting target gene expression, and, thus, inhibited the migration of breast CSCs [168]. The combinational application of curcumin

and EGCG reduced CSC population in breast cancer by targeting STAT3 and NF- κ B signaling pathways [169]. To improve the bioavailability of curcumin, analogs, and combinational therapy were developed. For example, difluoro-curcumin (CDF), in combination with 5-FU and oxaliplatin (Ox), inhibited cell growth, yet promoted apoptosis and disintegration of colon CSCs by down-regulating ABCG2 and attenuating EGFR, IGF-IR, and NF- κ B signaling pathways [170].

Quercetin from *Quercetum* (oak forest)

Quercetin (Table 2, 3) was a ubiquitous flavonoid that was originally identified from *Quercetum* (forest oak). It was also isolated from a wide range of fruits and vegetables. It showed inhibitory effects on various cancers. For example, quercetin down-regulated the expression of survivin and anti-apoptotic proteins in human glioma cells, resulting in caspase-dependent apoptosis [171]. The mechanism of action study suggested that quercetin inhibited cell proliferation by regulating Bcl2 and Bax expression [172]. Quercetin also affected CSC properties. Using an in vitro and in vivo pancreatic CSC model, quercetin reduced the capacity of self-renewal, ALDH1 activity, and apoptosis resistance. Quercetin, together with sulforaphane, had synergistic effects on the prevention of EMT [173]. Quercetin was an effective inhibitor of CSCs in head and neck cancers with the concentrations ranging from 25 to 100 μM . Further studies suggested that quercetin suppressed ALDH1 activity, and reduced self-renewal and migration ability of CSCs [174]. Other studies on tongue cancer-derived stem cells showed that quercetin promoted cell apoptosis by inhibiting p-Hsp27 expression, which led to the reversal of drug resistance triggered by the activation of p38 MAPK signaling pathway [175]. Recent study on $CD133^+$ cancer stem cells derived from human colorectal HT29 cancer cells suggested that quercetin increased cytotoxicity and apoptosis induction of DOX with an IC_{50} concentration of 75 μM . Quercetin, in combination with DOX, induced G_2/M cell cycle arrest in HT29 cells [176].

Resveratrol from *Polygonum cuspidatum*

Resveratrol (Table 2, 4) is a polyphenol molecule present in various fruits and foods, such as grape skins. The compound can also be found in *Polygonum cuspidatum* and is used in oriental folk medicine [177]. Grape seed extract, in combination with resveratrol, potentiated the cell apoptosis and suppressed the proliferation of human colon CSCs via the activation of p53-dependent pathway [178]. Resveratrol also suppressed the self-renewal ability of

Table 2 Natural products that have shown inhibition of cancer stem cells

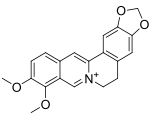
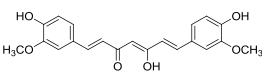
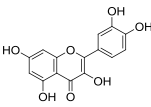
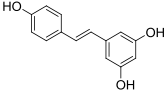
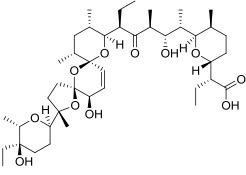
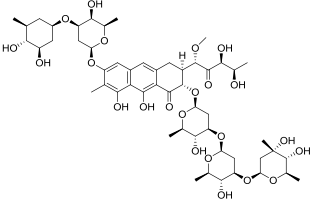
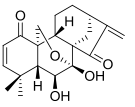
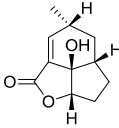
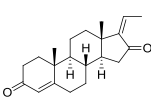
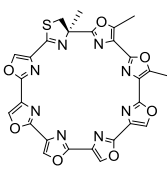
No.	Compound	Structure	Source	Biological mechanisms	CSC type
1	Berberine		<i>B. aristata</i> or <i>C. chinensis</i>	ROS G ₂ /M arrest Apoptosis NF-κB STAT3 E-cadherin Vimentin ABC transporters EMT SOX2 POU5F1 NANOG	Myeloma leukemia and murine melanoma cancer [162] Lung cancer [163,165] Pancreatic cancer [164]
2	Curcumin		<i>C. longa</i>	Wnt/β-catenin Notch Sonic hedgehog E-cadherin/β-catenin EMT STAT3 NF-κB micro-RNAs	Breast cancer [167] Colon cancer [171]
3	Quercetin		<i>Quercetum</i>	Apoptosis ALDH1 EMT p-Hsp27 Cell proliferation Bcl2 Bax Self-renewal Migration	Glioma cells [172] Pancreatic cancer [174] Head and neck cancers [175] Colorectal cancer [177]

Table 2 continued

4	Resveratrol		<i>P. cuspidatum</i>	Apoptosis Notch1 Wnt/ β -catenin MDR1 ABCG2 EMT Self-renewal Metastasis	Colon cancer [179] Pancreatic cancer [176] Nasopharyngeal carcinoma [181] Ovarian cancer [182] Breast cancer [183]
5	Salinomycin		<i>S. albus</i>	Autophagy-lysosomal pathway Wnt Apoptosis ALDH	Breast cancer [185] Gastric, glioma and pancreatic cancer [187] Osteosarcoma [190]
6	Mithramycin		<i>S. argillaceus</i>	ABCG2	Lung cancer and esophageal cancer [192]
7	Eriocalyxin B		<i>I. eriocalyx</i>	NF- κ B signaling Apoptosis	Ovarian cancer [193]
8	Galiellalactone		<i>G. rufa</i>	STAT3 pathway	Prostate cancer [194,195]
9	Guggulsterone		<i>C. mukul</i>	MUC4 JAK/STAT Src/FAK	Pancreatic cancer [196] Glioma [197]
10	Telomestatin		<i>S. anulatus</i>	Telomere <i>c-Myb</i>	Glioma [198]

The table lists the compound, its structure, the natural source of the compound, the biological mechanism or signaling pathway targeted by the compound, and the types of cancer stem cells that have been targeted by the compound

pancreatic CSCs which derived from human tumor and Notch1, MDR1 and ABCG2 overexpressed Kras^{G12D} mice [179]. In addition, resveratrol hampered stem-cell properties, including drug resistance, self-renewal ability, tumor initiation ability and metastasis potency, and EMT through the activation of p53 in nasopharyngeal carcinoma CSCs [180]. A recent study suggested that resveratrol, at 60 μM , also inhibited EMT, induced by CDDP treatment in ovarian cancer cell lines. It caused cell death in an apoptotic-independent manner [181]. Resveratrol also dramatically inhibited the proliferation of breast CSCs and induced the autophagy through the suppression of Wnt/ β -catenin signaling pathway [182].

Salinomycin from *Streptomyces albus*

Salinomycin (Table 2, 5) was recently isolated from *Streptomyces albus* [183]. It selectively inhibited the cell growth of CD44⁺/CD24⁻ breast CSCs by 20-fold relative to vehicle control. In addition, salinomycin reduced the tumor-seeding, tumor formation, and metastatic ability [184]. Salinomycin was found to suppress the autophagy-lysosomal pathway, which was crucial for the tumorigenicity of breast CSCs [185]. It was also proven that salinomycin was not only effective on breast CSCs, but also on gastric, pancreatic, and glioma CSCs in different ways [186]. In the gastric and pancreatic CSCs, salinomycin inhibited the proliferation of the CSC population by suppressing the Wnt signaling pathway, which was essential for the self-renewal capacity of gastric and pancreatic CSCs [187, 188]. Salinomycin killed glioma CSCs with an IC₅₀ value of 0.06 μM [186]. In a recent study on osteosarcoma stem cells, salinomycin selectively killed tumor stem cells with an IC₅₀ value of <5 μM . This inhibition effects also involved Wnt/ β -catenin signaling pathway [189].

Other CSC inhibitors

Mithramycin (Table 2, 6), a major product from *Streptomyces argillaceus*, exhibited remarkable cytotoxicity against various cancers [190]. It inhibited stem-cell signaling in lung and esophageal cancer cells and suppressed ABCG2 expression [191]. An *ent*-daurene diterpenoid from *Isodon eriocalyx* var. *Laxiflora*, eriocalyxin B (EriB, Table 2, 7), was shown to target p50 by inducing apoptosis via NF- κ B signaling inhibition. The compound was also an effective inhibitor of ovarian CSCs with a GI₅₀ of 0.5–1 μM [192]. A secondary metabolite from fungus *Galeilla rufa*, galiellalactone (Table 2, 8), was a promising therapeutic agent for both prostate cancer cells and prostate

CSCs by targeting the STAT3 pathway [193, 194]. Guggulsterone (GS, Table 2, 9), isolated from the plant *Commiphora mukul*, showed anti-proliferation activity against various cancers. In a study on pancreatic cancer cells, GS inhibited cell growth and metastasis by down-regulating MUC4 expression involved in cancer cell fate, invasion, and drug resistance [195]. Meanwhile, GS, in the cooperation with SANT-1, a novel inhibitor on glioma cells, can target stem and non-stem glioma cells [196]. Telomestatin (Table 2, 10), a macrocyclic compound from *Streptomyces anulatus* 3533-SV4, exhibited strong inhibition against glioma CSCs via telomere disruption and *c-Myb* inhibition [197].

Prospects

Natural products derived from microbes, plants, and marine organisms have played a dominate role in cancer drug discovery. The membrane transporters, a major contributor to MDR in cancer, have been demonstrated as one of the most important targets in cancer therapy. A growing number of natural products have been discovered to reverse MDR in cancers; however, problems with these compounds still remain. In the reversal of MDR in cancer, the effects on the normal cells, especially the cells expressing ABC transporter family, should be considered. High cytotoxicity from these compounds also limits their potential application in clinical practice. For example, cyclosporine A (CsA), a well-known P-gp inhibitor, reduced the efflux of DOX in tumor cells. However, it cannot be administrated for long period, because of the side effects it caused, including immunosuppression and severe nephrotoxicity. Therefore, searching for the drugs with specific activity against MDR is still a major barrier to the success of chemotherapy. Hunting for new therapeutics from nature, which hosts a vast resource of natural products that target MDR cells, is a potential strategy for cancer treatments. In the future, to overcome the MDR in cancer, the targets can be concentrated on ABC transporters, as they are widely expressed in cancers, but this does not exclude other targets that may be expressed in specific cancer cell types.

The existence of CSCs is another prominent challenge for cancer therapy. More and more evidence suggests that CSCs play a critical role in the aetiology of metastasis, the main cause of the mortality of cancer patients. A better understanding of the origins of CSCs and their molecular mechanisms will make important contribution to the treatment of CSCs. Though many products from nature were discovered to eliminate CSCs, they still displayed high toxicity against normal cells. For example, salinomycin was effective on many cancers, but it displayed

neurotoxic effects [198]. On the other hand, some of the drugs have physical and chemical property limitations, resulting in the failure of bioavailability. Curcumin had prominent anti-cancer properties, but was limited in clinical applications due to its insolubility in water and instability.

In this review, the cellular mechanisms of MDR and CSCs and the developed natural products against MDR cancer cells and CSCs were discussed with a view that we can obtain a better understanding of the mechanisms which will ultimately help in the development of new targets in the battle against cancer. To overcome these hurdles, a significant effort has to be devoted to the discovery of new agents that eliminate MDR cancer cells and CSCs.

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