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Natural Product Modulators to Overcome Multidrug Resistance In Cancer

Aysegul Cort and Tomris Ozben
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Multidrug resistance (MDR) is a condition that makes cells simultaneously unresponsive to different drugs, unrelated to their chemical structure and mechanism of action. MDR caused by the presence and overexpression of ABC transporters makes obstacles in cancer treatment and lower the effectiveness of chemotherapy. Natural products are investigated by many researchers as MDR modulators for their low toxicity and potent, selective behavior. When coadministered, MDR modulators compete with cytotoxic agents for binding to the active site of the membrane transporters and reduce drug efflux. Natural product-based drugs are important in struggling against drug resistance during cancer therapy. This review is focused on the potential mechanisms against drug resistance, the development of inhibitors for ABC drug transporters, natural product modulators, and nanoparticle drug delivery.

POTENTIAL MECHANISMS OF RESISTANCE

Multidrug resistance (MDR) is a condition that makes cells simultaneously unresponsive to several drugs, with unrelated chemical structure and mechanism of action. Resistance to single or multiple chemotherapeutic drugs is a major complication in clinical oncology and constitutes one of the most common treatment limitations in cancer patients. It occurs in patients with a variety of blood cancers and solid tumors, including breast, ovarian, lung, and lower gastrointestinal tract cancers. Tumors typically consist of a mixed population of malignant cells; some drug-sensitive, some drug-resistant. In some cases, cancer cells exhibit resistance to chemotherapy since their first exposure to an anticancer drug; this is defined as intrinsic MDR. Chemotherapy eradicates drug-sensitive cells, favoring the survival of drug-resistant cells. As the tumor begins to grow again, chemotherapy may fail because the tumor cells are now resistant (1). The term acquired MDR is used when resistance to chemotherapy occurs during the course of treatment or upon disease recurrence, following an initially successful chemotherapy (2).

There are several hypotheses explaining the emergence of drug resistance, including altered drug transport across the plasma membrane, genetic responses, enhanced DNA repair, modification of target molecules, access to target cells, metabolic effects, and growth factors. Some of the mechanisms used by cancer cells to resist cytotoxic drugs are also observed in normal cells as part of a defense mechanism against environmental carcinogens.

Therapy resistance is related to the presence of at least two molecular “pumps” present in tumor-cell membranes that actively expel chemotherapeutic drugs from the cell interior, avoiding in this way the toxic effects of the drug or the drug-caused toxic molecular processes taking place in the nucleus or in the cytoplasm. The pumps that confer chemoresistance in cancer cells are P-glycoprotein, the so-called multidrug resistance–associated protein (MRP). Because of their function and importance, they became the targets of several anticancer drugs.

The overexpression of membrane ATP binding cassette (ABC) transporters constitutes one of the main mechanisms of MDR (3). The physiological functions and localization of ABC transporters in human tissues affects the overall adsorption, distribution, metabolism, elimination, and toxicity of any drug class (4). ABC drug transporters, including P-glycoprotein (Pgp; ABCB1), MRP1 (ABCC1), and ABCG2 (BCRP; MXR) seriously affect cancer chemotherapy. ABC transporters contain a pair of ATP-binding domains, also known as nucleotide binding folds, and 2 transmembrane domains containing 6 membrane-spanning α-helices. The nucleotide binding folds contains 3 conserved domains: Walker A and B domains, found in all ATP-binding proteins, and a signature (C) motif, located just upstream of the Walker B site. The C domain is specific to ABC transporters and distinguishes them from other ATP-binding proteins. The molecules pump substrates in a single direction, typically out of the cytoplasm. For hydrophobic compounds, this movement is often from the inner leaf of the bilayer to the outer layer or to an acceptor molecule (5). ABC transporters use energy derived from
hydrolysis of ATP to actively transport anticancer drugs across biological membranes, preventing drug accumulation, and the reaching of the targets within a cancer cell.

Pgp (ABCB1)

Pgp is the first discovered member of the ATP-binding cassette (ABC) transporter, which acts as a physiological barrier and ejects toxins and xenobiotics from the cells (6). Pgp is primarily found in epithelial cells lining the colon, small intestine, pancreatic ductules, bile ductules, kidney proximal tubules, and adrenal gland (7), and it is overexpressed on the surface of many neoplastic cells (8). Pgp protects cells from toxic compounds, prevents them to enter the cytosol by extruding them to the exterior. Pgp enhances the secretion of metabolites and xenobiotics into bile, urine, and lumen of the gastrointestinal tract. Pgp in humans has 2 isoforms. The Class I isoform (MDR1/ABCB1) is a drug transporter, whereas the Class II isoform (MDR2/3/ABCB4) exports phosphatidylcholine into the bile (8).

Tumors of colon, kidney, pancreas, and liver carcinoma usually express high levels of Pgp and tend to be drug resistant (9). Pgp can bind anticancer drugs such as anthracyclines, epipodophyllotoxins, vinca alkaloids, and taxanes. This drug-binding activity results in the activation of one of the ATP-binding domains of Pgp and the subsequent hydrolysis of ATP, leading to a major change in the molecular shape of Pgp, causing expulsion of the drug; in this way the cell chemotherapeutic agent internalization is prevented, and chemotherapy becomes ineffective (10).

MRP1 (ABCC1)

MRP1 (ABCC1) has a 5-domain structure with a third NH₂-proximal membrane spanning domain with 5 transmembrane segments and an extracytosolic NH₂ terminus (11). MRP1 is an active ATP-dependent transporter of cysteinyl leukotriene LTC4; 17b-estradiol 17-(b-D-glucuronide), exo and endo glutathione (GSH) conjugates of the mycotoxin aflatoxin B1 (12). MRP1 causes the efflux of some xenobiotics (e.g., vincristine, daunorubicin) through a cotransport mechanism with reduced GSH (13). GSH enhances transport of some MRP1 substrates. It has been shown that GSH increases the potency of certain compounds to inhibit conjugated organic anion transport activity by MRP1. Efflux of hydrophobic natural product anticancer drugs agents such as vincristine from cells expressing MRP1 requires GSH (14–16). GSH is the most abundant nonprotein intracellular thiol containing compound and a key molecule in MRP1-mediated MDR (17,18). GSH stimulates ATP-dependent uptake and efflux of anticancer drug vincristine by MRP1 (19). Concurrently, cellular depletion of GSH decreases MRP1-mediated resistance to anticancer drugs. Similar results have been reported in vesicular transport assays of vincristine and daunorubicin (15,19,20). The formation of drugs—GSH conjugates catalyzed by the enzyme glutathione S-transferase (GST)—causes their subsequent removal from the cells (21). The transport of LTC4 is poorly inhibited by vincristine or verapamil alone, but in the presence of GSH, the extrusion inhibition is enhanced more than 20-fold (22,23). Akan et al. reported that N-acetylcysteinine (NAC), as a source for GSH synthesis, increased the resistance of human embryonic kidney (HEK293) and MRP1 transfected (293MRP) parental cells against vincristine and buthionine sulfoximine (BSO), as an inhibitor of GSH synthesis, decreased NAC-enhanced MRP1-mediated vincristine resistance, indicating that induction of MRP1-mediated vincristine resistance depends on GSH in both cell types (16). Many of the anticancer drugs that are substrates for Pgp are also substrates for MRP1 except taxanes, which are substrates for only ABCC1 transporters.

ABCG2 (BCRP; MXR)

ABCG2 (BCRP; MXR) is found in a variety of stem cells and human tissues, including placenta, liver, kidney, and intestine, protecting them from exogenous and endogenous toxins. Low-oxygen conditions induce ABCG2 expression in tissues and ABCG2 protects cells and tissues from protoporphyrin accumulation under hypoxic conditions by interacting with heme and porphyrins (24). It has been suggested that absence of ABCG2 activity may increase risk for developing protoporphyrin and diet-dependent phototoxicity (25), indicating the importance of drug transporters in protection from toxicity of food constituents (26). van Herwaarden et al. demonstrated that ABCG2 knockout mice have elevated plasma levels and decreased intestinal, fecal, and hepatobiliary excretion of the food carcinogen 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (27).

DEVELOPMENT OF INHIBITORS FOR ABC DRUG TRANSPORTERS

Inhibiting the function of ABC drug transporters by inhibitors or modulators is a desired method to ameliorate drug sensitivity in MDR cancer cells. The inhibition of ABC drug transporters in MDR cancer chemotherapy allows elevated drug penetration, distribution, accumulation, and restores drug sensitivity. ABC transporter inhibitors, also called MDR modulators, chemosensitizers, or MDR reversal agents, are able to reverse resistance against anticancer drugs (28–31). Inhibitors influence ABC transporters by specific interactions with proteins, changing intracellular ATP level that is the source of energy, or affecting membrane profile to increase permeability. Inhibitors can affect other biological targets by unspecific binding. However, the use of inhibitor drugs at high concentrations necessary to reach a sufficient inhibitory effect may cause toxicity.

The first-generation chemosensitizers has low activity and high toxicity, containing antagonists of calmodulin (e.g.,
trifluoperazine, chlorpromazine, prochlorperazine, clopenthixol, triflupromazine, flupenthixol, etc.), channel blockers (e.g., verapamil, felodipine, isradipine, nicardipine, nifedipine, bepridil, and diltiazem), immunosuppressant steroids (e.g., progesterone and tamoxifen), inhibitors of kinase C, cardiovascular drugs, indole alkaloids, and detergents (32,33). The therapeutic use of these first-generation inhibitors has been barred after Phase I clinical trials (34), with the exception of cyclosporin A, a commonly used immunosuppressant, which remains 1 of the most effective first-generation MDR modulators (35).

The second-generation chemosensitizers, with low affinity to ABC transporters, are cyclosporin D, diaryl imidazole, and valspodar. They are more specific toward MDR and therefore more potent and considerably less toxic. Verapamil analogues including dexverapamil, emopamil, gallopamil, and Ro11-2933 can reverse MDR in vitro to a degree equivalent to verapamil with comparatively lower toxicity (36). Other second-generation MDR modulators include PSC833 (valspodar), VX-710 (biricodar), and GF120918 (elacridar). PSC 833, without immunosuppressive or nephrotoxic action, is more potent than cyclosporine A in vitro (37). Several factors have limited the clinical use of second-generation compounds. These compounds are often inhibitors of other ABC transporters [e.g., MRP2 also named nonbile acid organic anion transporter (cMOAT)] (38–40). This characteristic is believed to be responsible for adverse effects occurring when second-generation compounds are combined with antineoplastic agents that are Pgp substrate. These second-generation inhibitors cause mainly neutropenia and other myelotoxic effects (41), they are also substrates of cytochrome P-450 (CYP). Inhibition of the metabolism of anticancer drugs by competing for CYP-mediated oxidative reactions may result in pharmacokinetic interactions with increased host toxicity from cytotoxic drug overexposure leading to severe side effects (42–47).

The third-generation inhibitor compounds have been developed using quantitative structure–activity relationship studies and combinatorial chemistry to design molecules with specific physico–chemical characteristics (e.g., lipophilicity, positive charge at neutral pH, and presence of aromatic rings), and potentially able to overcome the limitations of previous compounds (34,38,48–50). Third-generation MDR modulators include tariquidar (XR9576), zosuquidar (LY335979), ONT-093 (OC144-093), and laniquidar (R101933) and have increased specificity, potency, and fewer pharmacokinetic interactions. They act through noncompetitive inhibition of the pump and bind it with high affinity (30,33). Nanomolar concentrations of third-generation agents are sufficient to reverse drug resistance in cancer in vivo (47, 51). Newman et al. reported OC144-093 reversed almost completely doxorubicin resistance in BDF1 mice engrafted with a Pgp overexpressing murine P388/ADR ascite tumor model (52). However, some of phase III studies of third-generation compounds, including the Pgp inhibitor, have been stopped due to toxicity (53,54) or failure to demonstrate an advantage over the use of the cytotoxic agent alone (55). Although several Pgp inhibitors have been tested in controlled clinical trials, no satisfactory results have been obtained so far (56). The difficulty in finding an ideal inhibitor is often associated with problems in specificity, potency, and intrinsic toxicity.

Drugs inhibiting ABC transporters lead to toxicity in vital organs (e.g., brain, testis, liver, kidney, and intestine) because of diminished protection from xenobiotics. Furthermore, the variability in transporter protein expression levels and polymorphisms of ABC transporters among individuals render clinical trials related to MDR in cancer therapy extremely challenging (57). Mutations in the ABC transporters, presence of other drug efflux or uptake transporters or channels, and problems with solubility, penetration, and distribution diminish the effectiveness of these inhibitors (58,59).

### NATURAL PRODUCT MODULATORS

Recently, many researchers initiate to screen natural products as MDR modulators for putative low toxicity chemosensitizers (60). Biologically active components obtained from plants and fungi (flavonoids, stilbenoids, coumarins, carotenoids, diterpenes, and cucurbitane derivatives) have been used as MDR modulators after purification and molecular characterization because of their putative low toxicity. Several studies have demonstrated that herbal compounds could act synergistically with anticancer agents and reverse MDR in cancer cells. Tissue distribution, substrates (61), and natural product modulators of ABC transporters are listed in Table 1. These natural modulators are substrates for ABC transporters. During coadministration, MDR modulators compete with cytotoxic agents for binding to the active site of the transporters and reduce drug efflux (4). The anticancer action of most phytochemicals is multifactorial.

In a recent study, 2 ω-3PUFAs; docosahexaenoic acid and eicosapentaenoic acid overcame drug resistance in MDR cells toward the Pgp and MRP1 substrates, decreasing the transporters activity in response to chemotherapy (62).

The activity of ABC transporters is directly related to the amount of cholesterol in the plasma membrane. A significant fraction of Pgp, MR1, and BCRP is embedded in cholesterol rich domains of the plasma membrane (63). ω-3PUFAs lowered the endogenous synthesis of cholesterol, decreased Pgp and MRP1 activity in chemoresistant colon cancer cells, and increased their chemosensitivity to doxorubicin.

There is accumulating evidence that many of the flavonoids can interact with the major drug transporters in the body, leading to alterations in the pharmacokinetics of substrate drugs, and thus their efficacy and toxicity. Flavonoids have been found to modulate Pgp mediated cellular efflux (64,65). Flavonoids inhibit Pgp ATPase by interacting directly with the vicinal ATP-binding site which is one of the potential mechanisms of inhibition of Pgp mediated efflux by flavonoids.
<table>
<thead>
<tr>
<th>ABC transporter</th>
<th>Distribution</th>
<th>Substrates</th>
<th>Natural products</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRP1 (ABCC1)</td>
<td>All tissues</td>
<td>Doxorubicin, Epirubicin, Etoposide, Vincristine, Methotrexate, Teniposide, Cisplatin, Vincristine, Thiopurines, 6-mercaptopurine, 6-thioguanine</td>
<td>Cannabinoids, Cepharanthine, Curcumin, Ginkgo biloba extract, Myricetin, Quercetin, Stemona curtisii root extract</td>
</tr>
<tr>
<td>ABCG2 (BCRP; MXR)</td>
<td>Placenta, Intestine, Breast, liver</td>
<td>Doxorubicin, Daunorubicin, Mitoxantrone, Topotecan, SN-38</td>
<td>3'-4'-7-Trimethoxyflavone, 6-Prenylchrysin, Acacetin, Biochanin A, Cannabinoids, Chrysirin, Curcumin, Daiztein, Eupatin, Fumitremorgin C, Genistein, Ginsenosides, Harmine, Hesperetin, Kaempferol, Naringenin, Plumbagin, Quercetin, Resveratrol, Rotenoids, Silymarin, Stilbenoids, Tectochrysin, Terpenoids, Tetrahydrocurcumin</td>
</tr>
</tbody>
</table>
Many flavonoids have been demonstrated to reduce transport activity of MRP1 (including apigenin, baicalein, kaempferol, naringenin, luteolin, morin, quercetin, myricetin, silybin) (23,67–70). In an anticancer strategy called ‘collateral sensitivity, MRP1 overexpression may lead to intracellular GSH depletion, which triggers apoptosis of the cells (71). Anticancer drugs are extruded by MDR transporters and consume ATP. The repletion of ATP from ADP by mitochondrial oxidative phosphorylation produces reactive oxygen species (ROS), causing cellular redox imbalance or oxidative stress, thus leading to apoptosis induction (72). A positive correlation between ROS production and MDR transporter expression have been reported indicating high concentrations of ROS induce MRP1 (73). MRP1 expression has been found to be regulated by the PI3K/Akt pathway (74).

Genistein increased daunorubicin accumulation in a doxorubicin-resistant small-cell lung cancer cell line (67). Resveratrol inhibited the genistein-induced binding of retinoid X receptor alpha to the promoter sequence of MRP2 gene; this mechanism could potentially contribute to the inhibition of genistein-induced MRP2 expression by resveratrol. These studies suggest that naturally occurring phytochemicals can potentially interfere with each other’s regulatory function on the cancer chemoprevention-related genes through a competitive mechanism (75). Silymarin was found to be the most effective MDR modulator in MRP1-expressing HEK239 cells (69). Quercetin and silymarin are able to inhibit MRP4 ATPase activity. Most potent inhibitors of MRP1-mediated efflux of carboxyfluorescein derivatives are sophoraflavanones A and H (76), silybin and morin (77), acacetin (78), and 8-prenylnaringenin (79).

There are contradictory effects reported for flavonoids such as quercetin, kaempferol, and galangin in different multidrug resistant cell lines. Flavonoids increased adriamycin efflux from HCT-15 colon cells (80), however quercetin and its methoxylated derivative were reported to inhibit rhodamine-123 efflux and revert MDR in MCF-7 breast cells (81). The schematic diagram of the possible mechanisms for MDR is shown in Fig. 1.

Genistein, daidzein, and quercetin inhibits daunorubicin transport but stimulates rhodamine 123 transport in lung cancer cells (82). Quercetin binds to purified Pgp and inhibits its activity efficiently (83). Apigenin had a similar effect. Lower binding affinities were observed for naringenin, a flavanone, and isoflavone genistein. Rutin is a highly hydrophilic, 3-O-glucoarhamnosyl derivative of quercetin showed a very low affinity interaction (84).

Summarizing, the majority of 22 flavonoids under study increased intracellular accumulation of both daunorubicin and vinblastine; however, apigenin, galangin, luteolin, and rhoifolin were inhibitors of vinblastine, but stimulators of daunorubicin transport. Fisetin and myricetin stimulated efflux of both substrates, whereas naringenin decreased accumulation of vinblastine but not of daunorubicin (85). The mechanism underlying the MDR inhibition by flavonoids lies in the blockade of the drug binding site, interference with the ATP hydrolysis process, alteration in integrity of cell membrane lipids, and decrease in Pgp or and MRP1 expression (84).

FIG. 1. Natural product affected multidrug resistance (MDR) mechanisms. PI3K = phosphoinositide 3-kinase; Akt = protein kinase B; RXR = retinoid X receptor; MRP2 = multidrug resistance-associated protein 2.
Curcumin (diferuloylmethane) is the principal component of the popular Indian spice turmeric, which is a member of the ginger family (Zingiberaceae) and has potent antitumor, antioxidant, and antiinflammatory properties (86). The anticancer properties of curcumin are mainly due to its ability to block the transcriptional nuclear factor kappa beta (NFκβ), which is a master regulator of inflammation, cell proliferation, apoptosis, and MDR in cancer cells. Curcumin was demonstrated to restore drug sensitivity in cancer cells overexpressing the MDR-linked Pgp, MRP1, and ABCG2 by directly inhibiting their functions, which leads to the increased accumulation of anticancer drugs and enhanced apoptosis (87). Curcumin has also been used as a therapeutic agent for reversing resistance to chemotherapy by inhibiting NF-κB signaling pathway (88).

Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL; Apo-2 ligand), a member of tumor necrosis factor (TNF) family, is a Type II transmembrane protein (89) whose extracellular domain can be released as a soluble cytokine (90,91). TRAIL displays an important regulatory performance in apoptosis by interacting with transmembrane receptors, mainly TRAIL-R1(TR/1)/DR4 and TRAIL-R2/TR/2)/DR5 (92). Loss of the TRAIL function is associated with drug resistance in tumor cells. It has been demonstrated that Pgp overexpression actually enhances the sensitivity of cancer cells to TRAIL-induced apoptosis. Mechanistic analysis established that TRAIL engagement of DR5 but not DR4 induces hyperactive ATP hydrolysis by Pgp, with consequent depletion of cellular ATP potentiating death induction by canonical TRAIL/DR5 signaling (93). Curcumin enhances apoptosis-inducing potential of TRAIL and sensitizes TRAIL-resistant cells in vitro (94). Enhanced TRAIL-induced apoptosis by curcumin was reported in chemoresistant ovarian cancer cells (95). Ganta et al. reported the curcumin administration inhibited NFκβ activity and downregulated Pgp expression in resistant cells.

Paclitaxel and curcumin combination therapy enhanced the cytotoxicity by promoting the apoptotic response in resistant ovarian cancer cells (96). Yan et al. studied enhanced oral bioavailability of docetaxel by curcumin in rats via downregulation of Pgp and cytochrome P4503A (97). Despite curcumin’s poor bioavailability, Phase I studies showed that curcumin is well tolerated (98). Phase II studies showed treatment with curcumin clinically improved the outcome of patients with advanced pancreatic cancer (99).

The anticancer potency of diarylheptanoids from the bark of black alder was investigated in human non-small cell lung carcinoma cell lines. Diarylheptanoid compounds was shown to have potential for overcoming MDR since they increased accumulation of doxorubicin via modulating Pgp activity (100). It was concluded that diarylheptanoids, which possess better inhibitory activities and selectivity than curcumin, could be used as novel natural compounds with a potential for treating cancer.

Kim et al. reported that resveratrol enhanced doxorubicin-induced cytotoxicity in doxorubicin-resistant breast cancer cells. Concurrent treatment with resveratrol and doxorubicin significantly increased cellular accumulation of doxorubicin by downregulating expression levels of ABC transporter genes, MDR1 and MRP1. Diminished tumor volume was observed with the concurrent treatment of resveratrol and doxorubicin in a xenograft model (101).

Bitter melon extract (BME) was reported to decrease the expression of MDR proteins; Pgp, MRP2, BCRP, and suppressed doxorubicin efflux in colon cancer cells overexpressing the 3 efflux proteins individually, suggesting that BME is a potent inhibitor of MDR function. BME was shown to suppress PXR expression, a xenobiotic sensing nuclear receptor and a transcription factor that controls the expression of the three MDR genes promoter activity (102).

Icaritin, an active ingredient isolated from the medical plant *Herba Epimedium*, reverses MDR of HepG2/ADR human hepatoma cells via downregulation of MDR1 and Pgp expression (103). Pien Tze Huang, a well-known traditional Chinese medical formula, has been used in China and Southeast Asia for centuries as a folk remedy for various types of cancer. Pien Tze Huang treatment in HT-29 SP colorectal cancer cells markedly inhibited the mRNA levels of ABC transporters ABCB1 and ABCG2, thereby contributing to reverse MDR (104). Paclitaxel was approved as a mitotic inhibitor used to treat patients with lung, ovarian, breast, head, and neck cancer. A preclinical study demonstrated that Pgp inhibitor zosuquidar trihydrochloride increased penetration of paclitaxel into the brain (105).

Ganoderic acid, a major ingredient in *Ganoderma lucidum*, reduced the mRNA and protein expression level of ABCB1 by inhibiting the activity of ABCB1 promoter and also inhibited the expression levels of MRP1 and MRP2 (106). Pgp and MRP1-mediated MDR were reversed by gypenoside aglycon (107) and Kuguacin J isolated from *Momordica charantia* leaves and plant sterols (108). Natural bioactive compounds such as flavonoids, chalcones, terpenoids, isothiocyanates, and nonprenylated rotenoids, inhibited BCRP (109–111).

Tamaki et al. evaluated the inhibitory effects of herbal extracts and isoflavonoids on BCRP-mediated methotrexate (MTX) transport. The results indicated that extracts of soybean, gymnema sylvestre, black cohosh, passionflower, rutin, and all isoflavonoids strongly inhibited BCRP-mediated transport of MTX. The addition of a 5-hydroxyl or 6-methoxyl moiety potentiated the inhibitory activity (112).

Fungal toxin fumitremorgin C (FTC) is an effective ABCG2 inhibitor discovered from natural sources but is neurotoxic (113). FTC was converted into the more potent, specific, and less toxic analog Ko143, which is more appropriate for clinical trials (114).

Inhibition of drug metabolism enzymes could become a promising strategy for reversing MDR. Zou et al. showed that...
ginkgolic acids, dihydromethysticin, methysticin, hyperforin, and quercetin significantly inhibited human P450 isoforms (115). Quercetin increased the bioavailability of pioglitazone in rats by inhibiting CYP3A (116). Rosemary extract enhanced antitumor effect of 5-fluorouracil by downregulation of thymidine synthetase enzyme and TK1 genes, which are essential for the synthesis of dTMP related to 5-fluorouracil resistance (117). Agosterol A, which is polyhydroxylated sterol acetate isolated from marine sponge, showed strong activity by completely reversing the resistance against vincristine, colchicine, doxorubicin, and etoposide in KB-C2 and KB-CV60 cells. Agosterol A inhibited ATP-dependent active efflux of drug through Pgp and MRP1. This suggests that AG-A diminishes MRP1-mediated drug resistance by inhibiting directly the ability of the pump to transport drugs and reducing the levels of the cellular glutathione required for drug efflux (118). Twenty-one compounds were isolated from *Illicium simonsii* and screened for their potential to restore the sensitivity of adriamycin resistant breast cancer cells and 5-fluorouracil-resistant hepatocellular carcinoma cells. Their MDR reversal abilities were investigated. Compounds with lower polarity generally had stronger sensitizing ability to the Pgp overexpressed in adriamycin resistant breast cancer cells. On the other hand, higher hydrophilic compounds seemed to exhibit a stronger reversal effect to the MRP overexpressed in 5-fluorouracil-resistant hepatocellular carcinoma cells (119). It has been shown that miRNAs play a critical role in drug transport and MDR (120). Herbal ingredients can modulate multiple pathways that are related to MDR, resulting in a synergistic therapeutic response. Honokiol, an active component isolated from TCM magnolia, combined with human epidermal growth receptor 2 (HER-2) inhibitor lapatinib is able to overcome therapy resistance, which is frequently clinically observed in HER-2 overexpressing breast cancer and is believed to be one of the reasons for the resistance to several anticancer agents (121).

miR-495 regulates MDR by modulating copper-transporting P-type adenosine triphosphatase A (ATP7A) expression in non-small cell lung cancer suggesting that miR-495 has a potential for the treatment of multidrug resistant non-small cell lung cancer patients with high ATP7A levels (122). Dasyntinib, which is a Src family tyrosine kinase inhibitor, reversed drug-resistance in drug-resistant cell lines suggesting that the Src inhibitors are potentially useful as an anti-MDR agent for the treatment of malignant tumor cells (123). Sequence-specific Pgp gene silencing by RNA interference (RNAi) may provide a more effective approach for downregulation of specific protein targets due to high specificity, limited toxicity, immunogenicity, and relative ease in synthesis. RNAi can be implemented by delivery of synthetic small interfering RNAs (siRNAs) or by gene expression of short hairpin RNAs using gene expressing vectors (124).

HZ08, a novel tetrahydroisoquinoline derivate, was discovered modulating MDR. In a study conducted by Feng et al., RNAi to MDR1 was introduced and the interaction between HZ08 and some classic agents (verapamil, rhodamine 123) with clear binding sites were investigated. It was concluded that HZ08 is the substrate of Pgp and acts as a multiple target modulator to invert the efflux function of Pgp (125).

Poly(ADP-ribose) polymerases (PARPs) participate in defense of the genome as efficient DNA-break sensors and signaling molecules as part of a survival program. In replicating cells, limited damage to DNA induces PARP activity that allows the activation of DNA repair pathways through the recruitment of DNA repair factors. The decision of the cell to engage the apoptotic pathway after genotoxic stress takes place downstream of p53 activation, and the molecular determinants that switch between DNA repair and cell cycle arrest and apoptosis have not been yet fully understood. As a consequence of the apoptosis option, PARPs are cleaved by caspases and inactivated to avoid futile repair. PARP inhibitors potentiate the cytotoxicity of DNA damaging agents. In postmitotic cells, ROS damage DNA, which in turn activates PARP and triggers apoptosis inducing factor translocation to the nucleus. The resulting chromatinolysis exacerbates PARP activity and NAD\(^+\) depletion. PARP under these pathological conditions might be responsible for the inflammatory status of the tissue/organ. PARP inhibitors are known to prevent both apoptosis-inducing factor translocation and inflammatory injury (126). Several inhibitors of PARP have been synthesized and explored for their therapeutic profile against cancer, either as single agents or combination regimens (127,128). PARP inhibitors are currently being evaluated for the treatment of tumors bearing loss-of-function mutations of BRCA1 and BRCA2 (129,130), in particular breast, ovarian, and prostate cancers. PARP inhibition sensitized cancer cells to DNA-damaging agents and potentiated the clinical efficacy of ionizing irradiation, alkylating agent temozolomide, topoisomerase inhibitor camptothecin, as well as platinum compounds such as cisplatin and carboplatin (131–134). It was reported that PARP inhibitor 3-aminobenzamide downregulates expression of PARP-1 at transcriptional and translational levels in cisplatin-resistant ovarian cancer cells (135). In a recent study, Michels et al. reported unexpected findings that nonsmall cell lung carcinoma cells resistant to cisplatin overexpress PARP1, constitutively exhibit high levels of PARP enzymatic activity and rely on it for their survival, as PARP inhibition kills nonsmall cell lung carcinoma cells that harbor hyperactivated PARP1. CDDP-resistant cells respond more efficiently than their wild-type counterparts to PARP inhibitors. PARP inhibitors were suggested to be the most beneficial for nonsmall cell lung carcinoma patients relapsing after CDDP-based chemotherapy (136). Barber et al. investigated tumor material from patients who developed resistance to the PARP inhibitor olaparib. BRCA2 secondary mutations were identified in olaparib-resistant metastases. This study has important implications in regard to the management of patients with BRCA1 or BRCA2 mutations, the choice of therapy and also the development of biomarker assays that can predict response (137).
NANOPARTICLE DRUG DELIVERY IN CANCER

Nanoparticle carriers have shown to be of benefit in the treatment of cancer. They target tumors and deliver single or multiple drugs with synergistic effects via endocytosis, avoiding activation of efflux pumps in the cell membranes and drug resistance (138). There are various nanoparticle carriers used for combination therapies to overcome drug resistance such as liposomes, polymer-based nanoparticles and micelles, dendrimers, carbon-based nanoparticles, metallic and magnetic nanoparticles, and layer-by-layer particles (139).

Six nanoparticle-based cancer therapies have been clinically approved. Liposomal formulations of anthracyclines and cytarabine, albumin-bound nanoparticle, and polymeric nanoparticle formulations of paclitaxel are among the approved ones (140). Intracellular accumulation of drugs carried by nanoparticles was shown to increase significantly in contrast to free drugs, excluded significantly from the MDR cancer cells. Enhanced drug accumulation caused partial restoration of drug sensitivity in MDR cancer cells.

The addition of targeting ligands to liposomes, such as the mAb 2C5 with doxorubicin (141) and an anti-HER2 mAb with paclitaxel (142), are in the preclinical phase, whereas others are already undergoing clinical trials. Polyethylene glycol (PEG) addition to liposomes increases circulation time. Polymeric nanoparticles can covalently attach to or encapsulate therapeutic cargo. Capsules are formed spontaneously by mixing the drug with the polymers. Hydrophobic drugs are carried in solid core of nanospheres.

Drugs covalently bound to water-soluble polymers, which increase circulation time and limit toxicity to normal tissues (139). Polymers have been refined with the addition of PEG to avoid opsonization and increase circulation time, the use of targeting ligands, and the use of pH-sensitive or hypothermic polymer conjugates. Currently, FDA-approved 2 polymers, poly lactide (PLA) and poly (lactide-co-glycolide) (PLGA), which are polymeric biodegradable nanoplatforms used for synthesis of nanomedicines, and many more are undergoing clinical trials (143). Apte et al. reported that intravenous injection of PEGylated liposomal doxorubicin multifunctional immunoliposomes into mice with tumor xenografts, in comparison with the nonmodified liposomes caused a significant reduction in tumor growth and enhanced therapeutic efficacy of the drug in both drug-resistant and drug-sensitive mice, was obtained (144). Zhu et al. used poly (lactic-co-glycolic acid) nanoparticle (PLGA NPs) for codeelivery of doxetaxel as a model anticancer drug together with 0, 10, 20, and 40% vitamin E TPGS (d-alpha tocopheryl polyethylene glycol succinate). They found that the doxetaxel-loaded PLGA NPs formulation was 5.57-fold effective than the free doxetaxel and that the doxetaxel-loaded PLGA NPs of 10% or 20% TPGS further was 11.85- and 52.7-fold effective than the doxetaxel-loaded PLGA NPs containing no TPGS (145). Zeng et al. demonstrated that polyester-based hyperbranched dendritic-linear (HBDL)-based NPs carrying doxorubicin effectively overcame microsomal glutathione transferase 1-mediated drug resistance in breast cancer cells.

Doxorubicin-loaded HBDL NPs were translocated across the membranes of resistant cells via active endocytic pathways and transported to lysosomes, mitochondria, and the endoplasmic reticulum. A significantly lower amount of doxorubicin accumulated in the cytoplasmic compartments of the resistant cells treated with free doxorubicin (146). The results of this study suggest that HBDL NPs can modulate subcellular drug distribution by specific endocytic and trafficking pathways resulting in drug delivery that alters enzyme levels and cellular signaling pathways and increases induction of apoptosis. In a recent study, folic acid conjugated polyethyleneimine coated on the hollow mesoporous silica nanoparticle HMSNPs surfaces enhanced siRNA binding capability. In vitro pH-responsive drug/siRNA codeelivery experiments were conducted on HeLa cell lines with high folic acid receptor expression. The pH-responsive intracellular drug/siRNA release greatly minimized the prerelase and possible side effects of the delivery system. Doxorubicin and siRNA against the Bcl-2 protein were delivered simultaneously into the HeLa cells, leading to suppression of the expression of the anti-apoptotic protein Bcl-2 and enhancement of therapeutic efficacy (147).

Dendrimers are highly branched, star-shaped macromolecules with globular structures. They are defined by 3 components: a central core, branches of repeating units, and an exterior surface with functional surface groups. Hydrophobic drugs such as doxorubicin and paclitaxel are frequently attached covalently to dendrimers (139). Multiple clinical trials are ongoing using amphiphilic diblock copolymer forming micelles to deliver paclitaxel to treat breast, nonsmall cell lung cancer, and advanced pancreatic cancer (148). Carbon nanotubes (CNTs) have the ability to enter cells using “needle-like penetration” and deliver molecules into the cytoplasm. Carbon nanotubes have a very large surface per weight ratio providing attachment sites for ligands, as well as an internal cavity that can contain either therapeutic or diagnostic agents. They possess good thermal stability and are resistant to many chemicals. Carbon nanotubes have electrical and thermal conductivity, which might be beneficial in cancer therapy applications such as thermal ablations. Cheng et al. designed PLGA functionalized CNTs to reduce toxicity, provide attachment sites for proapoptotic caspase-3 (CP3), and tune the temporal release profile of CP3 within bone cancer cells. Their results showed that CP3 was able to attach to functionalized CNTs, forming CNT-PLGA-CP3 conjugates. They also observed that this conjugate efficiently transduced cells at doses as low as 0.05 μg/ml and suppressed cell proliferation up to a week with no further treatments (149).

Current approaches using nanotubes include incorporation of the drugs such as doxorubicin and paclitaxel, with nucleic acids including antisense oligonucleotides and short-interfering RNAs (siRNAs), and the use of nanotubes as contrast agents for imaging (150). The gold core of gold nanoparticles...
is considered to be nontoxic and the therapeutic drug can be released from the conjugate due to their photo-physical properties. PEG can be attached to the surface of metallic nanoparticles to increase stability and circulation time (148). In a Phase I clinical trial, TNFα bound to colloidal gold is under investigation to treat advanced solid tumors such as sarcomas and melanomas (151). Nanodrugs with an increased circulation time, precise multiple targeting mechanisms, enhanced drug accumulation at the tumor site, delivered into the cytoplasm and/or nuclei of cancer cells, also having the ability to carry drug combinations, are attractive treatment options in overcoming MDR (139).

CONCLUSION

Drug resistance may develop against chemotherapeutic agents used for the treatment of cancer, reducing the efficacy of a drug in curing or improving patient symptoms: in fact, drug resistance to chemotherapy remains a major challenge in the treatment of cancer. Overexpression of several ABC drug transporters has a significant impact on cancer chemotherapy, leading to the development of MDR and treatment failure. Considering the growing drug resistance (78), it is crucial to clarify the mechanisms causing multiple drug resistance and adopt new strategies to overcome drug resistance, and to discover new combination therapies for treating cancers. MDR develops against effective anticancer drugs. Many different compounds, including natural herbal compounds have been used alone or in combination with anticancer drugs and represent a useful means in overcoming MDR (152). The primary aim of drug development programs is to design drugs that act on single selective disease targets to obtain highly effective and safe drugs with minimal side effects (153). The target of current MDR studies is to discover novel compounds and strategies to inhibit the function or expression of ABC transporters reverse MDR and sensitize MDR cancer cells to anticancer drugs in cancer patients.

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REFERENCES


