Chapter

Overcoming
P-Glycoprotein-Mediated
Doxorubicin Resistance

Suree Jianmongkol

Abstract

Intracellular concentration of doxorubicin in target cancer cells is a major determinant of therapeutic success of doxorubicin-based regimens. As known, doxorubicin is a substrate of P-glycoprotein (P-gp), the drug efflux transporter in the ABC superfamily. High expression level of P-gp in cancer cells can prevent intracellular accumulation of doxorubicin up to its effective level, leading to doxorubicin resistance and treatment failure. Moreover, these P-gp-overexpressed cells display multi-drug resistance (MDR) phenotype. Regarding this, application of P-gp modulators (suppressor of P-gp activity and expression) is likely to reverse MDR and restore cell sensitivity to doxorubicin treatment. In searching for potential chemo-sensitizer against resistant cancer, a number of phytochemicals or dietary compounds have been studied extensively for their P-gp modulating effects. Furthermore, combination between doxorubicin and P-gp modulators (e.g., plant-derived compounds, siRNA) given through specific target delivery platforms have been an effective strategic approach for MDR reversal and restore doxorubicin effectiveness for cancer treatment.

Keywords: P-glycoprotein, doxorubicin-resistance, P-gp modulators

1. Introduction

Multidrug resistance (MDR) is one of the major factors contributing to a failure of doxorubicin for cancer treatment. Typically, the loss of cell sensitivity to chemotherapy is not limit to only doxorubicin and anthracycline derivatives. The MDR phenomenon evidently extends across various structurally-unrelated anticancer drugs, regardless of their molecular targets [1–3]. Hence, MDR development in cancer cells can simultaneously reduce the effectiveness of several cytotoxic drugs, leading to chemotherapeutic failure. Consequently, patients need higher doses of the anticancer agents to achieve therapeutic success. Either intrinsic or acquired resistance to doxorubicin-based chemotherapy has been attributed to various mechanisms including high expression of the drug efflux transporters, alteration of cell cycle checkpoints and apoptotic signals, increased drug detoxification and DNA repair processes [4–6]. Regarding this, MDR reversal can be one of the strategic approaches to enhance the efficacy, without increased adverse events, of doxorubicin.

This chapter focused on the most studied drug efflux transporter P-glycoprotein (P-gp) and its role in doxorubicin resistance in chemotherapy. In addition, some strategic approaches to conquer P-gp-based MDR in cancer treatment were also described.
2. The drug efflux transporter: P-glycoprotein

Drug transporters can be grouped, according to their transport direction, into uptake and efflux pumps. Most of the known efflux transporters particularly P-glycoprotein (P-gp or MDR1; encoded by \( \text{ABCB1} \)), multidrug resistance protein 1 (MRP1, encoded by \( \text{ABCC1} \)), multidrug resistance protein 2 (MRP2, encoded by \( \text{ABCC2} \)) and breast cancer resistance protein (BCRP; encoded by \( \text{ABCG2} \)) are members of the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily. The ABC transporters require ATP hydrolysis for their transport activity across plasma membrane in the secretive direction. These efflux transporters share similar structural assembly across plasma membrane, composing of a membrane-spanning \( \alpha \)-helix structure as a transmembrane domain (TMD) and a relatively hydrophilic ATP-binding site in nucleotide binding domain (NBD). High activity and expression of these ABC drug efflux pumps is a major contributing factor for development of MDR phenomenon in cancer cells \([1, 4]\).

Among the ABC efflux transporters, P-gp is the first and most studied transporter for MDR development in chemotherapy and drug-transporter-related interaction issues. This transporter was first identified from its involvement with multidrug-resistance in cancer cells. Particularly, overexpression of P-gp in cancer cells, either intrinsic or acquired, has been strongly associated with MDR occurrence, thereby P-gp becomes a promising target for development of chemosensitizers.

2.1 Overview of P-gp (structure, function, location, expression, and MDR)

P-gp (MW approximately 170 kDa) is a single polypeptide with 1280 amino acids arranging in two duplicated units of a 6 \( \alpha \)-helix structure hydrophobic TMD with linkage to a hydrophilic NBD (Figure 1) \([1, 2, 7]\). These two TMD with the total of 12 helices forms together into one channel as the membrane crossing passage. A substrate binds to the drug-binding site in the TMD whereas an ATP binds to the NBD. After ATP binding, ATP undergoes hydrolysis into ADP for energy to activate P-gp action through protein conformational alteration \([7, 8]\). This transporter, then, is able to move its substrates across lipid bilayer structure of plasma membrane to extracellular environment.

2.1.1 P-gp and its normal physiological functions

P-gp is constitutively located in the apical surface of either epithelial or endothelial linings of various normal tissues/organs such as adrenal glands, intestine, liver, kidney, pancreas, placenta, capillary vessels in the brain and testes \([2, 7–10]\). Some organs such as prostate, skin, heart and skeletal muscle have low constitutive expression of P-gp. It should be noting that expression level of P-gp varies in each organ. For example, the numbers of P-gp in colon and ileum are higher than those in jejunum, duodenum and stomach \([11, 12]\). The tissue distribution of P-gp indicates that this transporter normally serves as an intrinsic determinant of oral drug bioavailability and drug disposition \([13–18]\). Intestinal P-gp can influence the absorptive amount of its drug substrates, except those in BCS class I (i.e., high permeability and high solubility drugs such as verapamil), into the body after orally taken \([13, 19–21]\). The constitutive expression of P-gp at the mucosal surface in the lower gastrointestinal (GI) tract (i.e. jejunum, ileum, and colon) may prevent an uptake of its substrate, and perhaps also facilitate GI excretion. Moreover, the interplay between P-gp and the major phase I drug metabolizing enzymes (e.g. cytochrome P450, CYP450) can be anticipated due to their substrate similarity \([22]\).
As such, P-gp and CYP3A4 act in concert to affect metabolic biotransformation of their substrates such as paclitaxel in intestine and liver, influencing the oral drug bioavailability [22–24]. Localization of P-gp in the blood-organ barriers such as brain or testis prevents drug penetration into such organ systems such as brain, testes [13, 23, 25, 26]. The presence of P-gp on the brush border of nephron proximal tubule and hepatocytes involve with excretion of drugs and endogenous substrates into the urine and bile [13, 27]. To this end, P-gp can be considered as the protective mechanism against xenobiotics as well as pharmacokinetic influencer particularly on absorption, distribution and disposition.

2.1.2 P-gp expression and signaling pathways

Expression of P-gp at plasma membrane involves several cellular processes that linking to P-gp mRNA and protein expression. The regulatory mechanisms have been largely associated with (1) activation or inactivation of oncogenes (e.g., Ras, c-Raf) and transcriptional process, (2) MDR1 translation into P-gp and post translational modification, protein trafficking, and (3) P-gp turn over. It has been reported that the dysregulated microRNA levels (e.g., miR-21, -27a, -451, -130a, -298) could cause MDR development in various cancer cells [28–34]. For example, miR-130 was correlated to MDRI/P-gp overexpression, and cisplatin resistance in SKOV3/CIS cells [32]. Overexpression of miR-27a and miR-451 was linked to increased MDRI expression and MDR phenotype in resistant cancer cells A2780DX5 and KB-V1 [28].

Overexpression of P-gp particularly in MDR phenomenon has been evidently connected to up-regulation of MDRI gene through alteration of various signaling pathways and transcription factors. Example of the transcriptional factors involving in MDRI transcription are nuclear factor-κB (NF-κB) [35, 36], Y-box binding...
Advances in Precision Medicine Oncology

protein-1 (YB-1) [37, 38], activator protein-1 (AP-1) [39], and hypoxia-inducible factor-1 (HIF-1) [38, 40]. The activities of these transcription factors have been linked to various signal transduction pathways, particularly the two major cell survival signaling cascades i.e. (1) the mitogen-activated protein kinase (MAPK) [37, 41], and (2) the phosphatidylinositol 3-kinase (PI3K) pathways [42, 43]. It has been shown that hyperactivation of either MAPK/ERK1/2 or PI3K/Akt/NF-κB signaling pathways results in overexpression of P-gp in doxorubicin-resistant cells such as lung, breast and ovarian cancer cells [44–49]. An up-regulation of P-gp expression in vincristine-resistant human gastric cancer cells was associated with activation of the p-38/MAPK pathway [50].

After activation and translocation into nucleus, transcription factors such as NF-κB and YB-1 (Y-box binding protein) bind to MDR1 promoter region, leading to initiation of MDR1 transcription. Increase in YB-1 nuclear activity is related to P-gp-mediated development of MDR in several cancers including breast cancer, lung cancer, ovarian cancer, colorectal cancer, prostate cancer and osteosarcoma [38]. In response to cell stress such as hyperthermia, viral infection and chemical assault, the survival Akt and MAPKs signaling would be activated, and subsequently increase YB-1 expression and translocation into nucleus for its MDR1-transcription activity [51]. Doxorubicin is a known P-gp inducer in various cancer cells. Doxorubicin up-regulates MDR1 gene expression via the MAPK/ERK1/2 signaling that linked to activation of YB-1 in B-cell lymphoma [37]. Moreover, upregulation of P-gp has been reported after prolonged exposure to various functional unrelated compounds, leading to the loss of drug efficacy and safety [52]. Examples of the known P-gp inducers include anticancer (e.g., cisplatin, doxorubicin, etoposide vinblastine), antidepressants (e.g., trazodone, St. John’s Wort), anticonvulsants (e.g., carbamazepine, phenytoin), anti-HIV (e.g., saquinavir, indinavir, tenofovir), immunosuppressants (e.g., cyclosporine, tacrolimus), steroids (e.g., dexamethasone) [52–54]. It is worth noting that certain CYP450 inducers such as rifampin and St. John’s Wort are able to up-regulate P-gp expression, possible sharing through the PXR regulation [55, 56]. Prolonged exposure to rifampin and St. John’s Wort in human led to increased intestinal P-gp level, and increased digoxin absorption [57, 58]. Since, P-gp-mediated MDR in cancer is largely due to up-regulation of P-gp expression, better understanding of the signaling proteins and transcription factors will provide a promising targets in overcoming MDR for anticancer chemotherapy.

2.1.3 P-gp and multi-drug resistance in cancer

Overexpression of P-gp has been strongly correlated with chemo-resistance and cancer relapses in several cancer patients such as breast cancer, adult acute myeloid leukemia, pheochromocytoma patients, leading to poor prognosis from therapeutic failure in patients receiving chemotherapy [1, 59–62]. Accordingly, P-gp is intrinsically expressed in various cancer types, particularly those derived from tissues with high basal MDR1 expression levels such as colon, kidney and liver tissues. Being a transmembrane efflux pump, P-gp serves as a cellular defense mechanism against drug assault by limiting intracellular drug accumulation up to toxic threshold level. Regarding this, the susceptibility of cancer to anticancer drugs being P-gp substrate varies, depending on intrinsic expressed P-gp levels. Certain types of cancers may be classified as poor responder showing their unresponsiveness to chemotherapy regimens containing P-gp substrates. For example, prostate cancer appears to be better responder to chemotherapy, as compared to colorectal or renal cancers [63, 64]. Moreover, some cancers such as leukemia, lymphoma and breast cancer having low levels of intrinsic P-gp expression, and thus initially respond well to
chemotherapy. Later, after repeated treatment, the expression level of P-gp markedly increases, and those cancers display multi-drug resistance (MDR) phenotype [1, 65, 66]. This acquired MDR phenomenon can be viewed as cellular adaptive survival response to cytotoxic challenge.

2.2 Substrates and modulators

Examples of substrates and modulators of P-gp are listed in Table 1.

2.2.1 Substrates

Human ABC efflux transporters demonstrate their broad substrate specificity toward structurally diverse lipophilic compounds. Most of their substrates are weakly amphipathic and hydrophobic planar structure with aromatic ring and positively charged nitrogen atom [52, 54, 67, 68]. Examples of P-gp substrates are anticancer drugs (vinca alkaloids, anthracyclines, and epipodophyllotoxins), cardiovascular drugs (e.g., digoxin, quinidine, diltiazem, verapamil), anti-microbial agents (e.g., doxycycline, erythromycin, itraconazole), anti-viral drugs (e.g., indinavir), anticonvulsants (e.g., phenytoin), acid blockers (e.g., cimetidine), immunosuppressants (e.g., cyclosporine, tacrolimus), steroids (e.g., aldosterone, cortisol, dexamethasone), opioids (loperamide, morphine).

| Substrates (Anti-cancer drugs) | Inducers (Anti-cancer drugs) | P-gp modulators |
|-------------------------------|-----------------------------|-----------------|
| Actinomycin D                 | Daunorubicin                | Small molecule  |
| Colchicine                    | Docetaxel                   | Small molecule  |
| Docetaxel                     | Doxorubicin                 | Inhibitors      |
| Doxorubicin                   | Flutamide                   | Curcumin        |
| Daunorubicin                  | Paclitaxel                  | First generation|
| Epirubicin                    | Vinblastine                 | Cyclosporin A   |
| Etoposide                     | Vincristine                 | Verapamil       |
| Idarubicin                    |                             | Reserpine       |
| Imatinib                      |                             | Imatinib        |
| Methotrexate                  |                             | VX-710 (Biricodar)|
| Paclitaxel                    |                             | Verapamil       |
| Teniposide                    |                             | Reserpine       |
| Topotecan                     |                             | Imatinib        |
| Vinblastine                   |                             | NV-101          |
| Vincristine                   |                             | Sorafenib       |
| (Anti-cancer drugs)           | (Anti-cancer drugs)         |                 |
| (Inducers)                    | (Inducers)                  |                 |
| (P-gp modulators)             | Direct inhibitors           |                 |
| Substrates (Anti-cancer drugs) | Suppressors of expression   |                 |

Table 1. Selected substrates, inducers and modulators of P-gp.
2.2.2 Modulators

Modulators suppress P-gp activity through either (1) direct inhibition of P-gp function by either competitive or non-competitive inhibitors; or (2) suppression of P-gp expression levels by interferences with either transcription, translation/post-translation, and degradation processes.

2.2.2.1 Direct inhibition of functionally active P-gp

The direct inhibition of active P-gp can be attributed to the interaction between chemicals and P-gp at either TMB or NBD [67–69]. Any compound such as tyrosine Kinase Inhibitors interferes with ATP binding or hydrolysis in NBD site can reduce P-gp transport action [70]. Chemicals identified as small molecule P-gp inhibitors such as amiodarone, diltiazem, verapamil bind to substrate binding sites or allosteric sites in TMB, resulting in interference on substrate binding and transport. It has been reported that certain compounds such as cyclosporine A could exert their inhibitory action by interfering with substrate recognition and ATP hydrolysis [8, 67–69]. It is not surprising that these TMB type inhibitors and substrates share many common molecular features such as hydrophobic planar structure. In addition, due to the diversity in chemical structure of P-gp inhibitors, establishment of the structure activity relationship (SAR) of P-gp inhibitors is very challenging. The structure pattern of the inhibitors contains planar rings and basic nitrogen atom within an extended side chain of the aromatic ring. The presence of tertiary amino groups, in comparison with primary and secondary amine, increases the anti-MDR potency considerably. Furthermore, the presence of nitrogen atom in non-aromatic ring apparently increases inhibitory action of the compounds [71]. Examples of P-gp inhibitors are calcium channel blockers (verapamil, diltiazem), and various phytochemicals such as flavonoid and steroidal compounds (e.g., quercetin, resveratrol), indole alkaloids and polycyclic compounds (e.g., capsaicin, piperine, rhinacanthin C) [66, 72–74].

Ideally, the P-gp inhibitors should be potent and selective to P-gp function at target cells/tissues, with no systemic side effects. To date, there are four generations of small molecule inhibitors. The first generation inhibitors are known drug substrates of the ABC transporters such as verapamil, cyclosporine A, tamoxifen and quinidine [75]. They were not specifically designed to be P-gp inhibitors, and could not display good clinical outcomes in their MDR reversal activity. The clinical disappointment could be due to their weak inhibitory potency against the MDR transporters including P-gp, and their pharmacokinetic interactions with chemotherapeutic agents, leading to the need of high doses and intolerable adverse effects [1, 76]. Next, the second generation inhibitors such as valspodar (cyclosporine A derivative) were developed, based on structure activity relationships of the first generation compounds, in order to improve potency, specificity, and to reduce systemic toxicity. Although this group of inhibitors demonstrated their improvement in inhibitory potency, their clinical outcomes were still unsatisfied due to their pharmacokinetic interaction with the anti-cancer drugs via inhibition of cytochrome P450, and their severe toxicity [75, 77]. Subsequently, the third generation P-gp inhibitors such as elacridar, tariquidar and zosuquidar were developed in order to address the limitations of the second generation compounds. These inhibitors elicit no effect on CYP P450 metabolism, therefore they are unlikely to affect the plasma concentrations of anti-cancer drugs. They were also more potent and selective P-gp inhibitors, effectively working in nanomolar concentration range. However, the potent P-gp inhibitor tariquidar can be either substrate or inhibitor of P-gp depending on its given dose [78]. To date, the clinical efficacy for MDR reversal of this generation has yet completely satisfied, its effectiveness possibly also depends on given dosage and intrinsic tumor properties.
Currently, phytochemicals or natural compounds with MDR reversal activity have been subject of interest in searching for new effective chemo-sensitizer against cancer. This group of inhibitors obtained from natural sources is classified as the fourth generation inhibitor. Numerous phytochemical researches on pharmacological activities and pharmacokinetics have revealed that plant-based compounds elicit a broad spectrum of pharmacological actions such as anti-cancer, anti-oxidant, anti-microbial, anti-inflammation, etc. In addition, these plant-based compounds, depending upon its molecular structure, may interfere with P-gp and metabolizing enzymes, leading to the concerning issues in drug bioavailability and pharmacokinetic drug interactions. The advantages of the fourth generation inhibitors in part rely on their natural origin with long history of uses in dietary or health supplements and in traditional medicine. It may be able to presume that this group of inhibitors derived from known edible products possessed less toxicity and more tolerable than those of the previous generation compounds. Evidently, even vegetables (e.g., bitter melon), spices (e.g., black pepper, turmeric) or fruits (e.g., orange, grapefruit) also contain substances that could inhibit P-gp and other efflux transporters in the ABC superfamily [72–75, 77, 79–82]. Their competitive inhibition against the efflux transporters enhance cytotoxicity of anticancer drugs such as doxorubicin and vinblastine, leading to potential MDR reversal in various cancer cells. However, the inhibitory potency of these plant-based compounds against P-gp activity might be low. Their IC50 values obtained from the in vitro cell culture models appear to be in micromolar range. Thus, this group of inhibitors is unlikely a good MDR reversing agent through direct P-gp inhibition at MDR cancer cells in clinical setting. In addition, the interference of P-gp activity of these compounds in pharmacokinetic aspect may influence on P-gp-related ADME and bioavailability of chemotherapeutic drugs that concomitantly given. Nevertheless, the opportunities of further development into effective chemosensitizers cannot be excluded. Better understanding of QSAR may enable to facilitate chemical modification of these identified plant-based P-gp inhibitors to generate more potent and high selective P-gp inhibitors. Furthermore, several plant-based compounds (e.g., curcumin, resveratrol, quercetin) have been demonstrated their potential in down-regulation of P-gp and other key regulators in transporter-independent MDR mechanisms [75, 82–86].

In addition to small molecule inhibitors, monoclonal antibodies can be another alternative approach in inhibiting P-gp activity. Theoretically, any agents that specifically affect lipid-protein interactions or protein structure of targeted P-gp can be developed into P-gp inhibitor. Typically, monoclonal antibody can be developed to specifically recognize and bind to its target protein, leading to inhibition of changes in protein conformation. Regarding this, human P-gp-specific antibodies UIC2, MRK-16 and 4E3 reacted specifically to the extracellular loop of both halves of P-gp, and disabled P-gp transport activity [87]. Consequently, treatment cancer cells with these antibodies resulted in increased concentrations of anticancer drugs (e.g., vincristine, actinomycin D, doxorubicin, paclitaxel) within the cells, and improve drug effectiveness [87–91]. In athymic mice model, MRK16 was demonstrated its ability to significantly reduce tumor mass [92]. Further clinical studies of human P-gp-specific antibodies are needed to conduct in terms of safety and efficacy.

2.2.2.2 Suppressor of P-gp expression

In addition to direct inhibition, reduction of P-gp activity can arise from decrease of protein expression at plasma membrane. Interference on transcription and translation of MDR1 gene, resulting in reduction of P-gp expression, can be another approach to overcome MDR in cancer. Several innovative tools targeting at MDR transcription or mRNA including small molecules, antisense oligonucleotides, hammerhead ribozymes and RNA interference strategies have been employed.
2.2.2.2.1 MicroRNA and RNA interference (RNAi) technologies

Application of microRNA and RNAi technologies with either small-interfering RNA (siRNA) or small hairpin RNA (shRNA) to specifically silence MDR1 expression in cancer cells with MDR phenotype has been demonstrated their effectiveness in down-regulation of MDR1 and P-gp expression with paralleled increases in drug accumulation and improved sensitivity to treatment. MicroRNAs (miRNAs) are small non-coding RNA molecules that can inhibit ABCB1 mRNA translation processes \[93, 94\]. A number of miRNAs have been studied on their ability to down-regulate P-gp expression and restore cell sensitivity to P-gp drug substrates in drug resistant cells \[34\]. For example, miRNA-4539 could increase doxorubicin-mediated cell death in MDA-MB-231 breast cancer cells \[93, 94\].

The RNAi technologies involve either transient gene-silencing by siRNA or stable inhibition by MDR1 shRNA-transfected on plasmid DNA of MDR cancer cells. Treatment with siRNA against MDR1 increases drug-mediated cytotoxicity in various MDR cancer cells such as paclitaxel in MDR1 ovarian cancer cells and doxorubicin in doxorubicin-resistant breast cancer cells \[95\]. In addition, siRNA was able to significantly reduced size of doxorubicin-resistant xenograft in a mouse model \[96\]. MDR1 ShRNA transfected in taxol-resistant human ovarian cancer cell line A2780 effectively down-regulated P-gp expression, and enhanced paclitaxel-mediated toxicity in this cells \[97\].

Selective suppression of P-gp/MDR1 expression with either microRNA or RNAi technologies offers the novel approach to specifically combat P-gp-based MDR in cancer, and re-sensitize the MDR cells to chemotherapeutic agents. However, for their therapeutic applications, there are several challenges required especially the effective miR/RNAi delivery to target cancer cells, design of expression vectors for shRNA, systemic stability and degradation, and safety of patients.

2.2.2.2.2 Small molecules as P-gp down-regulator

Numerous small molecules particularly those in the fourth generation of P-gp inhibitors such as curcumin, ginsenoside, quercetin and resveratrol have been demonstrated their ability to reduce P-gp function in the MDR cancer cells via down-regulation of P-gp expression \[83–85\]. By targeting at the signaling pathways related to transcription process of MDR1, several plant-based compounds suppress P-gp expression in the resistance cells and improve chemo-sensitivity to anticancer drugs. For instance, the P-gp modulating effect of asiatic acid, ginsenoside, isoquinoline alkaloids (e.g. cephartamine, tetrandrine) resulted from their blockade of MAPK/ERK1/2 or PI3K/Akt pathways in MDR cancer cells \[86, 98–101\]. Another isoquinoline alkaloid berberine inhibited P-gp expression and enhanced doxorubicin-mediated toxicity in MCF-7 cells through down-regulation of AMPK-HIF1α signaling cascade \[102\]. Anti-MDR property of natural curcuminoids (e.g., curcumin, bisdemethoxycurcumin) involved with inhibition of human MDR1 gene expression in MDR cervical carcinoma KB-V1 cells \[103\]. In addition, certain compounds such as a natural marine product Et743 inhibit MDR1 transcription via blocking its promoter activation \[104\].

3. Doxorubicin and P-gp

Doxorubicin is one of the most effective cytotoxic anticancer drugs. This drug has been used for combating various types of cancers such as cancers of breast, ovary, prostate, stomach, thyroid; small cell cancer of lung; squamous cell cancer of
head and neck; multiple myeloma; Hodgkin’s disease; lymphomas; acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML). Unfortunately, the uses of doxorubicin can be limited because of its dose-related toxicity (e.g., nausea, vomiting, hair loss, leucopenia, cardiomyopathy, heart failure) and high MDR incidence [105, 106]. Despite the good clinical therapeutic responses are seen in patients receiving doxorubicin in the earliest stage of treatment, multi-drug resistance may later develop and lead to treatment failure.

One of the major mechanisms responsible for doxorubicin-induced MDR is up-regulation of MDR1/P-gp expression. Doxorubicin is an anthracycline derivative with a four-membered ring system containing an anthraquinone chromophore, and an aminoglycoside (Figure 1). This molecular structure accommodates its interaction with major MDR efflux transporters in the ABC superfamily proteins. It has been well established that doxorubicin and other anthracycline derivatives are P-gp substrates with ability to up-regulate P-gp/MDR1 expression after repeated exposure in various cancer cells such as breast and lung cancers as well as in vivo and in clinical settings [66, 107, 108]. For instance, lung perfusion with doxorubicin resulted in an increase of MDR1 RNA in patients with sarcoma pulmonary metastases [18]. The P-gp-overexpressed cancer cells would have intracellular doxorubicin concentration below its effective threshold level. Consequently, cancer cells increasingly survive from doxorubicin-mediated cytotoxicity. In this circumstance, titrating dose up to overcome MDR may not enable to achieve a successful outcome due to dose-limiting toxicity. Because the adverse effects of doxorubicin and other anti-cancer drugs are mostly concentration-dependent, increasing doses can produce higher degree of severity and unendurable adverse events, leading to patient’s intolerability and even fatal outcome. Addition of other cytotoxic drugs into doxorubicin-based regimens may not also enable to obtain a chemotherapeutic success, if those drugs are also substrates of the MDR transporters.

Generally, clinical efficacy of doxorubicin depends on its pharmacokinetics after systemic exposure influencing (1) the therapeutic concentration at target organs, and (2) the homogeneity of drug distribution in the cancerous tissues particularly solid tumor. In addition, it is very critical that doxorubicin accumulates within the targeted cancer cells at the level greater than its cytotoxic threshold to elicit its pharmacological actions.

3.1 P-gp effects on doxorubicin’s Pharmacokinetics aspect

Doxorubicin is poorly absorbed through GI with low bioavailability (approximately 5%) after orally taken, due to its instability in stomach acidic pH and CYP450 biotransformation in liver. In addition, doxorubicin can induce cytotoxicity in normal tissue. Currently, doxorubicin is commercially available for cancer treatment in injection dosage form. Due to its lipophilicity, doxorubicin moves through plasma membrane into the cells via passive diffusion, and its extent of tissues/cellular permeation and cellular retention can be limit by the existence of efflux transporters particularly P-gp. Apparently, doxorubicin is extensively distributed to several organs such as liver, heart, kidney after injection. Being the efflux transporters, P-gp has a significant impact on doxorubicin distribution to certain target tissues such as brain, testes [109, 110]. Certain P-gp inhibitors such as PSC–833, piperine capsaicin, resveratrol, silymarin and quercetin were reported their influence on the pharmacokinetics and tissue distribution of doxorubicin in animal models [85, 110]. Capsaicin was reported to significantly increase the extent of doxorubicin accumulation in mice brain after iv injection probably through inhibition of P-gp at blood brain barrier [110]. In addition, piperine and capsaincinc,
through P-gp inhibition, reduced drug excretion into bile and urine, leading to increased drug levels in liver and kidney [110].

3.2 P-gp effects on doxorubicin’s Pharmacodynamic aspects

Critically, overexpression of P-gp on the plasma membrane of cancer cells is a major determinant in preventing intracellular doxorubicin accumulation up to its cytotoxic level. Doxorubicin resistant cancer cells clearly display significant lower intracellular doxorubicin retention with more tolerable to doxorubicin exposure than their parental sensitive cells [65, 66]. Thus, P-gp can be a potential therapeutic target for either MDR reversal or bio-enhancing effect in cancer therapy. The presence of P-gp modulators clearly demonstrates their abilities to restore doxorubicin-mediated killing effect in various cancer cells by increasing intracellular level of doxorubicin [66, 111]. Several plant-based compounds such as limonin, quercetin, resveratrol, curcumin and rhinacanthin-C at their non-cytotoxic concentration have been reported to significantly enhance doxorubicin-mediated cytotoxicity in various cancer resistance cells through modulation of P-gp function [66, 112]. These phytochemical P-gp modulators may suppress P-gp function either by direct inhibition of activity or down-regulation of protein expression.

Moreover, the influence of P-gp on clinical resistance to doxorubicin-based treatment has been reported in cancer patients [113–116]. In order to improve drug efficacy and patient tolerability, several approaches targeting at the P-gp function and expression have been introduced to increase cellular doxorubicin drug level and restore drug sensitivity without the need of higher concentration or additional chemotherapeutic drugs in the therapeutic regimen.

4. Strategic approaches to overcome P-gp mediated resistance to doxorubicin

Taken that doxorubicin is a known substrate of P-gp, the drug efflux transporters in the ATP binding cassette (ABC) family. Hence, any approaches target at the function of these transporters can be presumed to increase therapeutic success for doxorubicin-based chemotherapeutic regimens. Regarding this, the strategies are as follows:

- Increases in dose of doxorubicin or number of cytotoxic drugs to achieve therapeutic success. This has not been a satisfactory approach due to drug toxicity and patients’ intolerance.

- Utilization of P-gp modulators to inhibit either function or expression.

- Development of better drug delivery platforms to bypass P-gp activity, leading to increase intracellular retention of doxorubicin within target cells.

The current MDR reversal strategy has been exploited P-gp modulators that either directly inhibit P-gp activity or down-regulate P-gp expression in order to restore cell chemo-sensitivity to doxorubicin [107]. With the encapsulation technology, P-gp modulators can be co-administered with doxorubicin in the same drug delivery platform, and enhance intracellular doxorubicin accumulation. This approach can be accomplished if the potent, non-cytotoxic P-gp modulators that specifically target at cancer cells are implemented. In addition, the P-gp modulators that also target at non-transporter based resistance such as activation of cellular
survival pathways can exert potentially synergistic impact on MDR reversal effect and better response to doxorubicin treatment. Collectively, the combined doxorubicin and P-gp modulators with multiple-hit targets is a promising strategy to achieve chemotherapeutic efficacy without the need of high dose or additional cytotoxic drugs in the therapeutic regimen.

4.1 Synergy with P-gp modulators

This approach aims to suppress P-gp activity at plasma membrane of target cancer cells. Several P-gp modulators in combination with anti-cancer drugs have been evaluated for safety and efficacy in clinical trials. The clinical outcomes from the first three generations of ABC inhibitors such as quinine, verapamil, cyclosporine-A, tariquitor, PSC 833, LY335979, and GF120918 were quite disappointed, partly because of their dose-limiting adverse events. Most of the P-gp inhibitors required high doses for their clinical MDR reversal effects. In addition, their interference on the P-gp or other ABC transporters at non-target tissues such as brain and kidney could adversely increase accumulation of cytotoxic drugs in these tissues.

The fourth generation of P-gp modulators which are mostly natural products have gained a great interest as potential chemosensitizers in MDR cancer treatment. The advantages of being natural products with long history of use are inclined to the known safety profiles in human and potential hit multiple targets that can restore cell sensitivity to doxorubicin. In addition to direct inhibition of P-gp activity, a number of the natural compounds at non-cytotoxic concentration elicit their chemosensitizing effects through down-regulation of MDR1 and signaling proteins in cell adaptive survival mechanisms. The higher degree of synergism between doxorubicin and a P-gp modulator can be anticipated with potential therapeutic success. Synergistic outcomes between doxorubicin and natural compounds such as resveratrol, quercetin, silymarin, gallic acid, curcumin, epigallocatechin-3-gallate have been demonstrated in various cancer cell models [82, 83, 103, 111, 117–120]. In addition to P-gp modulatory activity (inhibiting both P-gp function and expression), these natural compounds have a broad spectrum of pharmacological activities such as antioxidant, anticancer, anti-inflammation, possible through multiple signaling pathways. For example, the biological effects of curcumin have been related to multiple signaling pathways including NF-kB, Akt, MAPK, Nrf2, AMPK, JAK/STAT that involve in MDR1 expression, cell inflammation, and apoptosis [121]. Co-administration of doxorubicin and curcumin significantly improved doxorubicin-mediated cytotoxicity in vitro cell models and in vivo hepatic xenograft mice model, compared with doxorubicin alone [121–125].

In addition to chemical-based modulators, the uses of specific antibody against P-gp or RNA interference (RNAi) technology to silence P-gp expression may be effective approach to suppress P-gp activity and restore chemo-sensitivity to doxorubicin treatment. Clinical studies on these MDR reversing methods should be extensively conducted to support their uses and benefits in cancer patients.

4.2 Drug delivery system and formulation

This approach aims to develop targeted delivery platforms for improving the permeation of doxorubicin/P-gp modulators/chemo-sensitizers (e.g., antibodies against ABCB1, siRNA) into target cancer cells, leading to an increased intracellular doxorubicin concentration [3, 89, 96, 126–128]. Various nano-drug delivery platforms such as polymeric and solid lipid nanoparticle (SLNs), liposomes, micelles, mesoporous silica nanoparticles, nanostructured lipid carriers, dendrimers have been constructed to better targeting drug delivery to site of action. This approach
in couple with utilization of P-gp modulators can overcome MDR and enhance therapeutic efficacy of doxorubicin. Furthermore, with cancer-targeting ability, this target specific delivery would limit the adverse effect to normal tissues. With the encapsulation technology, nanoparticles (NPs) loaded with doxorubicin and P-gp modulators or other molecules (e.g., siRNAs) has been reported their effectiveness in target delivery into the cells. For examples, aerosol OT (AOT)-alginate NPs enhanced cellular delivery of doxorubicin in MCF-7 cells [129]. Lipid-modified dextran-based NPs loaded with doxorubicin and MDR1 siRNA significantly increased intracellular doxorubicin and reduced P-gp expression levels in osteosarcoma cell line, as compared to doxorubicin alone [130]. Doxorubicin-curcumin composite NPs (e.g., NanoDoxCurc, pegylated-DOX-CUR NPs) could enhance effects of doxorubicin both in vitro and in vivo models of DOX-resistant cancers (e.g., multiple myeloma, acute leukemia, prostate and ovarian cancers). In addition, doxorubicin-curcumin NPs did not cause cardiac toxicity and bone marrow suppression in mice model [131].

5. Conclusion

Doxorubicin is an effective anti-cancer drug that has high MDR incidence. High expression of an efflux transporter P-gp is one established mechanism responsible for the loss of drug effectiveness and MDR development. This can be due to the P-gp function in preventing intracellular accumulation of doxorubicin up to its effective level. Several approaches have been introduced in order to increase the efficacy of doxorubicin-based chemotherapy and overcome MDR. The combination of doxorubicin and non-cytotoxic P-gp modulators, particularly when given to the specific target cancer can be a promising approach to increase cancer sensitivity to doxorubicin through suppression of P-gp function. With the novel encapsulation technologies, it is very possible to develop the drug delivery platforms with specific targeted cancer cells as well as improvement of doxorubicin delivery into the cells. By these means, enhancement of doxorubicin-mediated cytotoxicity can be achieved with minimal dosing of the anti-cancer drugs. After clinically approval, it will provide a great benefit to patients receiving doxorubicin-based chemotherapy.

Acknowledgements

The author gratefully thanks IntechOpen Limited for the sponsorship program in publishing this work.

Conflict of interest

The Author declares no conflicts of interest.
Overcoming P-Glycoprotein-Mediated Doxorubicin Resistance
DOI: http://dx.doi.org/10.5772/intechopen.95553

Author details

Suree Jianmongkol
Department of Pharmacology and Physiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

*Address all correspondence to: suree.j@pharm.chula.ac.th

© 2021 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
References

[1] Robey RW, Pluchino KM, Hall MD, Fojo AT, Bates SE, and Gottesman MM. (2018). Revisiting the role of ABC transporters in multidrug-resistant cancer. Nature reviews Cancer 18(7): 452-464.

[2] Liu X. (2019). ABC Family Transporters. Adv Exp Med Biol. 1141: 13-100.

[3] Li W, Zhang H, Assaraf YG, Zhao K, Xu X, Xie J, Yang DH, and Chen ZS. (2016). Overcoming ABC transporter-mediated multidrug resistance: Molecular mechanisms and novel therapeutic drug strategies. Drug Resist Updat 27:14-29.

[4] Ji X, Lu Y, Tian H, Meng A, Wei M, and Cho WC. (2019). Chemoresistance mechanisms of breast cancer and their countermeasures, Biomedicine & Pharmacotherapy. 114: 108800.

[5] Mansoori B, Mohammadi A, Davudian S, Shirjang S, and Baradaran B. (2017). The Different Mechanisms of Cancer Drug Resistance: A Brief Review. Adv Pharm Bull. 7(3): 339-348.

[6] Kartal-Yandim M, Adan-Gokbulut A, and Baran Y. (2016). Molecular mechanisms of drug resistance and its reversal in cancer, Crit Rev Biotechnol. 36 716-726.

[7] Li Y, Yuan H, Yang K, Xu W, Tang W, Li X. (2010). The structure and functions of P-glycoprotein. Curr Med Chem. 17(8):786-800.

[8] Zhou SF. (2008). Structure, function and regulation of P-glycoprotein and its clinical relevance in drug disposition. Xenobiotica 38(7-8):802-832.

[9] Müller J, Keiser M, Drozdzik M, Oswald S. (2017). Expression, regulation and function of intestinal drug transporters: an update. Biol Chem. 398(2):175-192.

[10] Staud F, Ceckova M, Micuda S, and Pavé P. (2010). Expression and function of p-glycoprotein in normal tissues: effect on pharmacokinetics. Methods Mol Biol 596:199-222.

[11] Takano M, Yumoto R, and Murakami T. (2006). Expression and function of efflux drug transporters in the intestine. Pharmacology & Therapeutics. 109 (1-2): 137-161.

[12] Mouly S, and Paine MF. (2003). P-glycoprotein increases from proximal to distal regions of human small intestine. Pharm Res. 20(10):1595-1599.

[13] Schinkel AH. (1998). Pharmacological insights from P-glycoprotein knockout mice. Int J Clin Pharmacol Ther. 36(1):9-13.

[14] Murakami T. and Takano M. (2008). Intestinal efflux transporters and drug absorption. Expert Opin Drug Metab Toxicol. 4(7): 923-939.

[15] Ipar VS, Dsouza A, Devarajan PV. (2019). Enhancing curcumin oral bioavailability through nanoformulations. Eur J Drug Metab Pharmacokinet. 44(4):459-480.

[16] Mathur P, Rawal S, Patel B, Patel MM. (2019). Oral delivery of anticancer agents using nanoparticulate drug delivery system. Curr Drug Metab 20(14):1132-1140.

[17] Jain AK, Jain S. (2016). Advances in oral delivery of anti-cancer prodrugs. Expert Opin Drug Deliv. 13(12):1759-1775.

[18] Price DF, Luscombe CN, Edershaw PJ, Edwards, CD, and Gumbleton M. (2017). The differential absorption of a series of P-glycoprotein
substrates in isolated perfused lungs from Mdr1a/1b genetic knockout mice can be attributed to distinct physico-chemical properties: an insight into predicting transporter-mediated, pulmonary specific disposition. Pharm Res. 34:2498-2516.

[19] Yasir M., Asif M., Kumar A. and Aggarval A. (2010). Biopharmaceutical classification system: an account. International Journal of PharmTech Research. 2(3): 1681-1690.

[20] Wu C.Y. and Benet L.Z. (2005). Predicting drug disposition via application of BCS: transport/absorption/elimination interplay and development of a biopharmaceutics drug disposition classification system. Pharm Res. 22(1): 11-23.

[21] B Shekhawat P, B Pokharkar V. (2017). Understanding peroral absorption: regulatory aspects and contemporary approaches to tackling solubility and permeability hurdles. Acta Pharm Sin B. 7(3):260-280.

[22] Domínguez-Avila JA, Wall-Medrano A, Velderrain-Rodríguez GR, Chen CO, Salazar-López NJ, Robles-Sánchez M, González-Aguilar GA. (2017). Gastrointestinal interactions, absorption, splanchnic metabolism and pharmacokinetics of orally ingested phenolic compounds. Food Funct 8(1):15-38.

[23] van Waterschoot RAB, and Schinkel AH. (2011). A Critical Analysis of the Interplay between Cytochrome P450 3A and P-Glycoprotein: Recent Insights from Knockout and Transgenic Mice. Pharmacol Rev 63:390-410.

[24] Hendrikx J, Lagas J, Rosing H, Schellens J, Beijnen J, and Schinke AH. (2013). P-glycoprotein and cytochrome P450 3A act together in restricting the oral bioavailability of paclitaxel. Int J of Cancer 132(10): 2439-2447.

[25] Villanueva S, Zhang W, Zacchinati F, Mottino A, Vore M. (2019). ABC transporters in extrahepatic tissues: pharmacological regulation in heart and intestine. Curr Med Chem. 26(7):1155-1184.

[26] Abdullahi W, Davis TP, Ronaldson PT. (2017). Functional expression of p-glycoprotein and organic anion transporting polypeptides at the blood-brain barrier: understanding transport mechanisms for improved CNS drug delivery? AAPS J. 19(4):931-939.

[27] König J, Müller F, Fromm MF. (2013). Transporters and drug-drug interactions: important determinants of drug disposition and effects. Pharmacol Rev 65(3):944-966.

[28] An X, Sarmiento C, Tan T, and Zhu H. (2017). Regulation of multidrug resistance by microRNAs in anti-cancer therapy. Acta Pharmaceutica Sinica B. 7(1): 38-51

[29] Zhu H, Wu H, Liu X, Evans BR, Medina DJ, Liu CG, Yang JM. (2008). Role of microRNA mir-27a and mir-451 in the regulation of MDR1/P-glycoprotein expression in human cancer cells. Biochem Pharmacol. 76:582-588.

[30] Dong WH, Li Q, Zhang XY, Guo Q, Li H, Wang TY. (2015). Deep sequencing identifies deregulation of microRNAs involved with vincristine drug-resistance of colon cancer cells. Int J Clin Exp Pathol, 8(9): 11524-11530.

[31] Bao L, Hazari S, Mehra S, Kaushal D, Moroz K, and Dash S. (2012). Increased expression of P-glycoprotein and doxorubicin chemoresistance of metastatic breast cancer is regulated by miR-298. Am J Pathol 180(6): 2490-2503.

[32] Yang L., Li N., Wang H., Jia X., Wang X., Luo J. (2012). Altered
microRNA expression in cisplatin-resistant ovarian cancer cells and upregulation of miR-130a associated with MDR1/P-glycoprotein-mediated drug resistance. Oncol. Rep. 28:592-600.

[33] Lopes-Rodrigues V, Seca H, Sousa D, Sousa E, Lima RT, and Vasconcelos MH. (2014). The network of P-glycoprotein and microRNAs interactions. Int J of Cancer 135(2): 253-263.

[34] Xie Y, Shaap Y, Deng X, Wang M, and Chen Y. (2018). MicroRNA-298 Reverses Multidrug Resistance to Antiepileptic Drugs by Suppressing MDR1/P-gp Expression in vitro. Front Neurosci 12: 602.

[35] Ronaldson, P. T., Ashraf, T., Bendayan, R. (2010). Regulation of multidrug resistance protein 1 by tumor necrosis factor alpha in cultured glial cells: involvement of nuclear factor-kappaB and c-Jun N-terminal kinase signaling pathways. Mol Pharmacol. 77: 644-659.

[36] Kuo MT, Liu Z, Wei Y, Lin-Lee YC, Tatebe S, Mills GB, and Unate H. (2002). Induction of human MDR1 gene expression by 2-acetylaminofluorene is mediated by effectors of the phosphoinositide 3-kinase pathway that activate NF-kappaB signaling. Oncogene. 27: 1945-1954.

[37] Shen H, Xu W, Luo W, Zhou L, Yong W, Chen F, and Han X. (2011). Upregulation of mdr1 gene is related to activation of the MAPK/ERK signal transduction pathway and YB-1 nuclear translocation in B-cell lymphoma. Exp Hematol. 39: 558-569.

[38] Maurya PK, Mishra A, Yadav BS, Singh S, Kumar P, Chaudhary A, and Mani A. (2017). Role of Y Box Protein-1 in cancer: As potential biomarker and novel therapeutic target. J Cancer. 8: 1900-1907.

[39] Chen Q, Bian Y, and Zeng S. (2014). Involvement of AP-1 and NF-κB in the Up-regulation of P-gp in Vinblastine Resistant Caco-2 Cells. Drug Metab. Pharmacokinet. 29(2): 223-226.

[40] Doublier S, Belisario DC, Polimeni M, Annaratone L, Riganti L, Allia E, Ghido D, Bosia A, and Sapino A. (2012). HIF-1 activation induces doxorubicin resistance in MCF7 3-D spheroids via P-glycoprotein expression: a potential model of the chemo-resistance of invasive micropapillary carcinoma of the breast. BMC Cancer 12:4.

[41] Winder M, and Virós A. (2018). Mechanisms of drug resistance in melanoma. Handb Exp Pharmacol. 249:91-108.

[42] Shariati M, and Meric-Bernstam F. (2019). Targeting AKT for cancer therapy. Expert Opin Investig Drugs. 28(11):977-988.

[43] Sharma VR, Gupta GK, Sharma AK, Batra N, Sharma DK, Joshi A, and Sharma AK. (2017). PI3K/Akt/mTOR Intracellular Pathway and Breast Cancer: Factors, Mechanism and Regulation. Curr Pharm Des. 23(11):1633-1638.

[44] Christowitz C, Davis T, Isaacs A, van Niekerk G, Hattingh S, and Engelbrecht AM. (2019). Mechanisms of doxorubicin-induced drug resistance and drug resistant tumour growth in a murine breast tumour model. BMC Cancer 19(1):757.

[45] Fang XJ, Jiang H, Zhu YQ, Zhang LY, Fan QH, and Tian Y. (2014). Doxorubicin induces drug resistance and expression of the novel CD44st via NF-κB in human breast cancer MCF-7 cells. Oncol Rep. 31(6):2735-2742.

[46] Zheng HC. (2017). The molecular mechanisms of chemoresistance in cancers. Oncotarget. 6: 59950-59964.

[47] Yang SY, Miah A, Sales KM, Fuller B, Seifalian AM, and Winslet M.
Overcoming P-Glycoprotein-Mediated Doxorubicin Resistance
DOI: http://dx.doi.org/10.5772/intechopen.95553

(2011). Inhibition of the p38 MAPK pathway sensitises human colon cancer cells to 5-fluorouracil treatment. Int J Oncol. 38: 1695-1702.

[48] Liu S, Chen S, Yuan W, Wang H, Chen K, Li D, and Li D. (2017). PD-1/PD-L1 interaction up-regulates MDR1/P-gp expression in breast cancer cells via PI3K/AKT and MAPK/ERK pathways. Oncotarget 8(59):99901-99912.

[49] Liu Z, Zhu G, Getzenberg RH, and Veltri RW. (2015). The upregulation of PI3K/Akt and MAP kinase pathways is associated with resistance of microtubule-targeting drugs in prostate cancer. J Cell Biochem 116(7):1341-1349.

[50] Guo X, Ma N, Wang J, Song J, Bu X, Cheng Y, and Wei L. (2008). Increased p38-MAPK is responsible for chemotherapy resistance in human gastric cancer cells. BMC Cancer. 8: 375.

[51] Li S, Zhao Q, Wang B, Yuan S, Wang X, and Li K. (2018). Quercetin reversed MDR in breast cancer cells through down-regulating P-gp expression and eliminating cancer stem cells mediated by YB-1 nuclear translocation. Phytother Res. 32: 1530-1536.

[52] Elmeliegy M, Vourvahis M, Colabufo N, Berardi F, Perrone MG, Capparelli E, Cantore M, Inglese C, and Perrone R. (2010). Substrates, inhibitors and activators of P-glycoprotein: candidates for radiolabeling and imaging perspectives. Curr Top Med Chem 17:1703-1714.

[55] Hennessy M, Kelleher D, Spiers JP, Barry M, Kavanagh P, Back D, Mulcahy F, and Feely J. (2002). St John's Wort increases expression of P-glycoprotein: Implications for drug interactions. Br J Clin Pharmacol. 3(1): 75-82.

[56] Kota BP, Tran VH, Allen J, Bebawy M, and Roufogalis BD. (2010). Characterization of PXR mediated P-glycoprotein regulation in intestinal LS174T cells. Pharmacological Research 62: 426-431.

[57] Greiner B, Eichelbaum M, Fritz P, Kreichgauer HP, von Richter O, Zundler J, and Kroemer HK. (1999). The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. J Clin Invest 104(2):147-153.

[58] Dürr D, Stieger B, Kullak-Ublick GA, Rentsch KM, Steinert HC, Meier PJ, and Fattinger K. (2000). St John's Wort induces intestinal P-glycoprotein/MDR1 and intestinal and hepatic CYP3A4. Clin Pharmacol Ther. 68(6):598-604.

[59] Lili X, and Xiaoyu T. (2015). Expression of PKCα, PKCe, and P-gp in epithelial ovarian carcinoma and the clinical significance. Eur J Gynaecol Oncol. 36(2):181-185.

[60] Nam YS, Im KI, Kim N, Song Y, Lee JS, Jeon YW, Cho SG. (2019). Down-regulation of intracellular reactive oxygen species attenuates P-glycoprotein-associated chemoresistance in Epstein-Barr virus-positive NK/T-cell lymphoma. Am J Transl Res 11(3):1359-1373.

[61] Tong XZ, Wang F, Liang S, Zhang X, He JH, Chen XG, Liang YJ, Mi YJ, To KK, Fu LW. (2012). Apatinib (YN968D1) enhances the efficacy of conventional chemotherapeutical drugs in side population cells and
ABCB1-overexpressing leukemia cells. Biochem Pharmacol. 83(5):586-597.

[62] Lee TB, Park JH, Min YD, Kim KJ, and Choi CH. (2008). Epigenetic mechanisms involved in differential MDR1 mRNA expression between gastric and colon cancer cell lines and rationales for clinical chemotherapy. BMC Gastroenterol, 8: 33

[63] Linn SC, and Giaccone G. (1995). MDR1/P-glycoprotein expression in colorectal cancer. Eur J Cancer, 31(7-8): 1291-1294.

[64] Henrique R, Oliveira AI, Costa VL, Baptista T, Martins AT, Morais A, Oliveira AI, and Jeronimo C. (2013). Epigenetic regulation of MDR1 gene through post-translational histone modifications in prostate cancer. BMC Genomics. 14(898): 1-12.

[65] Mealey KL, Barhoumi R, Burghardt RC, Safe S, and Kochevar DT. (2002). Doxycycline induces expression of P-glycoprotein in MCF-7 breast carcinoma cells. Antimicrob. Agents Chemother. 46, 755-761.

[66] Chaisit. T., Siripong. P., Jianmongkol. S. (2017). Rhinacanthin-C enhances doxorubicin cytotoxicity via inhibiting the functions of P-glycoprotein and MRP2 in breast cancer cells. Eur J Pharmaco. 15, 50-57.

[67] Cantore M, Leopoldo M, Berardi F, Perrone R, and Colabufo NA. (2016). Design and Synthesis of New Selective P-gp Substrates and Inhibitors. Curr Pharm Des. 22(38):5774-5778.

[68] Kadioğlu O, Saeed ME, Valoti M, Frosini M, Sgaragli G, and Effertth T. (2016). Interactions of human P-glycoprotein transport substrates and inhibitors at the drug binding domain: Functional and molecular docking analyses. Biochem Pharmacol. 104:42-51.

[69] Leopoldo M, Nardulli P, Contino M, Leonetti F, Luurtsema G, Colabufo NA. (2019). An updated patent review on P-glycoprotein inhibitors (2011-2018). Expert Opin Ther Pat. 29(6):455-461.

[70] He M, Wei MJ. (2012). Reversing multidrug resistance by tyrosine kinase inhibitors. Chin J Cancer. 31(3):126-133.

[71] Wiese M, and Pajeva IK. (2001). Structure-Activity Relationships of Multidrug Resistance Reversers. Current Medicinal Chemistry 8(6):685-713.

[72] Dewanjee S, Dua TK, Bhattacharjee N, Das A, Gangopadhyay M, Khanra R, Joardar S, Riaz M, Feo V, and Zia-Ul-Haq M. (2017). Natural Products as Alternative Choices for P-Glycoprotein (P-gp) Inhibition. Molecules. 25;22(6):871.

[73] Joshi P, Vishwakarma, and Bharate S. (2017). Natural alkaloids as P-gp inhibitors for multidrug resistance reversal in cancer. Eur J of Med Chem 138:273-292.

[74] Tinoush B, Shirdel I, and Wink M. (2020). Phytochemicals: Potential Lead Molecules for MDR Reversal. Front. Pharmacol. 11: 832.

[75] Srivalli KMR, and Lakshmi PK. (2012). Overview of P-glycoprotein inhibitors: a rational outlook. Braz J of Pharm Sci. 48(3): 353-363.

[76] Chung FS, Santiago JS, Jesus MF, Trinidad CV, and See MF. (2016). Disrupting P-glycoprotein function in clinical settings: what can we learn from the fundamental aspects of this transporter? Am J Cancer Res 6(8):1583-1598.

[77] Darby RA, Callaghan R, and Mcmahon RM. (2011). P-Glycoprotein inhibition; the past, the present and the future. Curr. Drug Metab. 12: 722-731.
Overcoming P-Glycoprotein-Mediated Doxorubicin Resistance
DOI: http://dx.doi.org/10.5772/intechopen.95553

[78] Kannan P, Telu S, Shukla S, Ambudkar S, Pike V, Halldin C, Gottesman MM, Innis RB and Hall MD. (2011). The "specific" P-glycoprotein inhibitor Tariquidar is also a substrate and an inhibitor for breast cancer resistance protein (BCRP/ABCG2). ACS Chem Neurosci. 2(2):82-89.

[79] Karthikeyan S, and Hoti SL. (2015). Development of Fourth Generation ABC Inhibitors from Natural Products: A Novel Approach to Overcome Cancer Multidrug Resistance. Anticancer Agents Med Chem.15(5):605-615.

[80] Bansal T, Jaggi M, Khar RK, and Talegaonkar S. (2009). Emerging Significance of Flavonoids as P-Glycoprotein Inhibitors in Cancer Chemotherapy. J. Pharm. Pharm. Sci. 12: 46-78.

[81] Bhardwaj RK, Glaeser H, Becquemont L, Klotz U, Gupta SK, and Fromm MF. (2002). Piperine, a major constituent of black pepper, inhibits human P-glycoprotein and CYP3A4, J. Pharmacol. Exp. Ther. 302: 645-650.

[82] Zhang W, Han Y, Lim SL, and Lim L. (2009). Dietary regulation of P-gp function and expression. Expert Opinion on Drug Metabolism & Toxicology 5(7):789-801.

[83] Aires V, Colin DJ, Doreau A, Di Pietro A, Heydel JM, Artur Y, Latruffe N, and Delmas D. (2019). P-Glycoprotein affects chemoactivities of resveratrol against human colorectal cancer cells. Nutrients 11(9): 2098.

[84] Huang C, Cai T, Bai L, Huang Y, Li Q, Wang Q, Chiba P, and Cai Y. (2019). State of the art of overcoming efflux transporter mediated multidrug resistance of breast cancer. Transl Cancer Res 8(1):319-329.

[85] Reddy DR, Khurana A, Bale S, Ravirala R, Reddy VSS, Mohankumar M, and Godugu C. (2016). Natural flavonoids silymarin and quercetin improve the brain distribution of co-administered P-gp substrate drugs. SpringerPlus 5:1618

[86] Choi BH, Kim CG, Lim Y, Shin SY, and Lee YH. (2008). Curcumin down-regulates the multidrug-resistance mdr1b gene by inhibiting the PI3K/Akt/NF kappa B pathway. Cancer Lett. 259: 111-118.

[87] Vahedi S, Lusvarghi S, Pluchino K, Shafrir Y, Durell SR, Gottesman MM, and Ambudkar SV. (2018). Mapping discontinuous epitopes for MRK-16, UIC2 and 4E3 antibodies to extracellular loops 1 and 4 of human P-glycoprotein. Sci Rep 8(1):12716.

[88] Ma X, Zhao Z, Wang H, Liu Y, Xu Y, Zhang J, Chen B, Li L, and Zhao Y. (2019). P-glycoprotein antibody decorated porous hydrogel particles for capture and release of drug-resistant tumor cells. Adv Healthc Mater 8(13):e1900136.

[89] De Vera AA, Gupta P, Lei Z, Liao D, Narayanan S, Teng Q, Reznik SE, and Chen ZS. (2019). Immuno-oncology agent IPI-549 is a modulator of P-glycoprotein (P-gp, MDR1, ABCB1)-mediated multidrug resistance (MDR) in cancer: In vitro and in vivo. Cancer Lett. 442:91-103.

[90] Naito M, Tsuge H, Kuroko, Koyama T, Tomida A, Tatsuta T, Heike Y, and Tsuruo T. (1993). Enhancement of cellular accumulation of cyclosporine by anti-P-glycoprotein monoclonal antibody MRK-16 and synergistic modulation of multidrug resistance. J Natl Cancer Inst 85(4):311-316.

[91] Esser L, Shukla S, Zhou F, Ambudkar SV, and Xia D. (2016). Crystal structure of the antigen-binding fragment of a monoclonal antibody specific for the multidrug-resistance-linked ABC transporter human
P-glycoprotein. Acta Crystallogr F Struct Biol Commun 72(Pt 8):636-641.

[92] Iwahashi T, Okochi E, Ariyoshi K, Watabe H, Amann E, Mori S, Tsuruo T, and Ono K. (1993). Specific targeting and killing activities of anti-p-glycoprotein monoclonal antibody mrk16 directed against intrinsically multidrug-resistant human colorectal carcinoma cell lines in the nude mouse model. Cancer research 53: 5475-5482.

[93] Medarova A, Pantazopoulos P, and Yoo B. (2020). Screening of potential miRNA therapeutics for the prevention of multi-drug resistance in cancer cells. Sci Repo 10: 1970.

[94] Tormo E, Ballester S, Adam-Artigues A, Burgués O, Alonso E, Bermejo B, Menéndez S, Zazo S, Madoz-Gúrpide J, Rovira A, Albanell J, Rojo F, Lluch A, and Eroles P. (2019). The miRNA-449 family mediates doxorubicin resistance in triplenegative breast cancer by regulating cell cycle factors. Sci Repo 9: 5316.

[95] Wang Z, Lu B, Wang H, Cheng Z, and Yin Y. (2011). MicroRNA-21 modulates chemosensitivity of breast cancer cells to doxorubicin by targeting PTEN. Archives of medical research 42: 281-290.

[96] Nourbakhsh M, Jaafari MR, Lage H, Abnous K, Mosaffa F, Badii A, and Behravan J. (2015). Nanolipoparticles-mediated MDR1 siRNA delivery reduces doxorubicin resistance in breast cancer cells and silences MDR1 expression in xenograft model of human breast cancer. Iran J Basic Med Sci 18:385-392

[97] Xu H, Hong F, Li S, and Zhang P. (2012). Short hairpin RNA-mediated MDR1 gene silencing increases apoptosis of human ovarian cancer cell line A2780/Taxol. Chinese Journal of cancer research 24(2): 138-142.

[98] Wang P, Yang H L, Yang YJ, Wang L, and Lee SC. (2015). Overcome cancer cell drug resistance using natural products. Evid Based Complement Alternat Med. 2015, 767136.

[99] Cheng Q, Liao M, Hu H, Li H, and Wu L. (2018). Asiatic Acid (AA) Sensitizes multidrug-resistant human lung adenocarcinoma A549/DDP cells to cisplatin (DDP) via downregulation of P-Glycoprotein (MDR1) and its targets. Cellular Physiology and Biochemistry, 47(1), 279-292.

[100] Huang CZ, Wang YF, Zhang Y, Peng YM, Liu YX, Ma F, Jiang JH, and Wang QD. (2017). Cepharanthine hydrochloride reverses P-glycoprotein-mediated multidrug resistance in human ovarian carcinoma A2780/Taxol cells by inhibiting the PI3K/Akt signaling pathway. Oncology Reports, 38(4), 2558-2564

[101] Zhao BX, Sun YB, Wang SQ, Duan L, Huo QL, Ren F, and Li GF. (2013). Grape seed procyanidin reversal of P-glycoprotein associated multi-drug resistance via downregulation of NF-κB and MAPK/ERK mediated YB-1 activity in A2780/T cells. PLoS ONE, 8(8), e71071.

[102] Pan Y, Shao D, Zhao Y, Zhang F, Zheng X, Tan Y, He K M Li J, and Chen L. (2017). Berberine Reverses Hypoxia-induced Chemoresistance in Breast Cancer through the Inhibition of AMPK- HIF-1α. Int J Biol Sci. 13(6): 794-803.

[103] Limtrakul P, Anuchapreeda S, and Buddhasukh D. (2004). Modulation of human multidrug-resistance MDR-1 gene by natural curcuminoinds. BMC Cancer. 4:13.

[104] Jin S, Gorfajn B, Faircloth G, Scotto KW. (2000). Ecteinascidin 743, a transcription-targeted chemotherapeutic that inhibits MDR1
activation. 1. Proc Natl Acad Sci U S A. 97(12):6775-6779.

[105] AbuHammad S, and Zihlif M. (2013). Gene expression alterations in doxorubicin resistant MCF-7 breast cancer cell line. Genomics. 101: 213-220.

[106] Ichikawa Y, Ghanefar M, Bayeva M, Wu R, Khechaduri A, Naga Prasad SV, Mutharasan RK, Naik TJ, and Ardehali H. (2014). Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. J. Clin. Invest. 124: 617-630.

[107] Kumar, S, Kushwaha PP, and Gupta S. (2019). Emerging targets in cancer drug resistance. Cancer Drug Resistance, 2, 161-177.

[108] Xu F, Wang F, Yang T, Sheng Y, Zhong T, and Chen. Y. (2014). Differential drug resistance acquisition to doxorubicin and paclitaxel in breast cancer cells. Cancer Cell Int. 21, 142.

[109] Patel KJ, and Tannock I. (2009). The influence of P-glycoprotein in expression and its inhibitors on the distribution of doxorubicin in breast tumors. BMC Cancer 9:356.

[110] Kim TH, Shin S, Yoo SD, and Shin BS. (2018). Effects of phytochemical P-glycoprotein modulators on the pharmacokinetics and tissue distribution of doxorubicin in mice. Molecules 23:349.

[111] Eid, S.Y., El-Readi, M.Z., Fatani, S.H., Mohamed Nour Eldin, E.E., Wink, M., 2015. Natural products modulate the multifactorial multidrug resistance of cancer. Pharmacology and Pharmacy. 6, 146-176.

[112] El-Readi M Z, Hamdan D, Farrag N, El-Shazly, A., Wink, M. (2010). Inhibition of P-glycoprotein activity by limonin and other secondary metabolites from Citrus species in human colon and leukaemia cell lines. Eur J Pharmacol. 626: 139-145.

[113] Kato T, Mizutani K, Kameyama K, Kawakami K, Fujita Y, Nakane K, Kinimoto Y, Ehara H, Ito H, Seishima M, Deguchi T, Ito M. (2015). Serum exosomal P-glycoprotein is a potential marker to diagnose docetaxel resistance and select a taxoid for patients with prostate cancer. Urol Oncol. 33(9):385-e15-20.

[114] Lili X, and Xiaoyu T. (2015). Expression of PKC alpha, PKCepsilon, and P-gp in epithelial ovarian carcinoma and the clinical significance. Eur J Gynaecol Oncol. 36(2):181-185.

[115] Han L, Guo X, Bian H, Zhou Y, Li T, and Yang J. (2015). Changed expression and function of P-gp in peripheral blood CD56 + cells predicting chemoresistance in non-Hodgkin lymphoma patients. Cancer Biomark. 15(3):289-297.

[116] Kim JW, Park Y, Roh JL, Cho KJ, Choi SH, Nam SY, Kim SY. (2016). Prognostic value of glucosylceramide synthase and P-glycoprotein expression in oral cavity cancer. Int J Clin Oncol. 21(5):883-889.

[117] Zhao Y, Huan ML, Liu M, Cheng Y, Sun Y, Cui H, Liu DZ, Mei QB, and Zhou SY. (2016). Doxorubicin and resveratrol co-delivery nanoparticle to overcome doxorubicin resistance. Sci Rep. 6:35267.

[118] El-Readi MZ, Eid S, Abdelghany AA, Al-Amoudi HS, Efferth T, and Wink M. (2019). Resveratrol mediated cancer cell apoptosis, and modulation of multidrug resistance proteins and metabolic enzymes. PhytoMedicine. 55:269-281.

[119] Khan M, Maryam A, Mehmood T, Zhang Y, and Ma T. (2015). Enhancing Activity of Anticancer Drugs in Multidrug Resistant Tumors by Modulating P-Glycoprotein through
Dietary Nutraceuticals. Asian Pac J Cancer Prev. 16(16):6831-6839.

[120] Zhang S, and Morris ME. (2003). Effects of the flavonoids biochanin A, morin, phloretin, and silymarin on P-glycoprotein-mediated transport. J Pharmacol Exp Ther. 304(3):1258-1267.

[121] Satonaka H, Ishida K, Takai M, Koide R, Shigemasa R, Ueyama J, Ishikawa T, Hayashi K, Goto H, Wakisawa S. (2017). (−)-Epigallocatechin-3-gallate down-regulates doxorubicin-induced overexpression of p-glycoprotein through the coordinate inhibition of pi3k/akt and mek/erk signaling pathways. Anticancer Res 37(11):6071-6077.

[122] Lopes-Rodrigues V, Sousa E, and Vasconcelos MH. (2016). Curcumin as a Modulator of P-Glycoprotein in Cancer: Challenges and Perspectives. Pharmaceuticals 9:71.

[123] Wang J, Ma W, and Tu P. (2015). Synergistically Improved Anti-tumor Efficacy by Co-delivery Doxorubicin and Curcumin Polymeric Micelles. Macromol Biosci. 15(9):1252-1261.

[124] Rastegar R, Akbari Javar H, Khoobi M, Dehghan Kelishadi P, Hossein Yousefi G, Doosti M, Hossien Ghahremani M, Shariftabrizi A, Imanparast F, Gholibeglu E, and Gholami M. (2018). Evaluation of a novel biocompatible magnetic nanomedicine based on betacyclodextrin, loaded doxorubicin-curcumin for overcoming chemoresistance in breast cancer. Artif Cells Nanomed Biotechnol. 46(sup2):207-216.

[125] Zhao X, Chen Q, Li Y, Tang H, Liu W, and Yang X. (2015). Doxorubicin and curcumin co-delivery by lipid nanoparticles for enhanced treatment of diethylnitrosamine-induced hepatocellular carcinoma in mice. Eur J Pharm Biopharm. 93:27-36.

[126] Zhang J, Du Z, Pan S, Shi M, Li J, Yang C, Hu H, Qiao M, Chen D, and Zhao X. (2018). Overcoming multidrug resistance by codelivery of mdr1-targeting siRNA and doxorubicin using epha10-mediated pH-sensitive lipoplexes: in vitro and in vivo evaluation. ACS Appl Mater Interfaces. 10(25):21590-21600.

[127] Cavaco MC, Pereira C, Kreutzer B, Gouveia LF, Silva-Lima B, Brito AM, and Videira M. (2017). Evading P-glycoprotein mediated-efflux chemoresistance using Solid Lipid Nanoparticles. Eur J Pharm Biopharm 110:76-84.

[128] Yu J, Hu F, Zhu Q, Li X, Ren H, Fan S, Qian B, Zhai B, Yang D. (2020). PD-L1 monoclonal antibody-decorated nanoliposomes loaded with Paclitaxel and P-gp transport inhibitor for the synergistic chemotherapy against multidrug resistant gastric cancers. Nanoscale Res Lett 15(1):59.

[129] Chavanpatil MD, Khdair A, and Panyam J. (2007). Surfactant-polymer Nanoparticles: A Novel Platform for Sustained and Enhanced Cellular Delivery of Water-soluble Molecules. Pharmaceutical Research 24(4):8.3-810.

[130] Susa M, Iyer AK, Ryu K, Choy E, Hornicek FJ, Mankin H, Milane L, Amiji MM, and Duan Z. (2010). Inhibition of ABCB1 (MDR1) Expression by an siRNA Nanoparticulate Delivery System to Overcome Drug Resistance in Osteosarcoma. PLoS One. 5(5): e10764.

[131] Pramanik D, Campbell NR, Das S, Gupta S, Chenna V, Bisht S, Sysa-Shah P, Bedja D, Karikari C, Steenbergen C, Gabrielson KL, Maitra A, and Maitra A. (2012). A composite polymer nanoparticle overcomes multidrug resistance and ameliorates doxorubicin-associated cardiomyopathy. Oncotarget 3(6):640-650.