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Chapter

Overcoming P-Glycoprotein-Mediated Doxorubicin Resistance

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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 *ABCB1*), multidrug resistance protein 1 (MRP1, encoded by *ABCC1*), multidrug resistance protein 2 (MRP2, encoded by *ABCC2*) and breast cancer resistance protein (BCRP; encoded by *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 α-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 α-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].

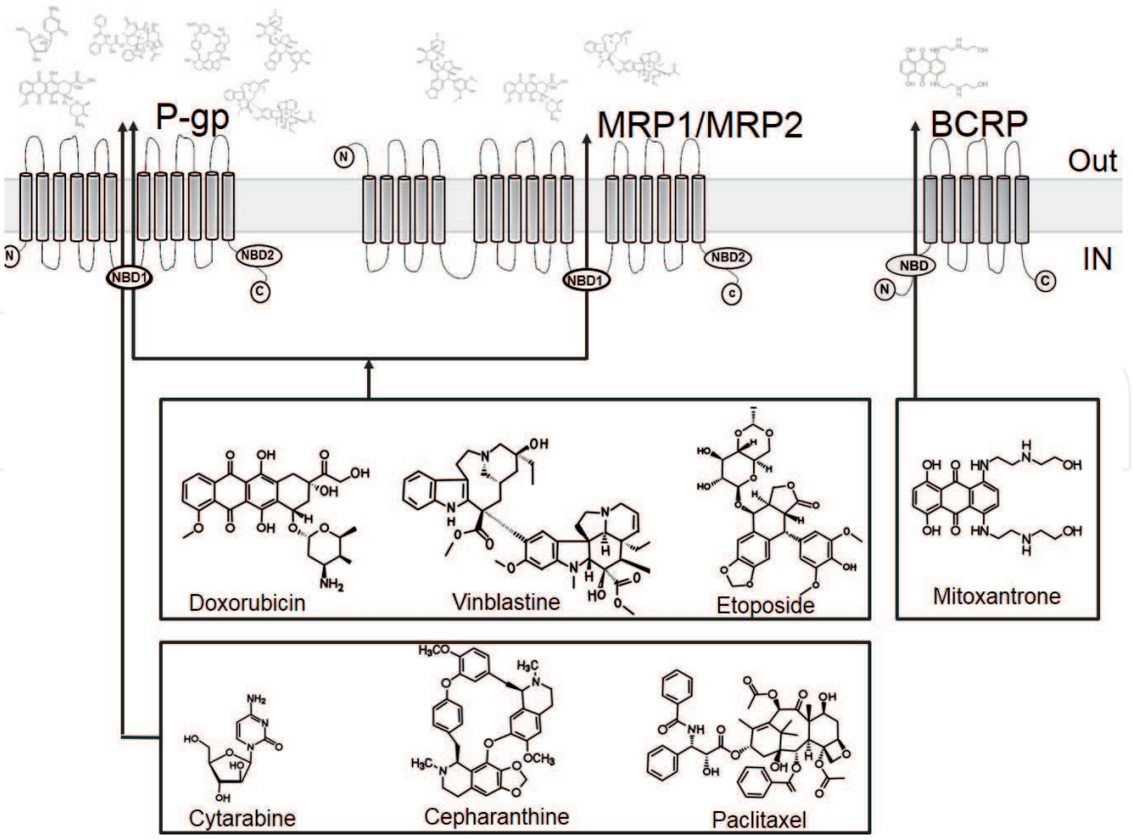

Figure 1.
The key ABC drug efflux transporters and their selected anticancer drug substrates.

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 MDR1/P-gp overexpression, and cisplatin resistance in SKOV3/CIS cells [32]. Overexpression of miR-27a and miR-451 was linked to increased MDR1 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 MDR1 gene through alteration of various signaling pathways and transcription factors. Example of the transcriptional factors involving in MDR1 transcription are nuclear factor-κB (NF-κB) [35, 36], Y-box binding

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-kB 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, indinarvir, 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, talinolol, diltiazem, losartan, verapamil), anti-microbial agents (e.g., doxycycline, erythromycin, itraconazole, rifampin), 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**	
		Direct inhibitors	**Suppressors of expression**
Actinomycin D Colchicine Docetaxel Doxorubicin Daunorubicin Epirubicin Etoposide Idarubicin Imatinib Methotrexate Paclitaxel Teniposide Topotecan Vinblastine Vincristine	Daunorubicin Docetaxel Doxorubicin Flutamide Paclitaxel Vinblastine Vincristine	Small molecule inhibitors *First generation* Cyclosporin A Verapamil *Second generation* VX-710 (Biricodar) Dexverapamil PSC833 (Valspodar) *Third generation* GF120918 (Elacridar) XR9576 (Tariquidar) LY335979 (Zosuquidar) *Fourth generation* Capsaisin Curcumin Limonin Piperine Quercetin Monoclonal antibodies MRK 16 MRK 17 UIC 2	Small molecule inhibitors Curcumin Dasatinib Dexverapamil Reserpine Imatinib Nilotinib Sorafenib Trifluoperazine Toremifene PSC833 (Valspodar) RNA interference *MDR1* small-interfering RNA (siRNA) Antisense oligonucleotides *MDR1* antisense oligonucleotides delivered via lysosomes

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 specific 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 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 significant 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. cepharantine, tetrandine) 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 cytotoxity. 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 capsaincin,

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' intolerability.

- 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 chemo sensitizing 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.

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Conflict of interest

The Author declares no conflicts of interest.

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