Review Article

State of the art of overcoming efflux transporter mediated multidrug resistance of breast cancer

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Contributions: (I) Conception and design: Q Huang, T Cai, L Bai, Y Huang, P Chiba, Y Cai; (II) Administrative support: None; (III) Provision of study materials or patients: None; (IV) Collection and assembly of data: None; (V) Data analysis and interpretation: None; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Abstract: Breast cancer is the leading cause of death from cancer in women worldwide. Chemotherapy represents one key treatment modality for the clinical management of breast cancer. However, ATP-binding cassette (ABC) transporter mediated active efflux of structurally and mechanistically different cytotoxic compounds results in multidrug resistance (MDR), eventually leading to failure of chemotherapy. The concept of combining anti-cancer drugs and transport inhibitors has been advocated as a concept for re-sensitization of resistant breast cancer to chemotherapy. Whether inhibition of efflux transporters may have the potential to improve therapeutic outcomes is discussed controversially. In this review we discuss challenges in the treatment of breast cancer, the role of MDR in development and the potential of natural products to overcome MDR.

Keywords: Breast cancer; multidrug resistance (MDR); ATP-binding cassette transporters (ABC transporters); inhibitors; natural products

Submitted Sep 02, 2018. Accepted for publication Dec 21, 2018.
doi: 10.21037/tcr.2019.01.19
View this article at: http://dx.doi.org/10.21037/tcr.2019.01.19

Introduction

Breast cancer represents a malignant disease, which seriously affects female physical and mental health. Although morbidity and mortality of breast cancer have declined over the past 20 years, it still represents the leading cause of cancer deaths in women worldwide (1). According to the World Health Organization fact sheet, 571,000 breast cancer related deaths were reported in 2015 (2). Chemotherapy has developed into an effective way of treating breast cancer, but multidrug resistance (MDR) frequently occurs in the course of cancer chemotherapy and eventually results in its failure (3). MDR refers to a condition, in which cancer cells acquire cross-resistance to anti-cancer drugs of different chemical structure and mechanism of action. The phenotype of MDR can have different reasons, including overexpression of drug efflux transporters, induction of detoxifying enzyme systems, suppression of apoptotic pathways and in a more narrow sense, mutation of target structures. Efflux transporter mediated MDR is typically caused by upregulation of members of the ABC protein family, primarily P-glycoprotein (P-gp) (ABCB1), multidrug resistance

protein (MRP; ABCC1) and breast cancer resistance protein (BCRP, ABCG2).

The concept of overcoming MDR caused by overexpression of these efflux transporters envisioned co-administration of standard chemotherapy regimens in conjunction with inhibitors of these efflux transporters. Candidate compounds from the arsenal of approved drugs in the market were initially identified serendipitously and first characterized in *in vitro* model systems in the early 1990's. Clinical use of these first generation P-gp inhibitors was limited by intrinsic pharmacological properties. Second generation inhibitors were thus designed to be devoid of these intrinsic pharmacological effects. These compounds, however, turned out to suffer from interfering anticancer drugs with changing pharmacokinetics at the level of cytochrome P_{450} isoenzymes. Only third generation inhibitors such as tariquidar, elacridar and zosuquidar, which did not show this interference, were ultimately used in numerous clinical studies. Disappointingly, third generation inhibitors, either failed to show a clinical benefit in the verum group, or they had severe side effects due to inhibition of P-gp in tissues, which under cytostatic therapy required P-gp function for cell survival. Frequently they led to bone marrow aplasia related deaths.

Generally, many novel classes of P-gp inhibitors showed adverse effects in preclinical studies, or they required drug concentrations, which could not be reached in patients (4). Although results from clinical trials were disappointing, our understanding of drug resistance has become more nuanced. A role of ABC transporters in the failure of drug delivery to tumors has unequivocally been demonstrated. Even low expression of P-gp was shown to lead to pronounced decreases in cellular accumulation of cytotoxic drugs (5). Thus, the main question to be asked is, if the concept of resensitization of tumor cells to drugs is invalid, or if clinical studies have failed because of conceptual shortcomings. It can certainly not be denied that many of the clinical studies have not shown a benefit for patients. While the role of multidrug efflux transporters in drug resistance is unequivocally established, further discussion on the aspect of MDR inhibition in cancer cell resistance is thus required. Apart from their role in MDR, ABCB1, ABCC1 and ABCG2 play an important role in drug disposition and drug-drug interactions (DDIs). Thus for biology, clinical pharmacology and drug development, MDR transporters remain a research field of major interest.

Natural products are produced by living organisms. They are not essential for survival, but nevertheless provide organisms that produce them with an evolutionary advantage. Many of the compounds that are used in the treatment of human diseases are natural products or derivatives of them. One remarkable example for the use of a natural product in medicine is the compound class of artemisinin first line drug treatment of malaria (6). Thus natural products are deemed indispensable for the pharmacological treatment of human diseases, as pharmacology heavily relies on the use of natural product drugs.

As results with the first three generations of inhibitors at the clinical trial stage were disappointing, researchers turned their attention to potent and relatively non-toxic natural products as inhibitors for blocking ABC transporters. In this review, we focus on the clinical management of breast cancer, MDR transporters and inhibitors that have been used in *in vitro*, mouse models and clinical studies. A short discussion of natural products, including alkaloids, saponins and flavonoids is included. These compounds may have the potential to overcome transporter mediated breast cancer MDR in a clinical setting without having the severe side effects observed in earlier clinical studies.

Clinical management of breast cancer

Breast cancer is treated by combined modality. This includes surgery, radiation therapy, chemotherapy, hormone therapy, and targeted therapy with biologicals. Different types of breast cancer may require a different extent of surgical treatment from breast-conserving surgery to total mastectomy and modified radical mastectomy. Radiotherapy to the region of the tumor bed and regional lymph nodes is often following surgery, in order to destroy tumor cells that may have escaped or been spread by surgery (7). Radiation can reduce the risk of recurrence by 50–66%, when delivered in the correct dose (8). Drug treatment involves hormone blockers, chemotherapy and targeted therapy with biologicals. Hormonal therapy represents one of the major modalities of medical oncology (9). It involves the manipulation of the endocrine system through exogenous administration of specific hormones, particularly steroid hormones or drugs, which inhibit the production or activity of such hormones. The selective estrogen receptor modulator (SERM) tamoxifen is currently first-line treatment for pre-menopausal women with hormone receptor (HR)-positive breast cancer (10). Aromatase inhibitors such as anastrozole or letrozole are given in postmenopausal women (11). Chemotherapy mainly works by destroying fast-growing or fast-replicating cancer cells,

either by causing DNA damage upon replication, or by mechanisms targeted at cell division (12). Chemotherapy can either be systemic or regional. Usually regimens contain combinations of different compounds, e.g., a combination of cyclophosphamide and doxorubicin, termed the "AC" regimen (13). However, medications may damage normal cells and thereby cause serious side effects. Thus targeted therapy with biopharmaceuticals or nanoengineered enzymes, which specifically identify and attack cancer cells, while minimizing damage to normal cells has evolved as a promising treatment strategy (14). The arsenal of targeted therapeutics currently includes monoclonal antibodies and small molecules [tyrosine kinase inhibitors (TKI), cyclin-dependent kinase inhibitors, mechanistic target of rapamycin (mTOR) inhibitors and poly ADP ribose polymerase (PARP) inhibitors. The anti HER2 monoclonal antibody trastuzumab has for example significantly improved the 5-year disease free survival of HER2-positive breast cancer (15). Recently, the FDA approved the cyclin dependent kinase 4/6 inhibitors ribociclib, and abemaciclib, which are administered in different combinations for the treatment of HR-positive, human epidermal growth factor receptor 2 (HER2)-negative advanced or metastatic breast cancer (16). Targeted therapy can also be combined with chemotherapy as adjuvant therapy (17). Treatments with biologicals was demonstrated to improve prognosis and quality of life for patients, but at the same time caused an increase in the number of patients that experience adverse drug effects. These include metabolic toxicity (hyperglycaemia, hypertriglyceridemia, hypercholesterolemia), dermatologic and cardiovascular toxicity and immune suppression (18-20).

In addition to the above treatments, high-dose chemotherapy and subsequent autologous stem cell transplantation represents a way of escalating dose regimens that lead to otherwise intolerable bone marrow toxicity (21). However, proof of a beneficial effect that exceeds standard chemotherapy regimens has yet to be provided.

Inhibitors of ABC transporters in overcoming breast cancer resistance

In vitro studies

ABC transporters thus have been documented to play an important role in MDR of cancer. Thus inhibitors may still be considered to enhance chemotherapeutic efficacy of standard anticancer drugs (22). To date, a larger number of different chemical inhibitor scaffolds have been reported. The majority has been characterized in vitro, while a larger number of compounds have also been studied in animal models. Selected compounds have progressed to the stage of clinical phase trials. First generation inhibitors included the calcium channel blocker verapamil, calmodulin antagonists, protein C kinase inhibitors, immune suppressive drugs such as cyclosporine A, antibiotics (e.g., erythromycin), antimalarial drugs (e.g., quinine) and steroid hormones (23-25). Verapamil was reported to not only increase the concentration of doxorubicin in drug-resistant human breast cancer cells (MCF-7/ADR) by inhibition of P-gp, but also to induce apoptosis of tumor cells (26). However, first generation P-gp inhibitors suffered from the limitation of having inherent pharmacological properties (27). Second-generation inhibitors were in part obtained by modifying first-generation inhibitors. PSC-833 was developed as a non-immunosuppressive analog of cyclosporine A with an improved safety profile and higher affinity. Studies showed that PSC-833 can be used as an efficient MDR modulator to reverse DOX-resistance in the human breast cancer cell line T47D/TAMR-6 (28,29). Another example for a second generation inhibitor of P-gp and BCRP was VX710 (biricodar). Second-generation inhibitors thus had higher potency, specificity, and lower cytotoxicity than the first generation inhibitors (30). However, second-generation inhibitors suffered from the initially unrecognized limitation that they interfered with pharmacokinetics (and thus cytotoxicity) of anticancer agents in co-administration protocols at the level of cytochrome P_{450} isoforms. In consequence, third generation modulators were developed to design out pharmacokinetic interference with anticancer drugs. These inhibitors were primarily directed against P-gp, including the derivatives of o-aminobenzamide tariquidar (XR9576), zosuquidar (LY335979), elacridar (GF120918) and laniquidar (R101933) (31-34). Compared to the first and the second generation inhibitors, third generation inhibitors exhibited a reversal potential of 200-fold or more at nanomolar concentrations, but did not show a significant influence on pharmacokinetics of co-administered chemotherapeutic agents (30,35), as these inhibitors are not metabolized by the cytochrome P_{450} isoform 3A4. LY335979 (zosuquidar) was found to not only inhibit P-gp, but also to down regulate its expression in breast cancer MCF-7/ADR and MT-3/ADR cells. In addition some tyrosine kinase inhibitors (TKIs) have been found to be not only substrates, but to also act as inhibitors of ABC transporters (36,37).

Inhibitor action in mouse models in vivo

Several breast cancer mouse models for preclinical intervention trials such as genetically engineered mouse models (PDX models, organoids, non-germline models) have been established to investigate the progression of breast cancer and drug resistance mechanisms. In particular genetically engineered mouse models have been used (38-40). The hereditary breast cancer K14cre; $Brca1^{F/F}$; $p53^{F/F}$ mouse model of spontaneous breast tumors shows that doxorubicin and docetaxel resistance was associated with up-regulation of the two P-gp or thologs in mouse Mdr1a and Mdr1b. This confirmed the relevance of even moderate increases of P-gp in drug resistance development *in vivo* (41). Resistance can be overcome by inhibiting P-gp. For example, long time treatment with the poly (APD-ribose) polymerase (PARP) inhibitor AZD2281did result in drug resistance, which could be reversed by co-administration of the P-gp inhibitor tariquidar. This suggests that P-gp inhibition provides a feasible strategy *in vivo* (5,42). In addition, anti-cancer drug resistance was also reported to be associated with increased expression of ABCG2 in the mouse model. Tumor-specific genetic ablation of the mouse isoform of ABCG2 significantly increased the overall survival of BRCA1 breast cancer mice treated with topotecan, confirming the correlation of ABCG2 expression and topotecan resistance (43). Indeed, the specific ABCG2 inhibitor Ko143 in combination with topotecan increased the overall survival of mice in the Brca1/p53 mouse model. The benefit, however, was modest and the study failed to demonstrate an increased accumulation of topotecan. Conversely the PEGylated SN38 compound EZN-2208 circumvents ABCG2-mediated topotecan resistance. ABCG2-expressing tumors were highly sensitive to EZN-2208. A significant increase in survival was observed, suggesting that PEGylation of Top1 inhibitors may be useful to circumvent transporter-mediated resistance and improve clinical efficacy (44). Therefore, we can conclude that breast cancer MDR is related to expression of P-gp and ABCG2 in *in vivo* studies, and the combination of inhibitors and chemotherapeutic drugs can overcome MDR and increase the sensitivity of tumor tissue to drugs *in vivo*. Furthermore, the use of mouse models may help clinicians to derive real-time genotype-specific drug response profiles and design more effective and durable patient-specific regimens (38,40). Although MDR1 acts a potential resistance mechanism in breast cancer of mice, the role of MDR1 in mediating resistance has not been as clearly implicated in human breast cancer (45). The inability to convert MDR1 data derived from a mouse model into humans may be due to the fact that the basal levels of MDR1 in rodents are higher than in humans, which converted to MDR1 expression in response to anticancer drug therapy in rodents (40). Another explanation is that due to methodological limitations, such as techniques for determining MDR1 expression, a relatively small increase of MDR1 expression in clinically important may not have been detected (40). To overcome the limitations of animal models in preclinical target characterization, human mammary epithelial organs can be used to validate data from animal models (46).

Clinical inhibitor studies

Although many studies have shown that multidrug transport inhibitors can work synergistically with anticancer drugs to reverse ABC transporter-mediated MDR *in vitro*, clinical results suggest that inhibitors do not benefit breast cancer patients (47). The epithelial tumors, breast cancer and advanced cancer of patients were selected to phase I studies. The safety, tolerability, pharmacokinetic and pharmacodynamics of MDR inhibitors co-administered with anti-breast cancer agents were assessed (48). Neurological or hematological toxicity emerged when P-gp inhibitors were administered in combination with anticancer drugs in phase I studies (49,50). Then phase II and III trials mainly studied in metastatic breast cancer patients. One randomized phase II clinical trial compared single-agent epirubicin treatment with or without lonidamine in metastatic breast cancer patients (51). The patients receiving lonidamine failed to show prolonged survival and reduced anthracycline-related toxicity (alopecia, nausea, vomiting, and stomatitis) compared to controls. Moreover, when different inhibitors were combined with the same anticancer drug, a significant improvement in the response rate could not be demonstrated (52,53). In a more recent phase III trial, both, relative improvement and absolute increase in response rate was shown for patients who received dofequidar plus cyclophosphamide, doxorubicin, and fluorouracil (CAF) (54). The 221 patients with metastatic breast cancer received six cycles of CAF (100 mg cyclophosphamide administered orally on days 1–14, 25 mg/m^2 adriamycin and 500 mg/m^2 fluorouracil administered intravenously on days 1 and 8) with or without 900 mg dofequidar administered orally on days 1–14). The treatment results showed the overall response rates of 42.6% and 53.1% (P=0.077) and median progression-free

survivals of 241 and 366 days (P=0.145) for CAF alone and CAF plus dofequidar, respectively. Again, these results did not reach statistical significance. Nevertheless, the search for inhibitors of ABC transporters is still ongoing. A recent report showed that HM30181AK, a novel P-gp inhibitor, prevents the efflux of various chemotherapeutic agents from intestinal epithelial cells to the gastrointestinal tract (55).

Potential natural sources to overcome breast cancer

More than 70% of reported MDR inhibitors identified to date are natural products, while those inhibitors, which have been used in phase III studies are synthetic compounds. Although some third generation inhibitors have entered phase III clinical trials, the results of relevant clinical trials are not ideal. The search for high-efficiency, low-toxic P-gp inhibitors from chemically synthesized source compounds has encountered insurmountable barriers in real-world research. Therefore, it is feasible to find efficient and low-toxic P-gp inhibitors from novel and diverse natural extracts. In the process of evolution, natural plants synthesize and secrete secondary metabolites (SMs) that are used to protect natural enemies (herbivores). These SMs are not directly involved in plant growth, differentiation and regeneration, but it has a toxic effect on herbivorous animals. In the course of natural evolution, herbivores express a related substance transport system with exogenous toxicants, such as P-gp or other transport proteins, in order to protect against such toxic SMs and eliminate harmful substances in food. Therefore, the academic community speculated that there must be related SMs in the natural plants that inhibit the activity of transporters such as P-gp (56). Most of the mechanisms of action of P-gp inhibitors from chemically synthesized sources have been reported to reverse tumor MDR by inhibiting the function of P-gp efflux substrates. One of the major candidate reversal drug resistance agents is a competitive substrate of P-gp in chemical structure and can be mediated by P-gp. However, natural extraction and isolation of organic products not only inhibit P-gp efflux function but some of the reported natural candidate agents such as Ramified curcumin hydrolyzed (57), Honokiol (58), Tetramethylpyrazine (59), Triptolide (60), can also inhibit the expression level of P-gp in drug-resistant tumor cells, thereby mediating the reversal of tumor MDR (61). The candidate reversal compounds of natural origin show better reversal of tumor resistance in structure and mechanism of action. Natural products can be a reliable candidate compound source for finding efficient and low-toxic P-gp inhibitors.

Natural products that are administered in treatment regimens have been in the environment of evolutionary successful organisms for a long time and upregulation of MDR transporters has been one successful strategy to ensure survival of other species in the presence of these natural toxins. MDR transporters thus represent an Achilles heel for cancer treatment, as their presence allowed survival of extant species. On the other hand, organisms that produce xenotoxic compounds may also have developed strategies to keep these toxins effective, by co-releasing inhibitors of MDR transporters. Thus research on identification of compounds with MDR inhibitory action from plants may be considered a promising strategy for the identification of novel scaffolds.

Natural products have been the single most productive source of leads for the development of drugs (40). They can act as MDR inhibitors as they compete with the efflux of cytotoxic agents by binding to the active site. It is hoped that the drug discovery process will be spurred by the identification of more effective inhibitors or modulators originating from natural sources. Materials extracted from plants are most diverse and often have complex chemical scaffolds. Importantly, many natural products have low toxicity and are well tolerated (62). Curcumin, ginsenoside and alkaloids are examples of natural produces with MDR inhibitory properties (63). In addition, these compounds can also help to increase oral bioavailability or tissue penetration of therapeutic drugs. This may represent an additional concept for a clinical application of these natural products (64). For instance, quercetin was able to increase the peak plasma concentration and oral bioavailability of the P-gp substrate doxorubicin by 1.3- to 2.4-fold in rats (61).

Notably, it should not be ignored that nearly 80% of all drugs approved by the FDA for cancer therapy during the past three decades were either natural products *per se* or based on a natural product scaffold. Others are mimetics of natural products in one form or another (65). Therefore, the interest of scientists in searching for candidates from natural sources that have a modulatory effect on the function of disease-associated ABC transporters persists. Below, we present a brief overview of potential natural products that can overcome ABC transporter mediated MDR in breast cancer (*Figure 1*).

Alkaloids

Alkaloids are a highly diverse group of compounds (66), which contain a ring structure and a nitrogen atom,

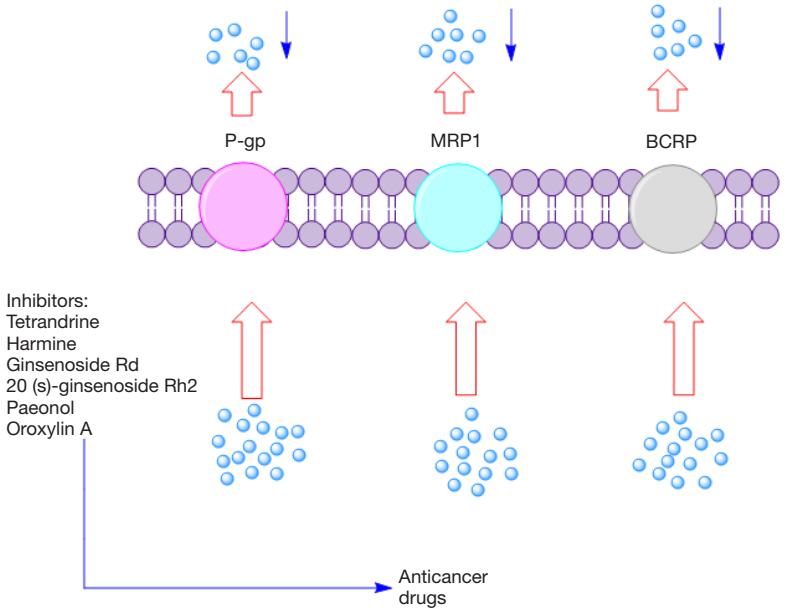

Figure 1 Natural products as potential reversing agents of MDR. MDR, multidrug resistance.

and mainly exist in higher plants such as Leguminosae, Ranunculaceae and Magnoliaceae. Some of the alkaloids have been used as anticancer chemotherapeutics, including camptothecin (CPT) (67) and vinblastine (68). It was suggested that the active alkaloid may act as a competitive inhibitor of P-gp and BCRP (69). Tetrandrine (Tet), a benzyl isoquinoline alkaloid, is a good candidate for the development of new MDR-reversing agents due to the potent inhibition of P-gp (70). Similarly, Berberine, another isoquinoline alkaloid isolated from Berberis species, could suppress P-gp by down-regulating the AMPK-HIF-1α signalling pathway to enhance DOX chemosensitivity in MCF-7 drug-resistant cells (71,72). Ma *et al.* studied the effect of harmine on the overexpression of BCRP in MDA-MB-231 breast cancer cells (73). Twenty μM harmine could inhibit BCRP more effectively than FTC at identical concentration. Results indicated that harmine was able to inhibit BCRP-mediated drug efflux and reverse drug resistance, and suggested that harmine may be used as a lead compound for the development of BCRP reversal agents. In addition, it is reported that more than 15 alkaloid compounds exhibit reversal activity to drug-resistant breast cancer cells recently. However, these compounds need further investigation to convert into successful drug candidate (74).

Saponins

Saponins are a class of compounds found in various plant species. The pharmacological effects of saponins include antiviral (75), anti-inflammatory (76) and antitumor activity. These effects are proposed to be related to their cholesterol binding properties, polarity and hydrophobicity (77). A recent report has shown that saponins and anticancer drugs in combination can increase the sensitivity of P-gp-expressing tumors (78). Ginsenoside is the main active constituent of ginseng, which are highly valued owing to its enhanced pharmacology effects such as immunostimulating, antioxidant, anti-cancer and antiaging activity (79). Ginsenoside Rh2, a major pharmacological active component of ginseng, could influence the MAPK/NF-kB pathway to down-regulate Adriamycin-induced ABCB1 expression in previous study (80). Moreover, Ginsenoside Rh2 could mediate the miRNA expression to reduce the drug resistance of breast cancer (81). Similarly, Saikosaponin D, one of the major triterpenoid saponins derived from Bupleurum, significantly down-regulated MDR1 mRNA and P-gp expression without altering the pharmacokinetic profiles of DOX to involve in the reversal of MDR for breast cancer (82,83). Conversely, another saponin compound, 20(S)-protopanaxadiol, stimulated the activity of ABCB1 ATPase to enhance the efficacy of substrate drugs in drug-resistant breast cancer cells rather than suppress the expression of ABCB1 mRNA or protein (84).

Flavonoids

Flavonoids are a class of naturally occurring polyphenols, which are widely distributed in the leaves, flowers and stems of higher plants. When grapefruit juice was reported to affect therapeutic efficacy of nifedipine (a CYP3A substrate) (85), interest in the relevance of flavonoids in ABC transporter mediated MDR flared. Not surprisingly, flavonoids were found to inhibit ABC transporters (86-88). Interestingly, curcumin has broad-spectrum modulatory effects on all three major ABC drug transporters: ABCB1, ABCC1 and ABCG2 (89,90). Curcumin could reduce breast cancer stem cells population for sensitizing breast cancer cells to mitomycin C both *in vitro* and *in vivo* by suppressing ABCG2 (91,92). However, usually curcumin loaded nanoparticles combination with chemotherapy drugs for effectively overcoming MDR due to the low aqueous solubility and poor stability of curcumin (93,94). Furthermore, paeonol, another component extracted from the root cortex of the Paeonia suffruticosa, could reverse paclitaxel resistance in breast cancer with a superior 8.2-fold reversal index (95). The study found that the compound down-regulated transgelin 2-mediated paclitaxel resistance by reducing the expression of P-gp, MRP1 and BCRP in MCF-7/PTX cells. Also, Silibinin (96) and Rutin (97) have the potential to sensitize chemo-resistant breast cancer cells. Remarkably, a recent investigation confirmed that flavonoids are avid inhibitors of BCRP, which suggested that their presence at high levels in the diet could cause food-drug interactions (98).

Discussion and conclusions

Breast cancer is the second leading cause of death from malignant diseases in women. Chemotherapy is one important treatment option to slow the progress of the disease, but the emergence of MDR represents an impediment to successful treatment and ultimately to its failure. Transporter mediated drug efflux represents a critical factor in the development of drug resistance. Thus, clinical studies addressed synergistic effects of anti-cancer drugs and transport inhibitors based on preclinical studies. *In vitro* and *in vivo* studies demonstrated a benefit of the combination of anticancer drugs and transport inhibitors. However, these findings to date did not translate into beneficial clinical outcomes. Why has the majority of clinical trials trying to combine anti-cancer drugs with MDR inhibitors not been successful? Several factors may explain these disappointing results: (I) combinations of anticancer drugs and P-gp inhibitors can kill bone marrow stem cells in patients that receive combination therapy. However, this side effect may be circumvented by autologous bone marrow transplantation; (II) although inhibitors have been shown to be effective against tumors, in the absence of complete tumor eradication, survival of the fittest will lead to recurrence; (III) P-gp only contributes to tumor cell survival *in vivo*, but other factors contribute to a resistance phenotype and these effects may be dominant; (IV) the tumor microenvironment plays a crucial role in drug uptake; (V) the polymorphic variants of ABC transporters may influence the efficacy of inhibitors; (VI) patient recruitment will also affect clinical research results.

Although clinical studies with transport inhibitors did not live up to expectations that were tied to the advocation of a most simple concept, results from animal studies may warrant further attention to it. A balanced view on matters may be possible in retrospect from a distance in time. In this review, we attempted to discuss and summarize results with a focus on natural compounds from plants (alkaloids, saponins and flavonoids). As mentioned above, MDR may be caused by factors other than drug efflux transporters. Non-toxic, potent and selective natural product modulators of ABC transporter mediated MDRs may be identified by high-throughput screening and a subsequent combinatorial chemistry approach with extended quantitative structure-activity relationship studies (62).

Acknowledgments

Funding: We acknowledge the financial support from the National Natural Science Foundation of China (81273707, 81173215), the Ministry of Education in the New Century Excellent Talents (NECT-12-0677), the Natural Science Foundation of Guangdong (S2013010 012880,2016A030311037), the Science and Technology Program of Guangzhou (2014J4500005,201704030141), the Science Program of the Department of Education of Guangdong (2013KJCX0021, 2015KGJHZ012), the Science and Technology Program of Guangdong (2015A050502027), and the 2017 International Science and Technology Cooperation Project of Guangzhou Economic & Technological Development Zone (2017GH16).

Footnote

Conflicts of Interest: All authors have completed the ICMJE

uniform disclosure form (available at http://dx.doi.org/10.21037/tcr.2019.01.19). The authors have no conflicts of interest to declare.

Ethical statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Huang Q, Cai T, Bai L, Huang Y, Li Q, Wang Q, Chiba P, Cai Y. State of the art of overcoming efflux transporter mediated multidrug resistance of breast cancer. Transl Cancer Res 2019;8(1):319-329. doi: 10.21037/tcr.2019.01.19

