MINI-REVIEW

Cancer Multidrug Resistance (MDR): A Major Impediment to Effective Chemotherapy

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Abstract

Multidrug resistance (MDR) continues to be a major challenge to effective chemotherapeutic interventions against cancer. Various types of cancers have been observed to exhibit this phenomenon, a strategy that involves cellular and non cellular mechanisms employed by cancer cells to survive the cytotoxic actions of various structurally and functionally unrelated drugs. The present article is a brief review of the fundamental mechanisms underlying the phenomenon of MDR in cancer cells and some novel approaches addressed at its inhibition, circumvention or reversal. The emergence of natural products as potential anti-MDR molecules is of particular significance. Since many of these are essential components of the human diet, they are expected to possess fewer side effects and may possibly represent a new generation of MDR modulators.

Key Words: Multidrug resistance - chemotherapy - mechanisms - natural modulators

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Introduction

The resistance of human tumor to multiple chemotherapeutic drugs has been recognized as a major reason for the failure of cancer therapy (Gottesman and Pastan, 1993).The phenomenon of tumor drug resistance became a hotspot of cancer research after the emergence of a novel type of resistance discovered by Juliano and Ling in 1976, when it was shown that a glycoprotein of 170 kD, called P-glycoprotein, correlated with the degree of drug resistance in several Chinese hamster ovary cell lines (Juliano and Ling, 1976). The phenomenon called multidrug resistance subsequently appeared as a major impediment to the curative treatment of a variety of malignancies.

Cancer multidrug resistance is defined as the cross-resistance or insensitivity of cancer cells to the cytostatic or cytotoxic actions of various anticancer drugs which are structurally or functionally unrelated and have different molecular targets (Gottesman,1993). Pharmacokinetic studies dealing with the absorption, distribution, metabolism and clearance of administered drug have been useful in elucidating the levels of drugs in cancer cells and it seems that there are two factors that are primarily responsible for multidrug resistance: i) Individual specificity with regard to variations in absorption, metabolism and delivery of drugs to target tissues. This factor is influenced by individual's genetic pattern which generates various cellular responses that obstruct the drug from reaching to threshold levels inside the cells required for its pharmacological action. ii) Tumor specificity in terms of origin, vasculature and tissue function. Tumors located in parts of the body where the drug is not accessible or tumors with compromised vasculature often show resistance to chemotherapy. The former specificity is linked to acquired resistance where the altered genetics of the cancer cells exhibit mechanisms that lead to MDR and the latter specificity is responsible for the inherent or natural resistance conferred to certain types of tumors which do not respond to standard chemotherapy drugs from the beginning.

Tumors derived from tissues, the physiological role of which requires high expression of transporter proteins exhibit intrinsic multidrug resistance to cytostatic agents even before chemotherapy is initiated. The MDR in tumors derived from other tissues appears phenotypically upon induction of genes coding for transporter proteins by a cytostatic agent resulting into acquired MDR during the course of the treatment. Past researches of about 35 years have thrown up various hypothesis related to the mechanisms of MDR development and also the modulators tailored to address this problem. The present article provides a general overview of mechanisms proposed to mediate multidrug resistance in cancer cells, counter strategies in practice and novel approaches having the potential as anti-MDR modulators for the future.

Mechanisms of Multidrug Resistance

Numerous mechanisms have been proposed to mediate multidrug resistance in cancer cells. Such mechanisms can be categorized as non-cellular or cellular based on

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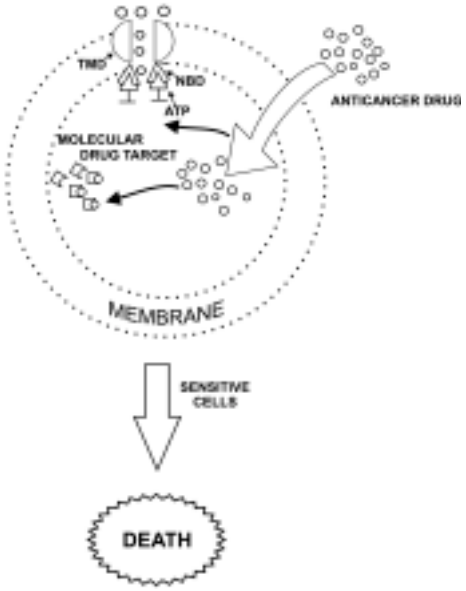

Figure 1. Schematic Presentation of Anticancer Drug Entry into a Cancer Cell via the Membrane. The drug accumulates to threshold concentrations and interacts with molecular targets leading to cell death. Such cells have normal expression of P-gp and are sensitive to chemotherapy

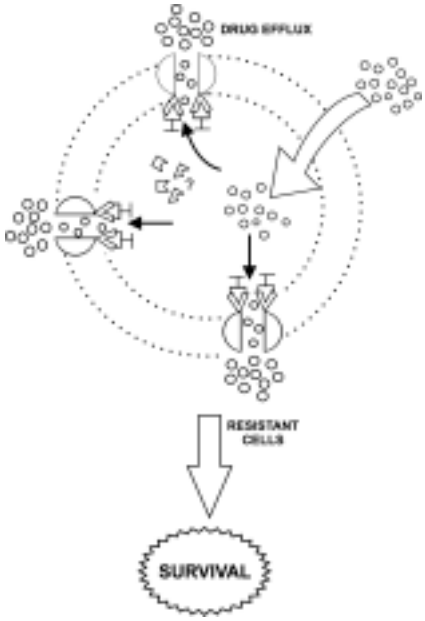

Figure 2. Schematic Presentation of the Phenomenon of MDR in Cancer Cells. P-gp actively extrudes the drug from the cell, thereby keeping the intracellular levels below the killing threshold. Such cells have high expression of P-gp and are resistant to chemotherapy.

the factors contributing to MDR development (Fan et al., 1994). Non cellular mechanisms involve factors that are extracellular such as limited vascular accessibility or cell growth environment whereas cellular mechanisms include enzymes and transport systems.

Non cellular MDR mechanisms

These mechanisms normally hold for certain types of cancers which show inherent or natural resistance to chemotherapy at the initial exposure to the drug. Transformation to the cancerous state requires the cells to grow beyond their natural boundaries and such a process should be assisted by a well structured vasculature. However, in certain solid tumors angiogenesis is compromised (Jain, 1987) leading to poor vasculatures that hinder the accessibility of the drug to the cancer cells thereby limiting the drug induced cytotoxicity. The growth environment in which cancer cells proliferate is markedly different from that of the normal cells. Lack of nutrition and hypoxia due to poor vasculature and the resultant lactic acid accumulation could confer resistance to cancer cells against drugs that act on actively dividing cells or the cellular uptake of which requires a pH gradient (Demant et al., 1990).

Cellular MDR mechanisms

Cellular MDR mechanisms may be classified as non classical / non transport based or classical / transport based mechanisms. Non-transport based cellular MDR mechanisms involve enzyme systems that limit the desired activity of the drug without altering its effective concentration inside the cell. Glutathione-S- transferase (GST) an important enzyme of xenobiotic metabolism catalyzes the biotransformation of organic molecules by conjugating them with polar molecules to facilitate their excretion. GST is known to mediate biotransformation of various anticancer drugs and its elevated level has been reported in various resistant cancer cell lines like MCF-7 (Batist et al., 1986; Hao et al.,1994). The overexpressed GST modifies the drug into end product with reduced activity and enhanced rate of excretion. The transport based, classical cellular mechanism of MDR involves the efflux of drug from the cell by various energy dependant membrane transport proteins, thereby limiting it from reaching to therapeutic concentrations inside the cell (Gottesman, 2002). ATP-binding cassette (ABC) transporters are a family of proteins that mediate MDR via ATP-dependent drug efflux pumps (Leonard et al., 2003). Overexpression of ATP-binding cassette (ABC) transporters has been shown to be responsible for MDR (Choi, 2005).Various transport proteins of the ABC superfamily have been characterized and include P-glycoprotein, multidrug resistance-associated protein-1 (MRP1), its homologs MRP2-6 and the breast cancer resistance protein (BCRP) (Riordan et al.,1985; Doyle et al,1998; Borst et al., 2000) which are overexpressed in malignant cells and serve to pump anticancer drugs out of the cell, resulting in lack of intracellular levels of the drug necessary for effective therapy.

Although these resistant proteins belong to the ABC superfamily, they are quite different with respect to gene locus, amino acid sequence, structure and substrate (Gottesman and Pastan, 1993). However the most studied and well characterized is the first discovered multidrug transporter, P-glycoprotein or MDR1 protein, encoded by the MDR1 gene (Chen et al., 1986; Ambudkar et al., 1992; Gottesman and Pastan, 1993) The expression of P-gp is usually highest in tumors that are derived from tissues that normally express P-gp, causing reduction in the intracellular drug concentrations which decreases the

cytotoxicity of a broad spectrum of antitumor drugs including anthracyclines (e.g. DOX), vinca alkaloids (e.g. vincristine), podophyllotoxins (e.g. etoposide) and taxanes (e.g. taxol)(Abolhoda et al.,1999). Pgp is normally expressed in the transport epithelium of the kidney, liver and gastrointestinal tract (Nakamura et al., 2000).

There are three known isoforms of P-GP, namely, class I, II and III. Rodent cells have all three P-GP genes, encoding classes I, II, and III P-GP, whereas human cells have two, encoding class I and III P-GP (Lee et al., 1993). All three classes of P-GP are inherently ex-pressed in several normal tissues. Specifically, in mammalian tissues, class I P-GP is present in intestinal lining epithelium, endothelial cells, bone marrow progenitor xenocells, peripheral blood lymphocytes and natural killer cells, whereas class II is present in the adrenal cortex. The class III P-GP is localized in hepatocytes, cardiac and striated muscle (Thiebaut et al., 1987; Chaudhary et a.l, 1993). It is a 170-kDa membrane protein glycosylated at the first extracellular loop. The Pgp molecule is composed of two halves, each consisting of transmembrane α helices and the cytoplasmic ATP-binding domain. The two half molecules are separated by a highly charged linker region which is phosphorylated at several sites by protein kinase C. Each half contains a highly hydrophobic domain with 6 transmembrane α–helices(TMDs) and a hydrophilic domain located at the cytoplasmic face of the membrane, nucleotide binding domain (NBD) The protein molecule also contains the substrate(s) binding domain(s). The transmembrane regions form the drug translocating pathway (Loo and Clarke, 2001), while the ATP-binding sites, exhibiting ATPase activity, provide the metabolic energy upon ATP hydrolysis enabling the active drug efflux (Higgins, 1992). After binding of the substrate, ATP hydrolysis induces, conformation changes in the protein molecule, that opens the central pore and allows transport of hydrophobic drugs directly from the lipid bilayer into the central pore of the transporter expelling the substrate(s) out of the cell. ABC transporters are essential for many cellular processes that require transport of metabolites or substrates across the cell membrane. They are therefore key elements in the discovery and development of drugs targeting MDR in cancer cells.

Modulators of MDR

The compounds having the ability to reverse the resistance against anticancer drugs are called MDR inhibitors, chemosensitizers or MDR modulators (Kellen, 2003). Modulators targeting P-gp directed MDR belong to a number of chemical classes and have been classified as the first, second and third generation of MDR reversal agents on the basis of their affinity for the transporter proteins and relative toxicity towards normal cells as marker of their side effects.

First-generation modulators included drugs that were not specifically developed for inhibiting MDR but were used for other pharmacological activities and coincidentally found to be effective in sensitizing the drug resistant tumors towards chemotherapy. These include verapamil (calcium channel blocker), quinine

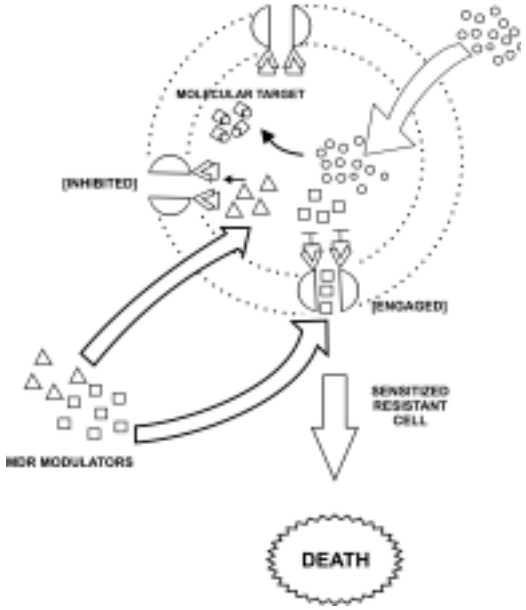

Figure 3. Schematic Presentation of MDR Reversal Strategies using MDR modulators. Modulators may bind to the nucleotide binding domain (NBD) or the transmembrane domain (TMD) of the P-gp directly inhibiting its function. Alternatively they as may be P-gp substrates which are actively transported into the cell, keeping the transporter protein engaged. In both cases, drug efflux is prevented, thereby sensitizing the resistant cells to chemotherapy

(antimalarial), cyclosporine A (immunosuppressant), tamoxifen(anti-steroid) and erythromycin (Ford and Hait ,1990).However, first generation modulators failed to create an impact on MDR, since their low affinity for the transporter proteins required high doses to achieve the desired effect. These drugs at higher doses resulted in adverse effects and enhanced toxicity to normal cells (Lampidis et al., 1986).The second generation modulators constituted drugs that were designed by modification of the first generation modulators and such modifications were aimed at reducing their adverse effects by eliminating their non MDR pharmacological activities, thereby making them specific for MDR. Some second generation drugs included valspodar (a non immunosuppressive analogue of cyclosporine A) and R verapamil (R enantiomer of verapamil, a weaker calcium channel blocker) (Hollt, 1992). Even these drugs failed to deliver the desired range of efficacy due to their low affinity for their target transporter proteins and therefore a need for a third generation of modulators with improved chemotherapeutic potentials was conceived. The third generation inhibitors are designed specifically for high transport affinity and low pharmacokinetic interaction. Inhibition of cytochrome P450 3A, which was responsible for many adverse pharmacokinetic effects with previous generation inhibitors, has generally been avoided in this generation of MDR modulators. These include tariquidar (anthranilamide derivative), biricodar (pipecolinate derivative), Annamycin (anthracycline derivative), mitotane (2,4-dichloro-diphenyldichloroethane derivatve), zosuquidar(dibenzosuberane derivative) and laniquidar (benzazepine derivative) (Liscovitch and Lavie,2002). These compounds exhibit effective MDR modulatory

potency, high affinity and selectivity for target MDR transporter(s) at low nanomolar range and subsequently low toxicity towards normal cells. These treatment strategies are aimed at either using P-gp inhibitors which can bind to transmembrane domain or nucleotide binding domain, thereby inhibiting the function of P-gp or more novel approaches are directed at engaging the P-gp transporters using ionic molecules that require transporter proteins to enter the cell. These molecules when administered along with the anticancer drug will keep the transporter proteins occupied and allow the drug to accumulate inside the cells to effective lethal concentrations. Figures 1, 2 and 3 gives a schematic representation of MDR in cancer cells and the strategies to reverse the phenomenon.

Natural Products as Potential MDR Modulators

Unfortunately, most of the agents from the first, second or third generation of MDR modulators suffer clinically from their intrinsic toxicity or from undesired effects on the pharmacokinetics of the accompanying anticancer drugs. These limitations have spurred on efforts to search for new and more effective compounds that could be effective at tolerable doses without any adverse effect. In this regard, recent researches showing natural products as potential MDR modulators are well appreciated. Since most of these natural compounds are essential components of human diet, it may be presumed that they would be least toxic even at higher doses. Limtrakul et al. (2007) have reported the reversal of MDR against vinblastine, paclitaxel and colchicines in KB-V1 cells (MDR human cervical carcinoma with high P-gp expression) by root extract of *Stemona curtisii* in a dose dependant manner. Drug-resistant KB-V1 cells have been shown to express P-gp at high levels on their plasma membrane (Schoenlein, 1994). *S. curtisii* root extract modulated p-gp activity and reversal of the MDR phenotype. In another study, Fong et al have shown that in multidrug resistant HepG2-DR and K562-DR cells with over-expressed P-glycoprotein, the extract of the rhizomes of *Alisma orientalis* showed a synergistic growth inhibitory effect with cancer drugs that are P-gp substrates including actinomycin D, puromycin, paclitaxel, vinblastine and doxorubicin. At the same toxicity levels the herbal extract was more effective than verapamil, a standard P-gp inhibitor, in enhancing cellular doxorubicin accumulation (Fong et al., 2007)

Lignans have also been reported to be effective inhibitors of multidrug resistance-associated protein 1 (MRP1) in HL60/Adriamycin MDR model (Ling et al., 2007). Breast cancer resistance protein (BCRP), an ABC transporter, which plays an important role in drug disposition has been found to be inhibited by flavonoids, a major class of natural compounds widely present in foods and herbal products(Shuzhong et al., 2005). A green tea polyphenol epigallocatechin gallate (EGCG) has been observed to enhance the efficacy of DOX and increased the DOX concentration in drug-resistant KB-A1 cells. EGCG modulated the function of P-gp in the resistant cell lines by binding to NBD2, there by preventing the binding of ATP and subsequent energy dependant efflux of the drug (Feng et al., 2005). Lobeline, a piperidine alkaloid from *Lobelia inflata* ,a medicinal plant has been shown to inhibit P-gp activity, leading to MDR reversal in resistant cells treated with doxorubicin and significantly such sensitization was achieved at non-toxic concentrations (Yonggang et al., 2008). Curcumin, a constituent of turmeric, downregulates mdr 1b gene (P-gp expression) in multidrug-resistant L1210/Adr cells at the transcriptional level via the phosphatidyinositol 3-kinase (PI3K)/Akt/nuclear factor-kB (NF-kB) signal cascade in the multidrug-resistant mouse leukemia L1210/Adr cell line (Byeong et al,2008). Polyphyllin D (PD) is a steroidal saponin found in a traditional Chinese medicinal herb *P. polyphylla*. Recent findings indicate that PD is a potent anti-cancer agent that can overcome the MDR and elicit programmed cell death in drug-resistant RHepG2 cells (Cheung et al, 2005). The molecular basis of the observed cytotoxicity of PD in HepG2 and R-HepG2 cells is presumably by the induction of apoptosis induced by mitochondrial fragmentation (Sung et al, 2007). Honokiol, a naturally occurring compound present in *Magnolia grandiflora*, a Chinese medicinal herb, has been demonstrated to combat cancer through mechanisms including inhibition of angiogenesis and induction of apoptosis and down-regulate the expression of P-glycoprotein at mRNA and protein levels in MCF-7/ADR, a human breast MDR cancer cell line. The down-regulation of P-gp was accompanied with a partial recovery of the intracellular drug accumulation (Dong et al., 2006).

Flavonoids have been considered to be a new class of chemosensitizers, which interact with both the ATP binding site and its vicinal steroid-interacting hydrophobic sequence of P-gp (Conseil et al.,1998). Chung et al (2007) have examined the effects of various flavonoids such as biochanin A, diadzein, fisetin, morin, naringenin, quercetin,and silymarin on P-gp function in human breast cancer cell lines, MCF-7 (sensitive) and MCF-7/ADR (resistant). The accumulation of daunomycin (DNM), a Pgp substrate, was greater in the sensitive cells compared to the resistant cells, while the efflux of DNM was higher in the resistant cells compared to the sensitive cells over a period of 2 h. The IC50 value of DNM in the resistant cells was about 22 times higher than that in the sensitive cells, indicating an over-expression of P-gp in the resistant cells, MCF-7/ADR. Biochanin A exhibited the greatest increase in DNM accumulation while DNM accumulation with quercetin and silymarin was similar to that of a well-known P-gp inhibitor, verapamil. biochanin A and silymarin significantly decreased the IC50 value of DNM, potentiating the cytotoxicity of DNM. The study suggests that biochanin A and silymarin appear to be potent and safe P-gp inhibitors that can increase the efficacy of chemotherapeutic agents when administered concomitantly.

On account of their routine intake and least toxicity, many of the natural products from fruits, vegetable, spices and other dietary supplements are currently being investigated for their anticancer activities and their role as MDR modulators would augment their effectiveness

against cancer. Screening of natural products for their MDR activity with associated benefit of no or low toxicity has thrown several such modulators, which in future hold promise for multidrug resistance therapy.

Conclusions

Chemotherapy is the most effective treatment for patients with cancer. The effectiveness however is seriously limited by the phenomenon of MDR. Anticancer drugs can fail to kill cancer cells for various reasons including variations in the absorption, metabolism and delivery of drug to target tissues and tumor location in parts of the body into which the drugs do not easily penetrate. Three major mechanisms have been proposed: first, decreased uptake of water soluble drugs such as folate antagonists and cisplatin ,which require transporters to enter the cells; second, various changes in cells that effects the capacity of cytotoxic drugs to kill cells such as reduced apoptosis; and third, increased energy dependant efflux of hydrophobic drugs where the intracellular drugs inside the resistant cancer cells are kept at sub-lethal level.

The most common of these mechanisms is the efflux of hydrophobic drugs mediated by energy driven ABC transporters such as P-glycoprotein, an integral membrane protein overexpressed in various malignancies. The broad substrate specificity and the abundance of ABC transporter proteins have been a major challenge towards attempts to circumvent ABC-mediated MDR *in vivo*.

Various generations of MDR modulators have represented novel and improved interventions, although not to the perfection. The perfect reversing agent would be the one which is efficient, devoid of unrelated pharmacological effects, shows no pharmacokinetic interaction with other drugs and restores the treatment efficiency of the anticancer drug to that observed in MDR negative phenotype. In this regard, recent studies have shown that natural compounds found in vegetables, fruits, plant derived beverages and herbal dietary supplements not only have anticancer properties, but may also modulate P-gp activity. P-gp inhibitors found in natural products, especially those found in traditional medicine and dietary supplements, have the potential to be developed as MDR reversing agents which could lead to more successful chemotherapy. Such elements from dietary sources posses the advantage of having least or no pharmacokinetic interactions with the anticancer drugs concomitant to their MDR modulatory activity. Furthermore, the likelihood of multiple alternative mechanisms for MDR also exists, thereby warranting further investigations regarding the mechanistic actions of novel modulators, for treatment as well as prevention of multidrug resistance in different types of cancer cells.

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References

Abolhoda A, Wilson AE, Ross H, et al (1999). Rapid activation of MDR1 gene expression in human metastatic sarcoma after in vivo exposure to doxorubicin. *Clin Cancer Res*, **5**, 3352–6.

Ambudkar SV, Lelong IH, Zhang J, et al (1992). Partial purification and reconstitution of the human multidrug-resistance pump: characterization of the drug-stimulatable ATP hydrolysis. *Proc Natl Acad Sci*, **89**, 8472-6.

Batist G, Tulpule A, Sinha BK, et al (1986). Overexpression of a novel anionic glutathione transferase in multidrug-resistant human breast cancer cells. *J Biol Chem*, **33**, 15544–9.

Borst P, Evers R, Kool M, et al (2000). A family of drug transporters: the multidrug resistance-associated proteins. *J Natl Cancer Inst*, **92**, 1295-302.

Byeong H Ch, Chang G K, Yoongho L, et al (2008). Curcumin down-regulates the multidrug-resistance mdr1b gene by inhibiting the PI3K/Akt/NFjB pathway. *Cancer Letters*, **259**, 111–8.

Chaudhary PM, Mechetner EB, Roninson IB (1992). Expression and activity of the multidrug resistance P-glycoprotein in human peripheral blood lymphocytes. *Blood*, **80**, 2735–9.

Chen CJ, Chin JE, Ueda K, et al (1986).Internal duplication and homology with bacterial transport proteins in the mdr1 (P-glycoprotein) gene from multidrug-resistant human cells. *Cell*, **47**, 381–9.

Cheung JY, Ong RC, Suen YK, et al (2005). Polyphyllin D is a potent apoptosis inducer in drug-resistant HepG2 cells, *Cancer Lett*, **217**, 203–11.

Choi CH (2005). ABC transporters as multidrug resistance mechanisms and the development of chemosensitizers for their reversal. *Cancer Cell Int*, **5**, 30.

Chung SY, Sung MK, Kim NH, et al (2005). Inhibition of P-glycoprotein by natural products in human breast cancer cells. *Arch Pharm Res*, **28**, 823-8.

Conseil G, Baubichon-Cortay H, Dayan G, et al (1998). Flavonoids: a class of modulators with bifunctional interactions at vicinal ATP- and steroid-binding sites on mouse P-glycoprotein. *Proc Natl Acad Sci USA*, **95**, 9831-6.

Demant EJ, Sehested M, Jensen PB (1990). A model for computer simulation of P-glycoprotein and transmembrane delta pH-mediated anthracycline transport in multidrug-resistant tumor cells. *Biochim Biophys Acta*, **1055**, 117–25.

Doyle LA, Yang W, Abruzzo LV, et al (1998). A multidrug resistance transporter from human MCF-7 breast cancer cells. *Proc Natl Acad Sci USA*, **95**, 15665-70.

Fan D, Beltran PJ, O'Brien CA (1994). Reversal of multidrug resistance. In: Kellen, J.A. (Ed.), Reversal of Multidrug Resistance in Cancer. CRC Press, Boca Raton, FL, pp. 93–24.

Feng Q, Dongzhi W, Qiang Z, et al (2005). Modulation of P-glycoprotein function and reversal of multidrug resistance by (–)-epigallocatechin gallate in human cancer cells. *Biomedicine & Pharmacotherapy*, **59**, 64–9.

Fong WF, Wang C, Zhu GY, et al (2007). Reversal of multidrug resistance in cancer cells by Rhizoma Alismatis extract. *Phytomedicine*, **14**, 160–5.

Ford JM, Hait WN (1990). Pharmacology of drugs that alter multidrug resistance in cancer. *Pharmacol Rev*, **42**,155-99.

Gottesman MM (1993). How cancer cells evade chemotherapy: Sixteenth Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res*, **53**,747-54.

Gottesman MM (2002). Mechanisms of cancer drug resistance. *Annu Rev Med*, **53**, 615-27.

Gottesman MM, Pastan I (1993). Biochemistry of multidrug

resistance mediated by the multidrug transporter. *Annu Rev Biochem*, **62**, 385–427.

Hao XY, Widersten M, Ridderstrom M, et al (1994). Co-variation of glutathione transferase expression and cytostatic drug resistance in HeLa cells: establishment of class Mu glutathione transferase M3-3 as the dominating isoenzyme. *Biochem J*, **297**, 59–67.

Higgins CF (1992). ABC transporters: from microorganisms to man. *Annu Rev Cell Biol*, **8**, 67-113

Hollt V, Kouba M, Dietel M, et al (1992). Stereoisomers of calcium antagonists which differ markedly in their potencies as calcium blockers are equally effective in modulating drug transport by P-glycoprotein. *Biochem Pharmacol*, **43**, 2601-8.

Jain RK (1987). Transport of molecules in the tumour interstitium: a review. *Cancer Res*, **47**, 3039–51.

Juliano RL, Ling V (1976). A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. *Biochim Biophys Acta*, **455**,152-62.

Kellen JA (2003).The reversal of multidrug resistance: an update. *J Exp Ther Oncol*, **3**,5-13

Lampidis TJ, Krishan A, Planas L, et al (1986). Reversal of intrinsic resistance to adriamycin in normal cells by verapamil. *Cancer Drug Deliv*, **3**, 251–9.

Lee CH, Bradley G, Zhang JT, et al (1993). Differential expression of P-glycoprotein genes in primary rat hepatocyte culture. *J Cell Physiol*, **157**, 392–402.

Leonard GD, Fojo T, Bates SE (2003). The role of ABC transporters in clinical practice. *Oncologist*, **8**, 411–24.

Limtrakul P, Siwanon S, Yodkeeree S, et al (2007). Effect of Stemona curtisii root extract on P-glycoprotein and MRP-1 function in multidrug-resistant cancer cells. *Phytomedicine*, **14**, 381–9.

Ling Li, Qiangrong Pan, Meng Sun, et al (2007). Dibenzocyclooctadiene lignans — A class of novel inhibitors of multidrug resistance-associated protein. *Life Sciences*, **80**, 741–8.

Liscovitch M, Lavie Y (2002). Cancer multidrug resistance: a review of recent drug discovery research. *Drugs*, **5**, 349–55.

Loo TW, Clarke DM (2001). Defining the drug-binding site in the human multidrug resistance P-glycoprotein using a methanethiosulfonate analog of verapamil, MTS-verapamil. *J Biol Chem*, **276**, 14972-9.

Nakamura M, Abe Y, Katoh Y et al (2000). A case of pulmonary adencarcinoma with overexpression of multidrug resistance associated protein and p53 aberration. *Anticancer Res*, **20**, 1921–5.

Riordan JR, Deuchars K, Kartner N, et al (1985) . Amplification of P-glycoprotein genes in multidrug-resistant mammalian cell lines. *Nature*, **316**, 817-9.

Schoenlein PV (1994). Role of gene amplification in drug resistance. In: Goldstein, L.J., Ozols, R.F. (Eds.), Anticancer Drug Resistance: Advances in Molecular and Clinical Research. Kluwer, Boston, MA, pp. 167–200.

Shuzhong Z, Xinning Y, Robert AC, et al (2005). Structure activity relationships and quantitative structure activity relationships for the flavonoid-mediated inhibition of breast cancer resistance protein. *Biochem Pharmacol*, **70**, 627–39.

Thiebaut F, Tsuruo T, Hamada H, et al (1987). Cellular localization of the multidrug resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci*, **84**, 7735–8.

Xu D, Lu Q, Hu X (2006). Down-regulation of P-glycoprotein expression in MDR breast cancer cell MCF-7/ADR by honokiol. *Cancer Letters*, **243**, 274–80.

Yonggang M, Michael W (2008). Lobeline, a piperidine alkaloid from Lobelia can reverse P-gp dependent multidrug resistance in tumor cells. *Phytomedicine* (in press)

Yu S, Chen TM, Tseng SY, et al (2007). Tryptanthrin inhibits MDR1 and reverses doxorubicin resistance in breast cancer cells. *Biochem Biophys Res Commun*, **358**, 79–84.