



## Introduction to P-Glycoprotein/ABCB1/MDR1 and their Modulator Extracted from Plant

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Received: 25-02-2017; Revised: 25-04-2017; Accepted: 05-05-2017.

### 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 significant. 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.

**Keywords:** Multidrug resistance, p-glycoprotein, chemotherapy mechanisms, MDR1, natural modulators, flavonoids.

### INTRODUCTION

The ATP-binding cassette (ABC) transporters constitute a super-family of membrane bound proteins, which transport a variety of compounds across the membrane against a concentration gradient, at the cost of ATP hydrolysis. It consists of 49 major transporters, categorized into seven families from ABCA-ABCG.<sup>1</sup> Substrates of the ABC transporters includes not only natural compounds like- lipids, bile acids, xenobiotics, bilirubin, glucocorticoids, peptides for antigen presentation, but also includes chemotherapeutic agents as well.<sup>2</sup>

Among the seven categorized families, ABCB1 family has eleven proteins named from ABCB1–ABCB11 (table 1). This review particularly focuses on ABCB1 (p-glycoprotein/mdr1) as this is the major protein over expressed, in case cells gets resistant.

#### Structure of ABCB1

ABCB1 gene is located on chromosome 7q21.12 (Gen Bank accession number NT\_007933).<sup>3</sup> It contains two transcriptional start sites: proximal promoter, (for constitutive expression) and distal promoter (for induced expression at the time of stress condition). In drug selected cell lines or tumor cells, both promoters are expressed and leads to the over expression of ABCB1.<sup>4</sup> The messenger RNA (mRNA) is 4872 base pairs in length, including the 5' un-translated region.

#### Protein structure of ABCB1

ABCB1 (MDR/PGY1) was the first human ABC transporter to be cloned and characterized.<sup>5</sup> All the eukaryotic ABC transporters have conserved structure, with one hydrophilic nucleotide binding domain (NBD), located in

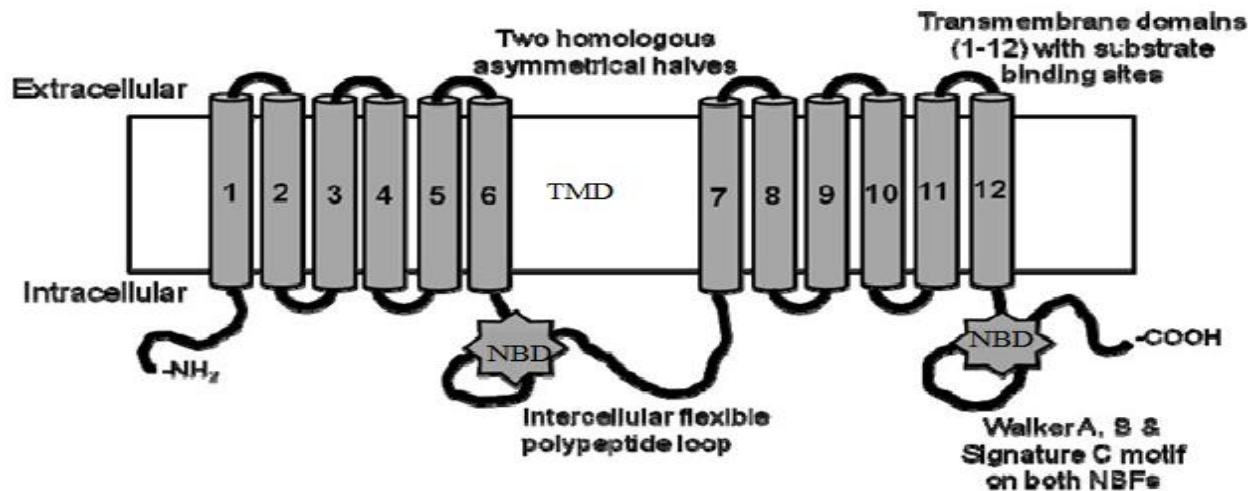
cytoplasm and one hydrophobic trans membrane domain (TMD). Half transporter consists of one NBD and one TMD, whereas full transporter needs dimerization of two such half transporters. ABCB1 includes both full (four in number) and half transporters (seven in number).<sup>6</sup> However, ABCA & ABCC contains full transporters, and ABCD & ABCG exclusively contain half transporters.

**Table 1:** A brief description of ABCB family

Symbol	Location	Function	Transporter type
ABCB1 (PGY1, MDR1)	7q21.12	Multidrug resistance	half-transporter
ABCB2 (TAP1)	6p21.3	Peptide transport	half-transporter
ABCB3 (TAP2)	6p21.3	Peptide transport	half-transporter
ABCB4 (PGY3)	7q21.12	PC transport	full-transporter
ABCB5	7p21.1	?	full-transporter
ABCB6 (MTABC3)	2q35	Iron transport	half-transporter
ABCB7 (ABC7)	Xq21-q22	Fe/S cluster transport	half-transporter
ABCB8 (MABC1)	7q36.1	?	half-transporter
ABCB9	12q24.31	?	full-transporter
ABCB10 (MTABC2)	1q42.13	?	half-transporter
ABCB11 (SPGP)	2q24.3	Bile salt transport	full-transporter

Functional protein in the plasma membrane comprises of two halves (one half having 610 amino-acids) joined by a 60 amino acid long linker peptide.<sup>7</sup> Each half comprises of six trans membrane segments in TMD and one ATP-binding site at NBD. TMD is involved in both drug binding and efflux, whereas ATP binding and hydrolysis occurs at

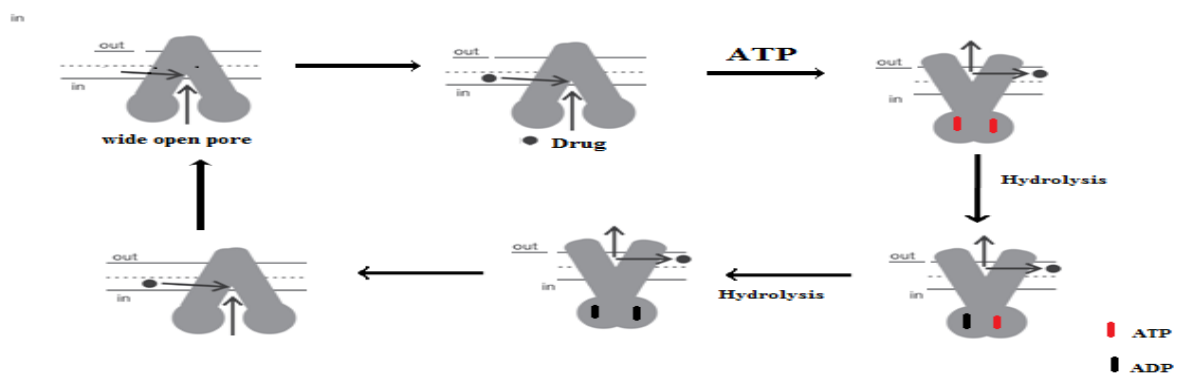
one of the NBD' sin an alternate manner.<sup>8</sup> Phosphorylation and N-glycosylation in the protein occurs post-transnationally.<sup>9,10</sup>



**Figure 1:** shows the 2-D structure of ABCB1/p-gp present as half transporter joined by linker peptide.

It has been studied that the transport of one drug molecule is accompanied by hydrolysis of two ATP molecules.<sup>8</sup> It has been found that ABCB1 can attain different conformations, so that it can recognize diverse group of compounds, indicating flexible nature of the transporter.<sup>11</sup> Substrate binding site lies in the internal cavity of the protein of about 6000 Å<sup>3</sup> (volume). This is

formed by trans-membrane helices and is accessible both from the cytoplasm and the inner leaflet of the lipid bilayer. This fact was also proposed by “vacuum cleaner” hypothesis that assumes that ABCB1 can recognize and bind its substrates within the membrane.<sup>12</sup> Moreover, ABCB1 can bind to more than one drug at a time and both drugs can influence each other transport.<sup>13</sup>



**Figure 2:** ABCB1/p-gp structure showing transport cycle.

Each NBD in ABCB1 contains consensus sequences: Walker A (GSSGCGKS), Walker B (ILLDEATSALD) and signature C motif (VSGGERKRV). Walker A and Walker B are separated by approximately 90–120 amino acids.<sup>14</sup> Highly conserved lys (K, underlined) residue present in Walker A motif is directly involved in the ATP binding whereas Walker B motif binds with the Mg<sup>2+</sup> with the help of asp (D, underlined) residue to stabilize NBD.<sup>15</sup> It has been proposed that signature C motif participate

to accelerate ATP hydrolysis by stabilization of transition state.

The transport cycle of the protein has already been proposed and involved different steps. Firstly, substrate molecule binds to the protein (from inside), followed by hydrolysis of the ATP. Hydrolysis induces a conformational change in both of the NBDs, which makes them approach closer to each other, finally resulting in another conformational change, where binding cavity becomes outward-facing (towards outer leaflet/extra

cellular environment of the cell membrane). In this stage, the affinity of the substrate is substantially reduced for the protein, resulting in release of the molecule outside the cell. P-gp ultimately returns back to the inward-facing conformation.<sup>17, 18</sup>

P-gp distribution is wide-spread in the body, including cells of the liver, colon, jejunum, kidney, pancreatic ductules, blood-brain barrier and adrenal gland.<sup>19-21</sup>

### Regulation of ABCB1

Studies on signaling pathways and transcriptional factors involved in the regulation of ABCB1 indicate the interaction of multiple pathways for activation and over expression of this protein. Ras/Raf/MAPK (mitogen activated protein kinase) pathway is associated with over expression of ABCB1 protein, as inhibitors of this pathway significantly reduces the survival of ABCB1 mediated MDR cancer cells.<sup>22</sup> The over expression of MAPK selectively activates downstream factor JNK, which binds and phosphorylates the NH<sub>2</sub> terminal activation domain of transcriptional factor c-Jun.<sup>23</sup> It has been found that reduced level of p-38, also a member of MAPK family, induces apoptosis in cancer cells.<sup>24</sup>

Evidences suggest that MAPK pathway also affect c-AMP dependent protein kinase A (PKA) and PI3K/Akt involved in activation of ABCB1.<sup>25-26</sup> PI3K/Akt pathways are downstream to PTEN, reduced level of this protein results in the development of MDR.<sup>27-28</sup> PTEN also maintains cellular PIP<sub>3</sub> at low level by removing the 3'-phosphate from PI3K generating PIP<sub>2</sub>. PIP<sub>3</sub> is upstream signal molecule of protein kinase B (PKB) pathway leading to the overexpression of ABCB1.<sup>28</sup>

In addition to this, ABCB1 can also be increased by TNF- $\alpha$ .<sup>29</sup> NF- $\kappa$ B transcription factor also involves in the activation of ABCB1 as NF- $\kappa$ B antisense RNA significantly blocks ABCB1 Promoter activity.<sup>30</sup>

### ABCB1 in Multi Drug Resistance (MDR)

MDR is defined as the simultaneous gain in resistance or insensitivity of a cancer cell to the cytotoxic and cytostatic action of various structurally and functionally unrelated drugs, even when exposed to a single drug. Cells exposed to toxic compounds/drugs can develop resistance by a number of mechanisms: cellular and non-cellular.<sup>31</sup> Non-cellular mechanisms include factors that decrease uptake of drugs, such as poor vascular accessibility or cell growth environment. However, cellular mechanisms involve enzyme systems and transport proteins so that there is increase in detoxification of drugs, alteration of target transport proteins and expulsion of the drugs out of the cell.<sup>32</sup>

Chemotherapeutic drugs commonly enter cells through the plasma membrane by passive diffusion. Till now, 49 ABC transporters have been identified in humans, with only twelve of them function as drug transporters.<sup>33</sup> However in majority of cancers, MDR seem to be the result of over expression of only three proteins: ABCB1 (p-

gp, MDR1), multidrug resistance-associated protein 1 (MRP1/ABCC1), and breast cancer resistance protein (BCRP/ABCG2).<sup>34</sup>

All the substrates recognized by this transporter are relatively hydrophobic in nature with neutral or cationic character under physiological conditions.<sup>35</sup> ABCB1 transports variety of substrates including anticancer drugs, anti fungals drugs, antibiotics, detergents, HIV protease inhibitors, and many other cytotoxic compounds.<sup>36</sup>

Tumours derived from ABCB1 positive cell shows over expression of this protein before chemotherapy treatment.<sup>37</sup> In other cancers, the expression of ABCB1 may be low at the time of diagnosis, but may be induced after exposure to chemotherapeutic agents, there by resulting in the development of MDR in those cells.<sup>37</sup> A variety of factors involved in the over expression of ABCB1. So MDR reversal is not possible by single drug. This is the reason why chemotherapy uses a combination of drugs and not a single drug.

### MDR modulation by ABCB1

Chemical compounds which reverse the resistance against anticancer drugs are called MDR modulators, chemo sensitizers, or reversers.<sup>38</sup> MDR modulator should inhibit drug efflux or increase accumulation of anticancer drugs inside the resistant cells so that cells again become sensitive towards drugs. An ideal modulator should be relatively non-toxic to the normal cells and should not interfere with other important cellular processes.<sup>39</sup>

A large number of modulators have been identified, although not specifically developed for ABCB1 inhibition but co-incidentally found to be effective in the reversal of MDR. These modulators can inhibit ABCB1 protein by number of mechanisms: competitive and non-competitive binding of modulators in the substrate binding pocket of protein, inhibits binding and hydrolysis of ATP.<sup>39</sup> The modulators can also induce biophysical changes in lipid bilayer in which the protein is embedded so that interfering with its transport activity.<sup>40</sup> These include verapamil (calcium channel blocker), quinine (anti malarial), cyclosporine A (immunosuppressant), tamoxifen (anti-steroid), and erythromycin.<sup>41</sup> Problem with most of these modulators is that they are non selective, less potent and also become transported by this protein.<sup>42</sup> Therefore, extremely high concentration of drugs are required to produce the same level of affect, which leads to the undesirable side effects such as cardio toxicity for verapamil or immuno-suppression for cyclosporine A.<sup>42</sup>

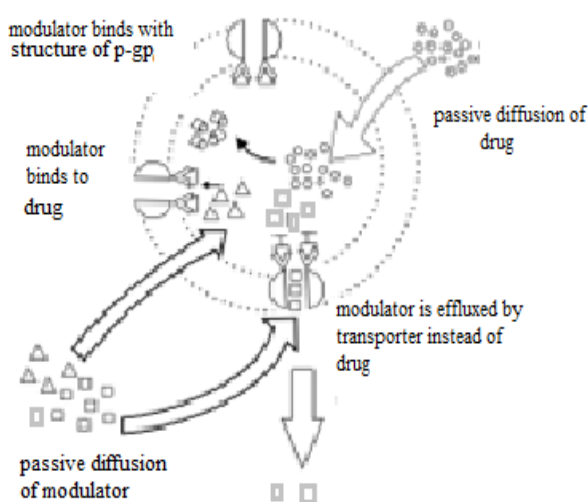
So these modulators are modified and such modifications were concentrated on reducing their adverse effects by eliminating their non MDR activities. Modified modulators involve valsopodar (a non immunosuppressive analogue of cyclosporine A) and R verapamil (R enantiomer of verapamil, a weaker calcium channel blocker).<sup>43</sup> Even these modulators also failed to deliver



desired effect due to their low affinity for ABCB1 protein at tolerable dose and co-incidentally found to be substrate for CYP3A so that clearance of xenobiotics present in the body was interfered with.<sup>44</sup>

Now these days, modulators are designed with high potency, high affinity and high selectivity even at low nanomolar range and relatively low toxicity towards normal cells. Modulators such as LY335979 and XR9576 are highly potent and selective in inhibiting ABCB1 protein. Even tariquidar (anthranilamide derivative) is undergoing clinical trials.<sup>45-46</sup>

### strategies used by modulator



**Figure 3:** Strategies of MDR reversal in ABCB1/p-gp using modulators.

For the effective modulation of ABCB1 protein expression, signal transduction pathways can be targeted by modulators, e.g. sildenafil, an inhibitor of cGMP-specific phosphodiesterase, was found to significantly reverse ABCB1 transporter mediated MDR. Another study showed that nilotinib, an inhibitor of p38/MAPK pathway, significantly increases the accumulation of doxorubicin (anti cancer drug) in synovial sarcoma. Clinical trials have been initiated for this combination of drug with modulator. Another class of compounds which is gaining rapid attention as ABCB1 modulators are natural plant products.<sup>47</sup>

### Plant products as MDR modulators

Unfortunately, most of the agents designed earlier suffer from their intrinsic toxicity or from undesired interference in metabolism. One of the advantages of plant products is that they are essential component of human diet; it may be assumed that they would not show toxicity at higher doses. It has been found that many plant products acts as potential MDR modulators. The first evidence of P-gp/ABCB1 inhibition by such components came from the grapefruit juice interactions with numerous drugs.<sup>48</sup> Since then there are frequent reports of pharmacokinetic interactions of herbs with drugs, causing either beneficial

or harmful effects.<sup>49-50</sup> The herb-drug interactions can be hazardous if they cause increased drug levels reaching above toxic threshold. On the other side, these effects may be exploited beneficially to improve pharmacokinetics of co-administered drug.<sup>51</sup> Increasing evidence from in vitro and in vivo studies indicated that the altered drug concentrations by co-administered herbs may be attributable to the induction or inhibition of hepatic and intestinal drug-metabolizing enzymes (CYPs), and drug transporters such as ABCB1.<sup>52-53</sup> Consequently this has raised concerns regarding their potential modulators of ABCB1 from herbal medicines.

Moreover, polyphenols are the most abundant integral component of our common diet. There are three main types of polyphenols (flavonoids, stilbenes, and lignans) that are classified by the number of phenol rings they contain and the binding properties of the ring structures.<sup>54</sup> Each class of polyphenols can be further sub classified by the interactions of their respective phenyl rings to carbon, oxygen, and organic acid molecules.<sup>55</sup> Flavonoids are divided into 6 subclasses: flavonols, flavones, isoflavones, flavanones, chalcones, dehydrosilybin. They are particularly abundant in vegetables, fruits, and plant-derived beverages such as wine and tea. The daily intake of total flavonoids from the average U.S. diet was estimated to be more than 1 g.<sup>56</sup>

In addition, a variety of flavonoid-containing dietary supplements and herbal products are now available in the market because of their proposed health-promoting activities, such as antioxidant, anti carcinogenic, anti-inflammatory, anti proliferative, anti angiogenic, and anti estrogenic (or estrogenic) effects and the lack of toxicity associated with this class of compounds.<sup>56-57</sup> In foods, flavonoids are often present as  $\beta$ -glycosides of aglycones and methoxylated forms. Upon ingestion, flavonoid glycosides are deglycosylated, the aglycones are metabolized into glucuronide, sulphate, and methoxylated conjugates.

### Structural activity relationship (SAR) for flavonoid-ABCB1 interaction

In general, the presence of the 5-hydroxyl group, the 3-hydroxyl group, and the 2, 3-double bond appears to be important for potent flavonoid- NBD interaction. In addition, isoflavonoids with ring B branched at position 3 instead of 2 have lower ABCB1 interaction activity.<sup>58</sup> Hydrophobicity of the substituent was an important parameter since alkoxylation up to 8–10 carbon atoms gradually increased the chalcone binding affinity<sup>59</sup>, and geranylation and prenylation also increases interaction.<sup>60-61</sup>

### Concentration required for effective modulation of ABCB1

The concentrations required for flavonoids to produce a significant modulation of ABCB1 activity seem to be, in general, 10  $\mu$ M or higher, which appears to be achievable in the intestine after ingestion of food and dietary

supplementation. For example, grapefruit juice contains 145–638 mg/L naringin<sup>62-63</sup>, equivalent to 250–1100  $\mu\text{M}$ , and orange juice contains 200–450 mg/L hesperidin<sup>64</sup>, equivalent to 330–740  $\mu\text{M}$ .

Although these flavonoid glycosides may not potently interact with ABCB1, their corresponding aglycones released from these glycosides in the intestine could be present in high enough concentrations to inhibit intestinal ABCB1, resulting in drug interactions. However, the main metabolites of flavonoids (glucuronides and sulfate conjugates) may not interact with ABCB1 because these metabolites are organic anions. So, systemic inhibition of ABCB1 by flavonoids or their metabolites may be, in general, insignificant after regular supplementation. Interaction could occur after administration of an extremely high dose, especially by intra venous injection.<sup>65</sup>

#### Mechanism behind flavonoid mediated ABCB1 inhibition

In general, P-gp can be inhibited by more than one mechanism. Modulators may interact with the steroid-binding site and substrate-binding site.<sup>66</sup> It has been shown that flavonoids genistein, epicatechin gallate, catechin gallate, epigallo catechin gallate and silymarin can directly bind to the P-gp substrate binding site.<sup>66-68</sup> Some flavonoids that are ABCB1 modulators have been

shown to be able to change membrane lipid packing order and thus change membrane fluidity or permeability.<sup>69</sup> The latter process involves either competitive binding to the substrate-binding site or interaction with other drug binding sites causing altered molecular conformation. Drugs such as cyclosporine-A inhibit transport function by interfering with both substrate recognition and ATP hydrolysis. Whereas, few others like cis(Z)- flupentixol (a thioxanthene derivative) prevent substrate translocation and dissociation due to allosterical changes produced in drug transporter.<sup>70</sup> So, it is possible that the observed effects of flavonoids on drug accumulation could be due to their nonspecific interaction with the cell membrane, resulting in increased passive membrane permeability.<sup>71</sup> Alternately, flavonoid-induced decrease in ABCB1 expression in ABCB1 positive cells could be another possibility.

Compounds inhibiting ATP hydrolysis could serve as better inhibitors, since they are unlikely to be transported by ABCB1, and these kinds of agents require low dose which is achievable at target site. Since some of the inhibitors known till now have been found to interact with the NBD to interfere the ABCB1 ATPase catalytic cycle causing stimulation and inhibition, further research would provide newer and better inhibitors with potent and specific activity.

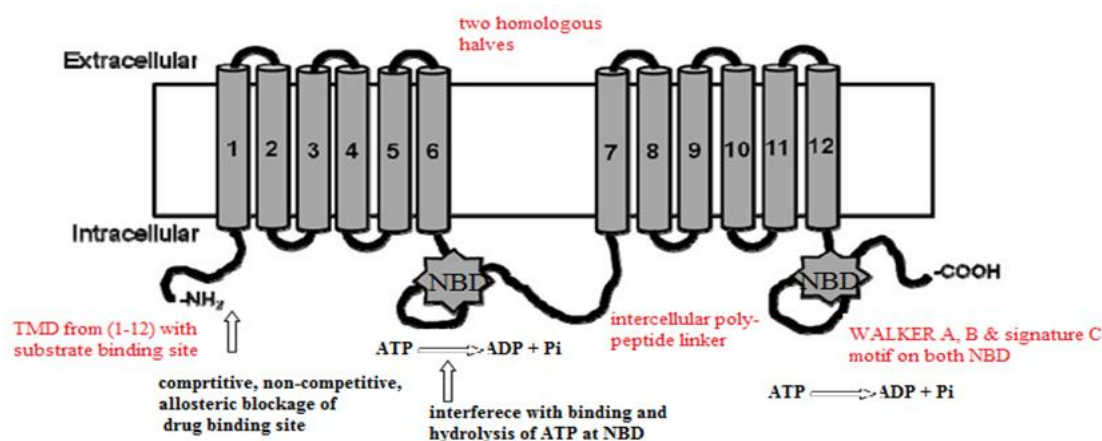


Figure 4: Mechanism of p-glycoprotein inhibition by plant products

#### Example of Natural products as ABCB1 modulator

Recent researches showing natural products as potential MDR modulators are well appreciated. It has been reported that root extract of *Stemona curtisii* modulates ABCB1 activity and sensitized the drugs including vinblastin, paclitaxel and colchicines against MDR cancer cells.<sup>72</sup> Another study shows that compound present in rhizome extract of *Alisma orientalis* binds at the substrate binding pocket of transporter protein.<sup>73</sup>

Several studies have suggested that catechins and the flavins found in tea can reduce the risk of various types of cancers.<sup>74</sup> Catechins have been found to inhibit ABCB1 activity in vitro.<sup>75</sup> A green tea polyphenol,

epigallocatechin gallate (EGCG) has been observed to increase the efficiency of doxorubicin in drug resistant cell as EGCG binds to NBD and prevent ATP binding of ABCB1 protein.<sup>76</sup> Another compound lobeline, a piperidine alkaloid from *Lobelia inflata* inhibits ABCB1 activity at non toxic concentration.<sup>77</sup>

Polyphyllin D (PD), a steroid found in *P. polyphylla*, is a potent anticancer agent probably induces apoptosis by mitochondrial fragmentation. One report shows that Honokiol, product of *Magnifolia grandiflora*, inhibits angiogenesis and simultaneously downregulate the expression of ABCB1 at mRNA level.<sup>78-79</sup>

It has been reported that curcumin can modulate the expression and function of P-gp.<sup>80-81</sup> Curcumin (also called turmeric) is a polyphenol compound extracted from root of *Curcuma longa*, which is also present in various medicinal preparations used for the treatment of inflammation, skin wounds, and tumors. Curcumin has been found to down regulates ABCB1 expression at transcriptional level by inhibiting PI3K/NF- $\kappa$ B signal cascade.<sup>80</sup> Curcumin is rapidly and extensively metabolized in the liver and intestine. Oral administration of curcumin usually results in relative low plasma concentration.<sup>82</sup> Moreover tetrahydro curcumin, one of the ultimate metabolites of curcumin has been found to be effective modulator of ABCB1.<sup>83</sup>

Silymarin has been found to increase daunomycin accumulation only in ABCB1 positive cells, but not in ABCB1 deficient cells.<sup>84-85</sup> Silymarin (also called milk thistle) is the active component of *Silybum marianum* that protects liver and kidney cells from toxic effects of drugs, including chemotherapy.<sup>84</sup> Milk thistle is a flavonoid, which interact with both the ATP binding site and substrate binding pocket of ABCB1 protein.<sup>85</sup>

Furthermore, active component of Ginseng have been tested for the effects on ABCB1 using several multidrug resistant cell lines. Ginenosid has been shown to enhance the accumulation of R-123, inhibit vinblastine efflux, and reverse the resistance to doxorubin and vincristine in multidrug-resistant KBV20C cells in a dosedependent manner, but has no effect on parental KB cells.<sup>86</sup> It has been found that treatment of drug-resistant cells with rosemary extract increased drug sensitivity by blocking the binding of substrate drug to ABCB1. The most important constituents of rosemary are caffeic acid and its derivatives such as rosmarinic acid. Rosemary extract has been found to increase the intracellular accumulation of doxorubicin and vinblastine, in drugresistant MCF-7 human breast cancer cells which express ABCB1 protein, but not affecting accumulation or efflux of doxorubicin in wild type MCF-7 cells.<sup>81, 87</sup>

## 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 dependent 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 over-expressed 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 chemotherapy successful. Such elements from dietary sources possess 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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**Source of Support: Nil, Conflict of Interest: None.**

