Review Article

Human P-glycoprotein Mediates Multidrug Resistance in Cancer Chemotherapy: A Brief Review

Abstract:

Multidrug resistance (MDR), as is well known, is regarded as the primary factor in cancer therapy failure. A common mechanism of MDR in anticancer drugs is the expression of P-glycoprotein (P-gp), a class of ATP-dependent membrane transport efflux pumps called adenosine triphosphate (ATP)-binding cassette (ABC) transporters. It pumps xenobiotics outside the cell and plays part in typical physiological detoxification and host defense activities. This transporter is distributed in gastrointestinal mucosa epithelial cell surfaces, blood-tissue barriers, hepatic biliary epithelium, proximal tubules of the kidney, and the adrenal cortex. P-gp is known to be responsible for MDR because of its over-expression in malignant cells. It functions as an efflux pump lowering the concentration of drugs intracellularly, thus decreasing the effectiveness of cancer chemotherapy. Although using multiple anticancer medications is a good strategy, Cancerous cells are able to develop MDR. A number of chemically synthesized P-gp inhibitors were investigated to overcome MDR in clinical studies. Additionally, certain natural compounds have been observed to modulate P-gp. This review discusses the role of P-gp in cancer MDR and challenges for inhibiting P-gp in the context of overcoming MDR mediated by P-gp. It is concluded that the discovery of selective, safe, and potent inhibitors of P-gp remains necessary.

Keywords: Multidrug resistance (MDR); ATP-binding cassette (ABC); P-glycoprotein (P-gp); P-gp expression; cancer chemotherapy; P-gp inhibitors

1. Introduction

Cancer is among the worst hazards to health of people in the twenty-first century which has been linked to unhealthy diets, cigarettes and alcohol use, ageing, population expansion, chronic infections, and environmental pollution [1]. The current rise in cancer cases places a heavy economic and social burden on society. Since Goodman [2], Farber et al. [3], and collaborators first introduced chemotherapy for the treatment of lymphosarcoma and leukemia before more than seventy years, chemotherapy has been extensively used to treat cancer. [4]. Drug resistance can develop following the initial treatment which is referred to as acquired drug resistance [5]. The phenomenon known as MDR occurs when several malignant cells become resistant to a variety of structurally distinct anticancer medications [6, 7]. MDR refers to a tumor's capacity to simultaneously exhibit resistance to several structurally and functionally unrelated anticancer drugs [8]. It is considered as one of the challenges to the effective clinical use of several chemotherapy drugs. Without prior chemotherapy exposure, MDR develops in cancer cells as a result of epigenetic and genetic changes which impact chemotherapy sensitivity. During chemotherapy, MDR can also develop in cases of cancer cells that were initially sensitive to anticancer drugs. Additionally, it is understood that a mixture of drug-sensitive and drug-resistant cells typically make up malignancies [9, 10]. Cells that are resistant to drugs start to predominate the cancer cells when drug-sensitive cells are selectively killed during treatment. Numerous investigations on strategies to modulate MDR have been conducted as a result of the significant impact of chemotherapeutic drug resistance.

2. MDR

MDR is still a major obstacle to successful cancer treatment. Data shows that more than 90% mortality of cancer patients is due to MDR [11]. In cancer chemotherapy, MDR refers to a cancer cell's capability for survival against a variety of anticancer medications where resistance to one chemotherapeutic agent is associated with resistance to agents that have completely different structures and mechanisms of action [12]. This defense mechanism has been developed by living organisms against toxic substances in the environment as they should prevent the harmful effects of cytotoxic substances through efflux pumps. Chemotherapy should permeate from the blood to tumor tissue cancer. Drug absorption in these cells is decreased as a result of MDR mechanism that may be created by increased drug release outside the cells [13].

During cancer treatment, overexpression of the efflux pump in tumor cell is a crucial MDR regulator [14]. The transport of cancer chemotherapy across the membrane is mediated by a family of ATP-dependent transporters. These transporters are consisting of 2 transmembrane domains (TMDs) and 2 cytoplasmic domains which bind to ATP thus called ATP-binding cassette (ABC) [13]. P-gp binds to chemotherapeutic drugs after ATP hydrolyzed then P-gp structure of has been modified. Then, the anticancer agent releases extracellularly. After the 2nd hydrolysis of ATP, the transporter recovers to its primary structure, enabling the drug to exit outside the cell [16, 17].

Research works on cell lines of tumor have recognized transporters that act to facilitate drug release extracellularly as a major mechanism for MDR. There are at least forty-eight structurally associated transporters called ABC-family [18], and subfamilies include subfamily (B) which includes P-gp and subfamily (C) which includes multidrug resistance-associated protein (MRP) transporters. P-glycoprotein binds to a wide range of substrates, especially which have hydrophobic domains and positive-charged-areas [19]. MRP1 expression has been reported in cancerous cell lines that resemble stem cells in some characteristics [20], supporting the idea that tumor stem cells generally express those drug-efflux transporters [21, 22].

3. P-gp: an overview

Burchenal et al were the first researchers who documented a drug resistance case in a mouse model of leukemia to 4-aminomethylpteroylglutamic acid [23]. Then, after few years, HeLa cells and Chinese Hamster Ovary (CHO) cells showed the same kind of resistance to the antibiotic actinomycin D, according to two other studies [24, 25]. The concept of MDR was thus emerged; however, it was not fully understood until the ATP-dependent efflux of daunomycin, another antibiotic, was seen in resistant cells of Ehrlich ascites carcinoma which displayed cross-resistance to anticancer agents vinca alkaloids [26]. A 170 kDa efflux pump was discovered as a result of surface-labelling experiments in CHO cells which exhibited colchicine resistance and also cross-resistance to a number of amphiphilic molecules [27]. This pump was named P-gp, or the permeability-glyco-protein, refers to its capability of altering the drug permeability rate; the higher the expression of P-gp the higher the degree of drug resistance. After MDR phenotype was genetically examined and post multiple cloning experiments in both animal and human cell lines, it was found that ABCB1 gene is responsible for producing the P-gp multidrug transporter, [28-32].

P-gp transports a variety of structurally varied molecules and possesses a wide range of drug specificities. As a result, drug accumulation inside cells is reduced, which in turn reduces medication efficacy. P-gp is known to be the first discovered and best studied MDR transporter. It is considered as a possible target to prevent cancer MDR [33]. Reduced responses to chemotherapy and bad prognosis in a variety of cancer forms were linked to higher expression of P-gp in carcinogenic cells. Leukemia and breast cancer are initially expressed P-gp at low levels and progressed after receiving chemotherapy, showed upregulation of P-gp [34]. A variety of chemotherapeutic agents which are essential for protocols of chemotherapy are liable to efflux mediated by P-gp [7, 35-37].

P-gp is an efflux pump which transport compounds actively outside the cell against the concentration gradient using ATP [38]. In normal cells, P-gp has the capability to directly affect the pharmacokinetics and the toxicity of medical products, influencing both bioavailability and efficacy [39]. This point should be considered when developing a new medication. Therefore, to check for possible P-gp substrates, screening during the early phases of drug production is encouraged by the Food and Drug Administration [40].

In humans, there are 2 gene family of P-gp, named MDR1 and MDR3 [41]. In contrast to MDR3, which shows a limited expression, human MDR1 is distributed to a large extent and can efflux a variety of medications out of the body cells. However, highest expression of MDR3 is in the canalicular membranes of hepatocytes [42]. Recently, it has been noted that human MDR3 contributes to medications transport thus it plays no role in MDR and has no relevant pharmacological impact [43]. Since MDR1 is widely expressed in human body cells and is considered among the most vital ABC-transporters for substrate efflux, it has pharmacological significance. It has been determined to be the primary cause of cancer MDR [41-43].

4. Localization and expression of P-gp

P-gp is localized and highly expressed on surface of the epithelial cells in different tissues like renal proximal tubules, adrenal cortex, mucosa of the digestive system, biliary epithelium of the liver and the blood-tissue barriers (Figure 1). The latter comprise the blood-brain barrier's endothelial component, the placenta, the endometrial tissue, and testicular tissue [44-46].

P-gp expression was investigated in cellular and animal models, as well as the human intestine [47]. P-gp is conveniently located to top villi of enterocytes to identify its substrate and pump it

back into to the lumen of the intestinal (Figure 1). P-gp levels in the small intestine are not uniform, and it has been observed that the transporter is expressed differently in different epithelial villi cells, with columnar cells expressing it more strongly than the crypt [48]. The expression of P-gp is not equal along the intestine, but instead increases from the stomach to the colon [45].

P-gp is overexpressed in cancer cells, causing these cells to efflux chemotherapeutic medicines out of them, lowering their concentration inside the cancerous cells [49]. However, P-gp expression levels in the kidney and adrenal cortex have been shown to be at least as high as those detected in several MDR cancerous cell lines [45, 50]. Intestinal P-gp expression was first shown in the Caco-2 cells (human colon cancer cells) [51-54] and in P-gp knock-out mice as well [55].

P-gp localization clearly suggests a key role for P-gp as efflux pump, which serves as a major gatekeeper by limiting the penetration of chemotherapy into the cancer cells.

5. Function of P-gp

P-gp is suggested to act as a cleaner by expelling anticancer medications from the cell inner membrane [56]. In addition to its function in cancer, P-gp has an essential role in physiological processes that normally detoxify the body and protect the host by transporting a variety of substrates [7]. P-gp protects our body against xenobiotics, medications or toxins by ejecting them out of the cell. Moreover, P-gp protects bone marrow’s hematopoietic-progenitor cells against chemotherapy's cytotoxic effects [57].

P-gp that is expressed in the epithelia of the gut plays a role on the oral bioavailability of medications because of its capability to decrease their permeability and increase their excretion [55]. P-gp, which is found in the apical membrane of vascular endothelial cells, is thought to be a vital piece of the blood-brain barriers, which prevents hazardous chemicals from entering the brain [58, 59].

P-gp has three main functions. First, it prevents drugs from entering the body after oral administration because it is found in the intestinal cell’s apical membrane. Second, once substrate have entered the bloodstream, P-gp eliminates them through urine and bile because it is found in the hepatic canalicular membrane and the apical surface of the renal proximal convoluted tubule cells, respectively. Third, P-gp prevents drugs from entering vital tissues, particularly the blood-brain barriers [60]. The bioavailability and distribution of medications are reduced largely by P-gp. The therapeutic level and bioavailability of the medication are therefore not met.

Paclitaxel was shown to be absorbed orally in P-gp-deficient mice more readily than in wild-type mice, demonstrating that P-pg prevents drug absorption by expelling medicines to the lumen of intestine. Studies utilizing P-gp inhibitors and substrates like cyclosporine [61] and docetaxel [62] have revealed that similar effects may possibly occur in humans. P-gp has also been demonstrated to serve as an excretory protein in the gut; after injecting mice that expressed P-gp with digoxin, a significant dosage amount was released within 90 minutes into the lumen of intestine after administration. This secretory effect was not observed when P-gp was inhibited or when P-gp knockout mice were used [63]. This result was also confirmed in humans [64].

6. Structure of P-gp and mechanism of substrates efflux

P-gp, ABC transporter 170-kDa with 1280 amino acids, is an ATP-dependent efflux pump and is encoded by MDR1 gene [65]. It is made up of two symmetrical cassettes, one with an amino (N) and one with a carboxyl (C) terminus [66]. Each consists of six TMDs that are connected to one another by a polypeptide chain that is 80 amino acids long and has an ATP-binding motif [67]. It is found that human P-gp have 4 domains, 2 of which are hydrophobic (TMDs) cross the membrane 6 times per domain i.e., twelve times per molecule of P-gp, through putative α-helices [68, 69]. The other 2 domains are hydrophilic (NBDs), and they are located at the cytoplasmic face of the cell membrane. (Figure 2). The protein appears to consist of two homologous parts, each of which contains a TMD and an NBD, and it was created through gene duplication. There is a lot of evidence that supports a catalytic cycle in which the two NBDs alternately hydrolyze ATP, despite the fact that the processes of substrate translocation and ATP binding by the P-gp are unclear [70]. The drug binding sites are found to be located on the TMDs [35]. Researchers supposed that ATP binding and/or hydrolysis alters the conformation of molecules, which is transferred from NBDs to TMDs, which therefore facilitates the translocation of solutes (Figure 3). There is indirect evidence that P-gp undergoes conformational changes after nucleotide binding [71–74]. Once ATP binds to P-gp cytoplasmic side and triggers its hydrolysis, the solute is excreted extracellularly and ATP molecule released phosphate [75]. A new molecule of ATP binds to the subsequent ATP binding site after the release of adenosine diphosphate (ADP). Reset of the protein will take place following ATP hydrolysis [76].

The most recent structural determinations have started to elucidate the molecular process through which P-gp mediates MDR. The precise drug transport pathway has not yet been fully elucidated

in the context of a changing conformational landscape for P-gp, even though P-gp structures in combination with actual anticancer drugs have not yet been discovered. The binding sites at P-gp are correlated with the ligands structure and activity, which also should be assessed with various medications and drug-bound structures.

P-gp has a large and diverse drug-binding domain that includes several, overlapping binding sites and can accommodate both small and large molecules as well as multiple compounds simultaneously. The disclosed mouse P-gp configurations further link individual TMD helices rotation and translation to the opening and closing of the two P-gp halves, resulting in a continuous change in surface topology [77].

In the pharmaceutical industry, determining drug's P-gp susceptibility has evolved as an crucial stage in the creation of new medicines [7, 78]. Documentation of drug-P-gp interactions is now mandated by US Food and Drug Administration (FDA) and European Medicines Agency (EMA) for approval of any new drug. [79].

7. P-gp inhibitors

P-gp transports a variety of substrates that vary from one another in both structure and functionality. P-gp substrates typically seem to have an amphipathic and lipophilic character [80]. They are basic nitrogenous compounds forming many hydrogen bonds [81]. They include various anticancer drugs [82-84] and many other classes of drugs with at least 480 substrates already recognized and this number is continually growing [85]. As mentioned before, P-gp serves to protect the body’s cells from toxins by preventing their entry. However, since P-gp is overexpressed in cancerous cells, impeding drug access, the effect of P-gp should be prevented in such conditions for chemotherapeutic efficacy [86] by inhibiting the P-gp-anticancer drug interaction [87]. Over the years, a number of P-gp inhibitors have been reported. When co-administered with an anticancer agent, the P-gp inhibitors increases the total concentration of important therapy inside the cancerous cell [88]. To suppress the P-gp function, many inhibitors have been explored [89, 90]. The main challenge of this approach is the absence of non-toxic and strong inhibitors. Instead, medication research efforts are currently focused on discovering of novel substances or ways to avoid P-gp action.

Co-administering P-gp transport substrates as cancer chemotherapeutic agents with P-gp inhibitors has been believed to be a therapeutic strategy to overcome MDR during cancer treatment by

inhibiting efflux of drugs mediated by P-gp. This combined therapeutic approach has been hypothesized to block P-gp thus decreasing the efflux of anticancer drugs and increasing their bioavailability [91]. Research efforts have been made to find safe, effective and specific P-gp inhibitors. The development of P-gp modulators has attracted a lot of attention in the past with the objective of overcoming P-gp-mediated MDR in chemotherapy. Due to failures of these modulators, excitement has largely subsided. The causes behind these failures are complicated and not entirely obvious. [92, 93]. One clear reason is their toxicity to healthy tissue. To reduce the unwanted toxic effect of P-gp inhibitors to healthy cells, these modulators should be delivered specifically to cancer cells. Low potency and specificity of most known P-gp modulators is another serious issue.

Older P-gp inhibitors have not been produced primarily to block MDR and instead have distinct pharmacological activity. Verapamil, for example, is a calcium channel inhibitor with a P-gp binding affinity of only about 10 μM and was one of the early P-gp modulators examined clinically [94]. Cyclosporine A, an immunosuppressive drug, was initially investigated as a low affinity P-gp inhibitor in clinical research [95]. Due to the limited affinity of such agents, large dosages had to be used, which had harmful effects and even toxicities. In general, identified P-gp inhibitors have lower specificity and potency than the majority of medications used clinically.

Most P-gp inhibitors are created to alter its functionality [96]. P-gp modulators have been found to act either through competition with binding sites or interference with hydrolysis of ATP. In addition to the two existing mechanisms, an allosteric mechanism for P-gp inhibition was recently identified [97].

Prior to being transported to the extracellular space, substrates are first bound to the P-gp [9 8]. P-gp binding pockets are thought to be low specific and high flexible, making it possible to bypass MDR-related problems when treating cancer [99]. The inhibitors of P-gp are divided into four major generations [97] (Table 1).

The 1st generation P-gp inhibitors are active pharmacological substances that involved in treatment of some diseases. They compete with the anticancer agents for the efflux pump (P-gp). The calcium channel blocker, verapamil, is the prototype inhibitor of P-gp [100] that can accumulate many chemotherapies intracellularly in many cell lines of cancers [101-103]. Research works discovered that P-gp inhibitors include other calcium channel blockers like, diltiazem [102], bepridil [104],

and isradipine [94]. Cyclosporin-A is a commonly used immunosuppressive drug that is considered as an important first-generation P-gp inhibitors [105-107]. In leukemia, MDR was reversed upon using verapamil. These inhibitors have low P-gp affinity, demanding high doses thus inducing devastating toxicities or deleterious adverse effects [108, 109]. Reserpine, yohimbine, toremifene, quinidine, and tamoxifen are also examples of P-gp modulators from 1st-generation class [110]. These modulators were replaced by second-generation class because of their low efficacy and their toxic effects [110].

The 2nd generation P-gp inhibitors are pharmacologically inactive but induce P-gp inhibition. These inhibitors are obtained by alteration in the chemical structure of the first-generation class members to attain low toxicity, high selectivity and potency. Dexverapamil (R-enantiomer of verapamil), emopamil, gallopamil or Ro11-2933 are considered more potent and less toxic than verapamil regarding the activity for inhibiting P-gp [111-114]. The non-immunosuppressive analog of cyclosporine-A, valspodar (PSC 833), is the most potent and most often used in vitro as MDR reversal agent [115, 116]. Novartis manufactured PSC 833 from cyclosporine-A by methylating amino acid in the lateral chain and oxidizing alcohol. It has a potency that is 5–20 folds more than that of cyclosporine-A [117, 118]. PSC 833 interacts with pharmacokinetics of anticancer medication, increasing the toxicity of these medications which necessitates decreasing the dosage [119]. We can conclude that second-generation of P-gp inhibitors are better than first-generation, but they have some properties restricting their utilization as MDR reversal agents. These modulators can inhibit metabolism of cancer chemotherapeutics, inducing toxicities which needs reduction of anticancer dosage. To diminish this issue, researchers have begun to draw their attention to P-gp modulators the 3rd generation P-gp modulators.

The 3rd generation P-gp inhibitors are discovered to alleviate the problems associated with the previous generations. The 3rd P-gp blockers are advantageous since they are less toxic, more selective and effective against MDR [120]. Moreover, they do not interact pharmacologically with anticancer agents, and they were created to be 200-times more potent than the earlier generations of P-gp modulators. These include OC144-093 [105], zosuquidar (LY335979) [121], XR9051 [122] and elacridar (GF120918) [123]. Inhibitory activities are mostly produced by the inhibitors' chemical structure. The tariquidar's heterocyclic ring, which is adjacent to the antranilamide ring, promotes the inhibitory activity [124]. In conclusion, these modulators have acceptable toxic

levels; nevertheless, clinical testing is still pending to determine how well they work in combination with anticancer medications.

The 4th generation P-gp inhibitors (natural P-gp inhibitors) are now being discovered to alleviate the toxicity seen with the synthetic inhibitors. Natural products were found to overcome P-gp mediated MDR and to exhibit antineoplastic activities [125]. Major classes of plant-derived compounds like flavonoids, stilbenes, coumarins, terpenoids, alkaloids and saponins (Figure 4) are well investigated. Examples of natural P-gp modulators that are incorporated to deal with cancer include curcumin, quercetin, piperine, capsaicin, and limonin [126]. Natural molecules are relatively new participants in P-gp inhibition field with full of promise outcomes, however the toxicity issue persists because of targets non-specificity and substrate pharmacokinetic alterations. Quercetin can inhibit P-gp [127-130] as well as the metabolizing enzyme, CYP3A4 [131], thus it could affect the pharmacokinetics of the anticancer drugs and induce toxicity. Numerous newly found natural compounds have already been investigated for their activity on ABC transporters using various models. With natural compounds, there is a good chance of success, but further study is required to find new candidate of natural origin with optimum activities.

8. Conclusion and perspectives

It is concluded that P-gp inhibitors, if combined with cancer chemotherapy, have been demonstrated to be an excellent approach to inhibit MDR in cancerous cell. Major paradigm shifts in the realm of cancer are brought about by P-gp-mediated multidrug resistance. Despite the challenges presented by the discovery of MDR modulators, clinical anticancer drug resistance continues to be a major concern, thus researchers should keep working to find solutions. Researchers are planning to improve the response of cancer patients to anticancer drugs by inhibiting P-gp thus preventing the MDR. P-gp is structurally different from many receptors or enzymes in that it is lacking a clearly defined site for drug-binding, which makes it challenging to develop highly effective P-gp-specific inhibitors. The main challenge is the absence of high potent and selective P-gp inhibitors; as a result, it is urgently necessary to find effective candidate that can potently and selectively inhibit P-gp. It is expected that specific, potent and safe P-gp inhibitors will be developed in the nearest future to overcome MDR mediated by P-gp and successfully treat malignant tumors. Discovering new safe and potent MDR reversal agents will enhance the efficacy of common antineoplastic drugs, especially in the incurable terminal stages of tumors. The use of

phytochemicals-loaded nanostructures to target P-gp could be promising to reverse MDR during cancer therapy. All strategies should be applied in clinical trials to adjust the dosage of P-gp inhibitors for getting the optimal efficacy of anticancer drugs as well as preventing toxicity in patients with cancer.

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Table 1: Classic P-gp inhibitors examples sorted by generation.

1st generation	2nd generation	3rd generation	4th generation
Verapamil	Doxverapamil	OC144-093	Flavonoids
Diltiazem	Emopamil	Zosuquidar (LY335979)	Stilbenes
Bepridil	Gallopamil	XR9051	Coumarins
Isradipine	Ro11-2933	Elacridar (GF120918)	Terpenoids
Cyclosporin-A	Valspodar (PSC 833)		Alkaloids
			Saponins

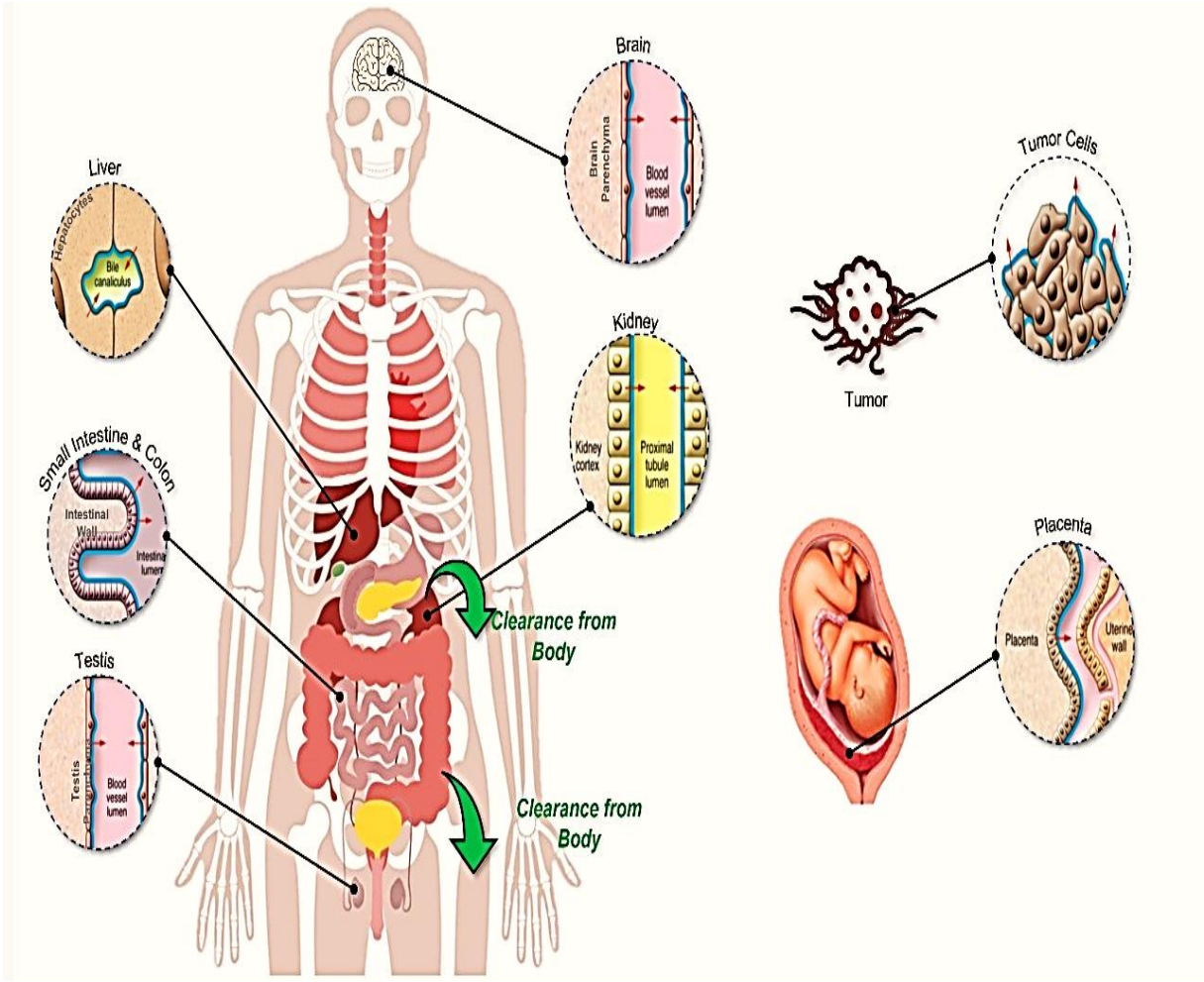

Figure 1: Localization of P-gp (blue lines) in different parts of human body. The direction of P-gp-mediated transport is indicated by the small arrows. The net body excretion of P-gp substrates is indicated by green arrows indicate. Expression of P-gp in tumor cells contributes to MDR.

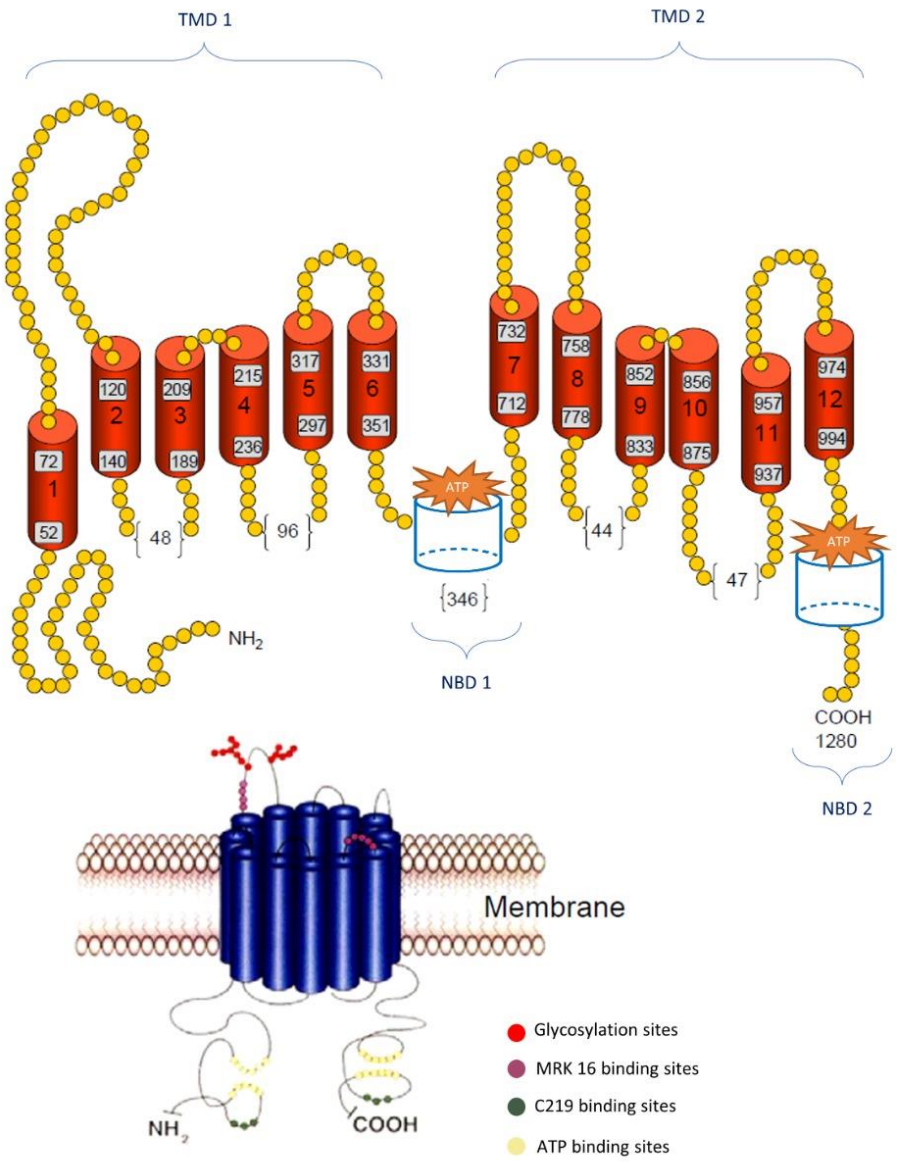

Figure 2: Schematic representation of 170-P-glycoprotein depicting the 12 transmembrane domains and the glycosylation as well as ATP-binding sites (see Insert). It has of 2 halves each of which has 2 TMD and 2 NBD. TMDs are consisted of 6 membrane α-helices and have sites for drug-binding and specify translocation through the cell membrane. NBDs link energy associated with ATP binding and hydrolysis which is necessary for drug active transport.

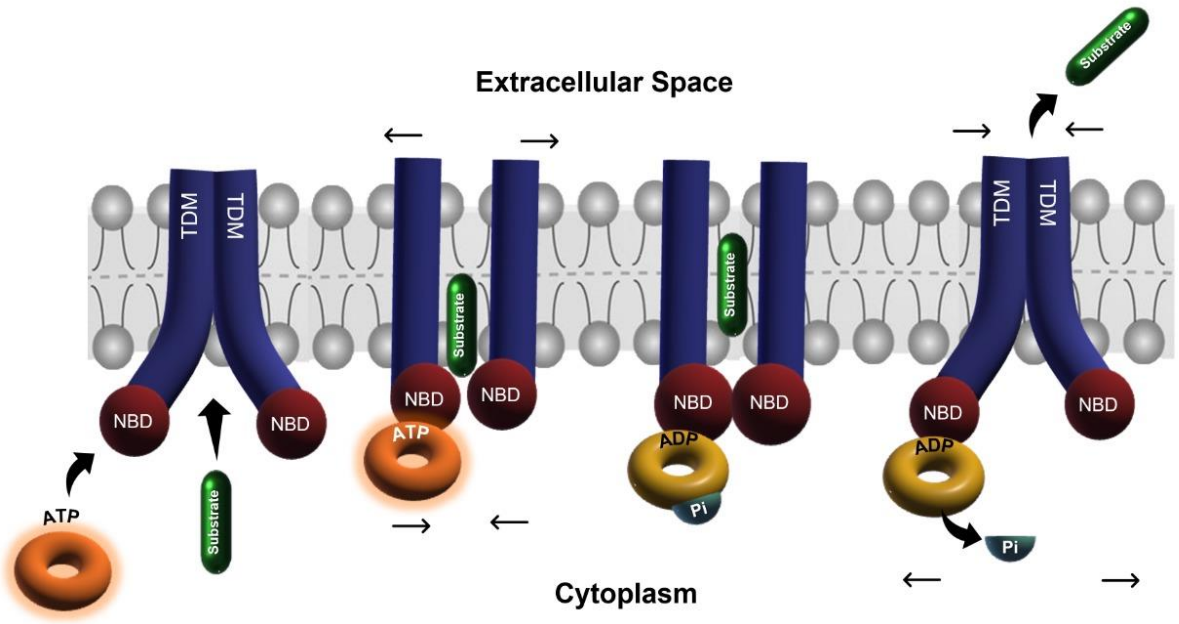

Figure 3: Substrate efflux mechanism through P-gp: **A:** The substrate passes through the membrane and attaches to the substrate-binding pocket (SBP) of the P-gp, which causes two ATP molecules to bind to the NBD, thus NBDs undergo dimerization. B: This causes a conformational change resulting in an outward-facing morphology that causes the substrate to be released extracellularly.

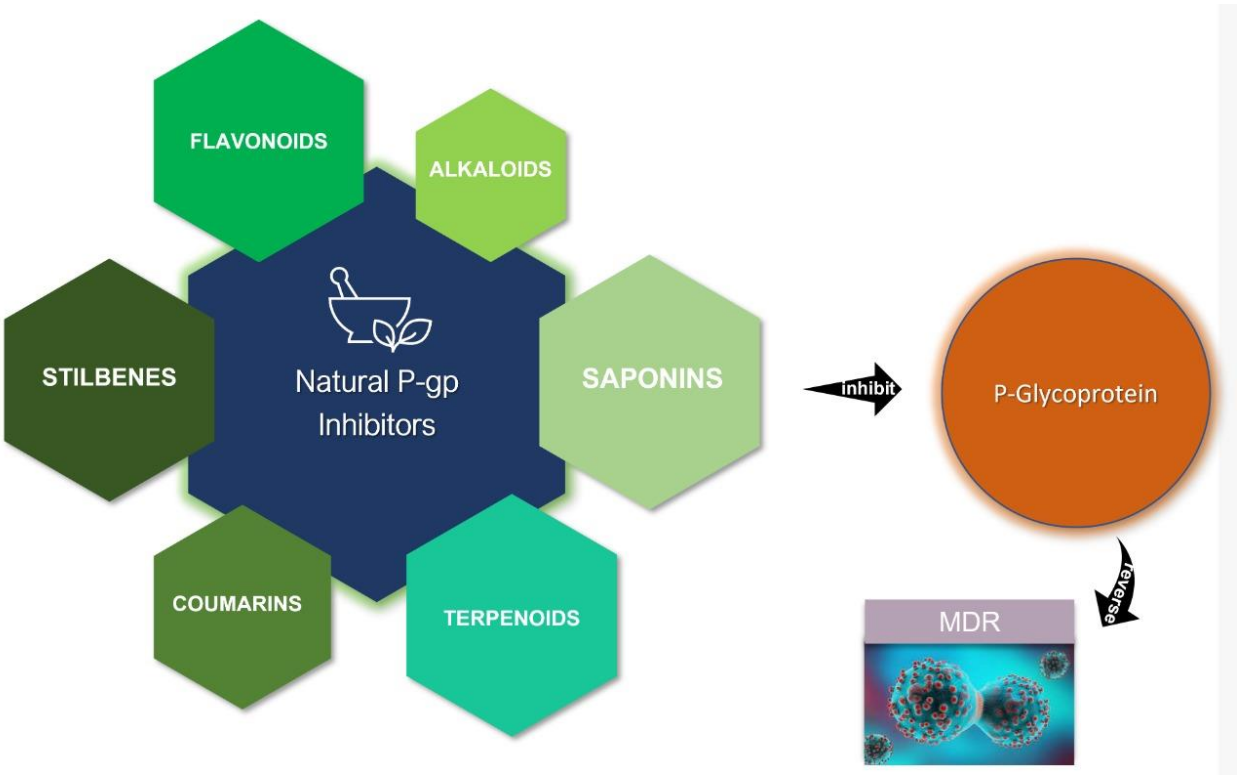

Figure 4: Major classes of plant derived compounds. Flavonoids, stilbenes, coumarins, terpenoids, alkaloids and saponins are natural P-gp modulators that can inhibit P-gp and reverse the MDR in cancer.