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New insights into the mechanisms of multidrug resistance in cancers

N. Montazami[1*], M. Aghapour[2*], S. Farajnia[1] and B. Baradaran[1]

[1] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[2] Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

Corresponding author: Behzad Baradaran, Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. Email: behzad_im@yahoo.com
* These authors contributed equally to this work.

Abstract

Drug resistance is one of the major obstacles in the treatment of various cancers**.** Since chemotherapy serves as a most beneficial method for the repression of tumor progression and due to its desirable cell death potency in tumors which reducing metastasis, failure of such a pivotal treatment lead to tumor recurrence and consequent mortality. Multidrug resistance, the principal mechanism by which many cancers develop resistance to chemotherapy drugs, is a major factor in the failure of many forms of chemotherapy. MDR1 overexpression is one form of the multidrug resistance (MDR) phenotype, which can be acquired by patients initially responsive to chemotherapy. In this review, we briefly look inside the recent mechanisms of chemotherapeutic resistance, the MDR1 gene expression in tumors and some novel inhibition-based approaches.

Key words: Chemotherapy, Drug resistance, Cancer.

Introduction

Multidrug resistance protein 1 (MDR1) is one of the well-known transporter of ATP-Binding Cassette (ABC) transporter protein family. Although, MDR1 gene is expressed in normal tissues, its overexpression has been linked to drug resistance in cancerous cells. The activity of drug efflux pumps (p-Glycoprotein or P-gp-ABCB1) in plasma membrane increases, once MDR1gene is overexpressed in neoplastic tissues. P-gp is found in epithelial surface of normal tissues such as small intestine and pancreas. Recently, many researches have been done to overcome difficulties caused by MDR1 gene overexpression. One of the promising strategies for promoting drug delivery is applying small interfering RNA (siRNA) in order to silence drug resistant genes. In this review, we provide some insights into drug resistance mechanisms and discuss recent advances in case of P-gp inhibition approaches.

Surprisingly, P-gp, as an energy-dependent efflux pump or transporter, plays a central role in multidrug resistance cancer cells (1, 2).

P-gp is one of the first members of ATP-binding cassette (ABC) transporter by extruding toxins and xenobiotics out of the cells. This unique transporter is expressed by two different linked genes in mouse, MDR1a and MDR1b, while in human MDR1 gene is responsible for P-gp production (3).

In 1976, new type of drug resistance modulator has been found by Juliano research team. They discovered a close link between the amount of 170 kDa surface glycoproteins and intensity of drug resistance in Chinese hamster ovary cells (1).

Multidrug resistance (MDR) is a process in which tumor cells show a cross-resistant nature against cytotoxic anticancer drugs that have a multiple molecular targets and are functionally or structurally different. The most extensively characterized MDR mechanisms are drug efflux transporters including ABC membrane transporter. Among MDR genes, P-gp plays a key role (4). In addition, there are some other members of MDR-ABC transporter like DrrB, MsbA, LmrA, LmrCD, Sav1866 and recently identified VcaM and BmrA that regulate multiple drug transportation in bacterial membrane. Significant homology of these efflux proteins to human P-gp has provided an appropriate p-gp studying model and improved the understanding of MDR mechanisms (5-7).

Cancer cells elude chemotherapy

Potential of a patient's cancer for responding to a specific therapy can result from one of two general causes: Specific genetically changes in tumor cells and host susceptibility;

Elderly patients have rapid drug metabolism and insufficient uptake that overall lead to limited drug delivery to the tumor site. It could as same as what happened in bulky tumors due to taking too much space or high molecular weight biological agents such as immunotoxins (8), and different factors related to host tumor microenvironment characterize its responses including specific monoclonal antibody and drug metabolism by non-tumor cells which could affect drug transition within both host and tumor cells interacting to each other (9).

All type of cancer expresses wide spectrum of drug-resistance genes, which demonstrate large amount of diversity (10). Drugs usually transfer inward or export outward of cells in three distinct ways (Figure1). Each of these influx-efflux mechanisms has been determined to have physiological significance based on detailed case studies on the potential of resistant mutants in which defect in these pathways have been observed.

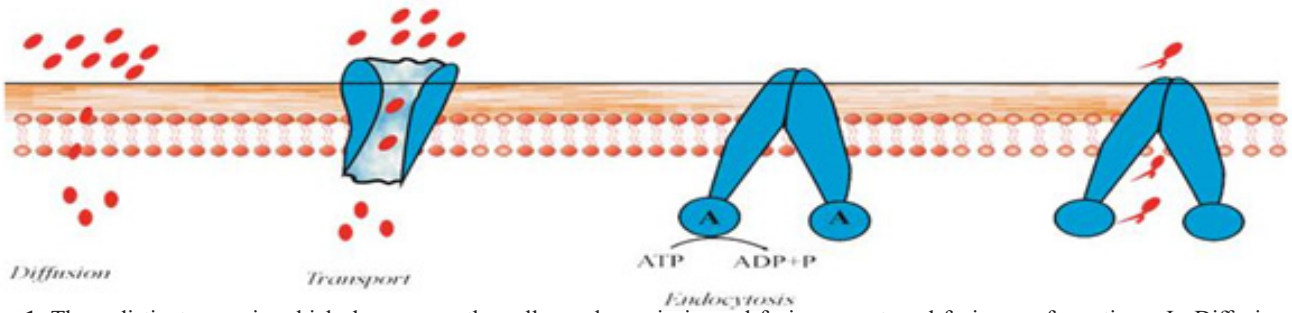

Figure1. Three distinct ways in which drugs cross the cell membrane in inward-facing or outward-facing conformations**;** In Diffusion, drugs moving from areas of high concentration to areas of low concentration. In Transport, substances move into or out of cells down their concentration gradient through protein channels in the cell membrane. Endocytosis is active transport which directly uses energy to transport molecules across a membrane.

The family of ABC transporters

ABC transporters, discovered after ATP binding cassette domain, are conserved proteins that usually translocate compounds across cellular membrane. These kinds of transporters mainly constitute of two transmembrane domains (TMDs) and two nucleotide binding domains (NBDs) (11).

Although several members of ABC superfamily have distinctive functions involving the transport of particular substrates, it is becoming increasingly clear that the complex physiological network of ABC transporters has a pivotal role in toxin elimination process of the body. This role is revealed by the tissue spread of ABC transporters, which are substantially expressed in important pharmacological check point sites, such as the brush border membrane of intestinal cells, the apical surface of renal proximal convoluted tubules, blood-brain barrier (BBB) associated epithelium and the biliary canalicular membrane of hepatocytes (12).

P-gp which is encoded as a transmembrane permeability glycoprotein constitutes of 1280 amino acids. It is further subdivided into two uniform TMDs and two more uniform NBDs (13-15).

The TMDs are made up of twelve transmembrane helices or segments which with NBDs altogether placed in the cytoplasm, forming an active pore lead to expulsion of cytotoxic drugs out of the cells. Transmembrane segments 1, 4, 5, 6, 10, 11, and 12 play significant roles in binding of substrates to P-gp, make it possible to recognize variety of substrates and target specificity as well (16-19).

Modulation of P-gp drug binding cavities results in unique transport models for each drug. The major mechanism of MDR which interfere with cancer chemotherapy is related to well-studied ATP-dependent efflux transporters called P-gp (1, 2).

The expression of P-gp has been detected in wide variety of cancer cells, such as colorectal and liver cancers, leukemia and myeloma cancers, ovary tumors and fibrosarcoma (20). P-gp mediated drug binding lead to activation of TMD helices which consequently go thorough ATP hydrolysis process, giving conformational alterations to the P-gp. Eventually, this process end to release of cytotoxic agent into extracellular space. Hydrolysis of second ATP molecule is required for resetting of efflux pump to its initial state, so that it can go back to square one (21, 22).

Although, the exact mechanism of remaining ABC family transporters is not fully understood, it is thought that ABC would serve as a promoter for initiating transport-mediated activity of the other members of this superfamily.

After finding the P-gp and characterizing its extensive expression in various human cancers, it has shown that many multidrug-resistant cancers, including gastric carcinoma, rarely encode P-gp gene.

Deely and colleagues cloned the other member of ABC family, termed as MRP1 (stands for Multidrug resistance associated protein 1) by utilizing lung cancer cell line as a MDR model (23). Unlike these two members of ATP binding cassette family, there is an additional ABC half-transporter for anticancer drugs called Mitoxantrone resistance protein (also known as MXR, BCRP, ABC-P and ABCG2) which only has single NBD followed by one membrane-spanning domain (MSD) with six predicted TMD (see Figure 2) but is thought to function as a dimer (24).

ABC transporter family in addition to MXR family members have been implicated in cancer chemotherapy resistance and drug transportation into the human cells (Figure2) (25).

Polymorphisms of MDR1 gene

Human P-gp is encoded by the multidrug resistance 1 (MDR1) gene located on chromosome 7q21, and is highly expressed on the Golgi membrane, cell membrane and transporting epithelia of different human normal tissues including, liver, kidney, colon, pancreas, uterus, placenta and also in specialized endothelial cells in the testis and brain (26-29).

Among the early researches on the polymorphisms

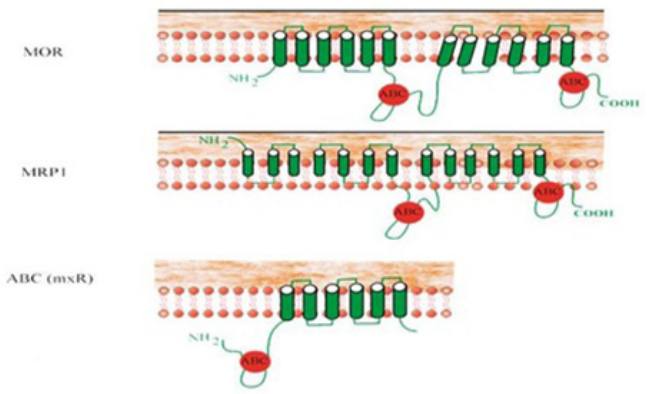

Figure2. The family of ABC transporter.

of MDR1 gene in human beings, Roninson *et al* have designated two new distinct genomic clones of MDR, named MDR1 and MDR2, using genomic DNA cross-hybridization. Extensive researches has been done on the pharmacodynamics and kinetics of MDR1, but only recent studies have identified several different single-nucleotide polymorphisms (SNPs), which are defined as a single base-pair variation in the human MDR1 coding region.

There are several reasons why gathering information on MDR1 SNPs is of a great importance, and among them the focus of many recent studies are: (1) defining the relationship between the MDR1 activity and SNPs positions will promote our knowledge about conformation-function interaction (2) if altered pharmacokinetics and any correlations between polymorphisms are discovered, the same approach will add to our knowledge about the role of P-gp in chemotherapy-resistant cancer cells; (3) since MDR1 is a very well-conserved gene (30), studying its SNPs will improve our understanding about the evolutionary development of this gene; (4) evidence of a connection between specific pharmacological changes and MDR1 gene polymorphisms may make it possible to predict individual sensitivity to the many drugs which are substrates of MDR1.

The MDR1 gene mainly composed of 28 exons ranging in size from 49 to 587 bp, and the cDNA spans 4.5 kb (31), three insertion/deletion variants and more than 50 SNPs have been mapped in the MDR1 gene. The schematic location of aforementioned SNPs which affect the MDR1 coding sequence (exon) has been illustrated in figure 3.

The data assembled in Figure 3 are based on the SNPs reviewed here, on the published intron/exon boundaries (32), and on the predicted 2-D structural model of P-gp, according to studies from our lab (33).

As shown in Figure3, some of those distinct polymorphisms resulted in amino-acid changes are located in exons 2, 5, 11, 21, and 24. Interestingly, amino acids which positioned in transmembrane domains are encoded by 5, 21, and 24 exons. Exon 2 is placed in the first intracellular domain of P-gp and the other one lies downstream to the ATP-binding site (exon 11).

When we compared each of these polymorphisms and original-type of MDR1, we found no difference in expression levels or cell-surface localization, and no significant changes in the transport function of the P-gp with these polymorphisms (34).

In humans, class I and III isoforms (MDR1 and MDR3) of P-gp with 80% amino acid homology have been identified (35). Either isoforms were found to be positioned on the chromosome 7 long arm and to be linked within 330 kb (36, 37). However, until now, no possible chemo-resistance function related to the human MDR3 gene and its consequent products have been observed (38).

The MDR1 gene expression in normal tissues

Fojo et al. (1987b) has reported overexpression of MDR1 gene in normal liver, kidney, jejunal, rectal, adrenal tissues and occasional lung, using slot blot quantitation. Other organs and tissues (heart, skin, subcutaneous tissue, skeletal muscle, spleen, bone marrow, ovary, lymphocytes, esophagus, stomach, and spinal cord) have little or no obvious expression levels (39).

P-gp was identified on the small biliary ductules and biliary surface of liver hepatocytes, in the apical surface of renal proximal tubules, in the luminal surface of pancreatic small ductules epithelial cells. High levels of P-gp were also found on the epithelial surfaces of both the colon and jejunum.

Moreover, P-gp is expressed in the capillary endothelial cells of the human brain, indicating its pivotal role in the blood-brain barrier (40, 41).

The MDR1 gene expression in tumors

Based on previous studies, the bulk techniques (Northern-, Western- or dot blotting, and RNase protection

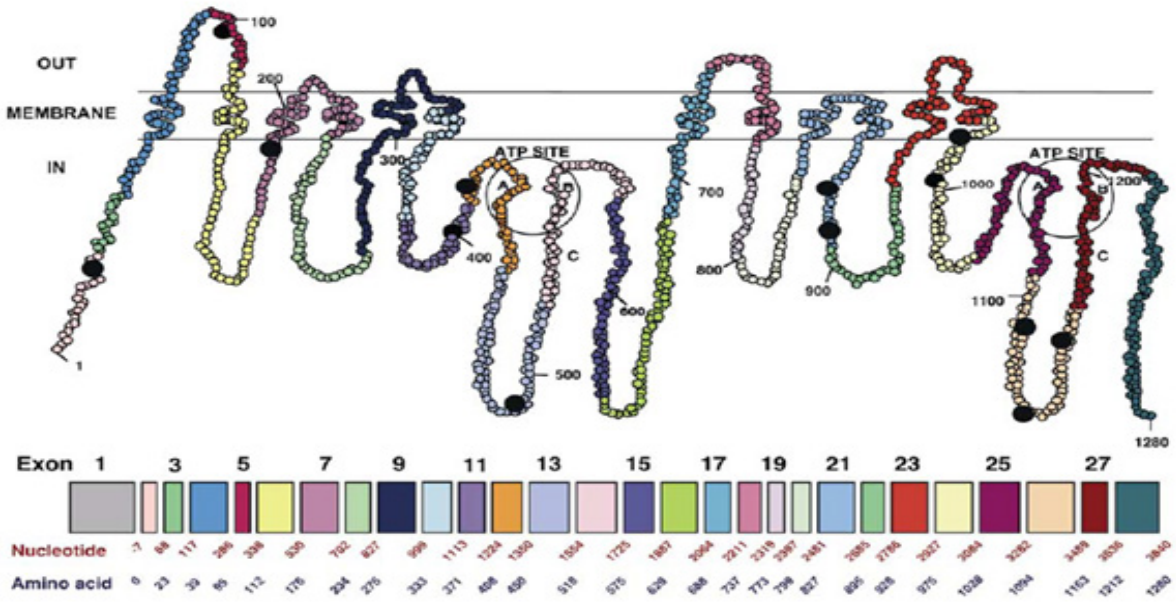

Figure3. Schematic representation showing the MDR 1 SNPs distribution, which affects the coding sequence of P-gp on the predicted 2-D structural model; At the bottom, 28 exons of the MDR 1 gene has been illustrated, and the P-gp region that encoded by a given exon is also highlighted in a same color on the predicted 2-D P-gp structure. The location of the reported SNPs has been shown with black-filled circles. P-glycoprotein: from genomics to mechanism, 2003 Nature Publishing Group.

assay) are the most employed methods for measuring the expression levels of the MDRl gene in human tumors**.** In present study, we have randomly categorized expression levels of solid tumors into three groups.

There have been several controversial reports on MDRl expression of all three groups, which partly could result from methodology (42-46).

Group I introduces tumors that arise from tissues normally expressing moderate to high MDRl levels such as colon kidney, liver, colon pancreas and adrenal.

Those tumors that occasionally have high mostly moderate MDR1 expression levels and also quite often lack expression fall into group II. This group includes the neuroblastomas, breast tumors, soft tissue sarcomas and the haematological malignancies.

Generally, drug response of group II tumors is much greater than first one, therefore achieving a proper response would be reachable.

The MDR1 expression levels were mostly undetectable or quite low in group III tumors. Remarkable results are obtained with ovarian tumors, which belong to this group. The first report on P-gp expression in human tumor cell line is related to ovarian cancer (47). In case of group III tumors, Chemotherapy would be effective, but acquired chemoresistance is the rule rather than the exception.

The MDRl expression levels can involve wide range from low to high and even in untreated tumors are occasionally high levels are observed (20, 48, 49).

In 1/3 of the patients with acute myeloid leukemia and about half of patients at first relapse stage, expression of MDR1 gene has been observed; the MDR1 expression level solely can explain resistance based on *in vitro* assays of P-gp transporting function and also be related to the possibility of relapse (50).

Although chemotherapy has a pivotal role for the treatment of breast cancer, the drug resistance still is one of the unsolved growing concerns. There is a great numbers of P-gp in the intestine (51, 52) which transports various substrates to the intestinal lumen (53). Hence, act as a strong gastrointestinal barrier that protects the lining cells against toxic agents and possible carcinogens.

Regardless of therapy plans, the chance of early chemotherapies for treating metastatic breast cancer is relatively high and the recovery process often last only a few months following treatment (54).

The correlation between ABC transporters and drug resistance in breast cancer has been investigated by measuring expression and protein levels using different molecular and probe-based techniques. The expression levels have been scored and linked to treatment response and outcome.

MDR regulation pathway is mediated by vast variety of proteins which consequently means that there is a high redundancy in this case.

The effects of chemotherapy drugs on gene expression of single ABC transporters also have been assessed, alongside functional assays of ABC-mediated drug transport (55).

Due to complexity of the mechanisms involved, the exact role of the ABC transporters in breast cancer MDR is not fully established yet. Although a number of clinical studies have claimed that high levels of tumor ABC transporters are associated with tumor progression, there is no obvious evidence indicating any relationship between expression levels and tumor sensitivity to drugs or patient response (56).

Cancer stem cells (CSCs) also express transmembrane ABC transporters, such as ABCG2 and MDR1,(57) which render them drug resistant (58). Based on previous findings, the levels of carcinoembryonic antigen (CEA) in drug-resistant human colorectal adenocarcinoma cells was two times higher than normal (59).

Tumor heterogeneity of drug-resistant colon cancer cells results in developing both P-gp and non P-gp mediated mechanisms of resistance. Colon or kidney tumor cells which expressing high levels of P-gp, resist to drugs that are not exposed to P-gp mediated transport, suggesting that 'intrinsically resistant' cancer is also protected by non-Pgp mediated mechanisms (60).

P-gp expression has been observed in more than half of pre-treated soft tissue sarcoma (STS); therapy with doxorubicin achieved even more expression levels (61).

Pharmacological inhibition of P-gp with a variety of drugs e.g. verapamil (a calcium channel blocker agent) (62, 63), and tyrosine kinase inhibitors was used (64). Thyroid hormones, T_3 and T_4, are known to induce P-gp gene transcript and its function (65-67). Tetrac is another therapeutic agent which antagonizes the effects of thyroid hormones on the cell surface integrin αvβ3. Maintaining time of chemotherapeutic agent, which also known as P-gp substrate, desirably increased, once cancer cells subjected to Tetrac (68). Therefore, 'intracellular retention time' of anticancer drugs in response to Tetrac reflected in decreased P-gp efflux or likely increased influx transporters (69).

P-gp inhibitors to overcome MDR

Clinical trials have been designed to attenuate P-gp function also highlighted its significance as a unique transporter (70).

First-generation of P-gp inhibitors, such as quinine, cyclosporine and verapamil which were already licensed for other therapeutic indications, exploited in early trials. In general, these compounds were toxic or inefficient at doses required to suppress P-gp function. It was shown that quinine can promote remission and survival rate in P-gp positive Myelodysplastic syndrome (MDS) patients with high-dose chemotherapy (71), indicating that successful modulation of P-gp is achievable.

The second generation of inhibitors, including valspodar (non-immunosuppressive analogue of cyclosporine A), biricodar, dexverapamil (D-isomer of verapamil), dexniguldipine and dofequidar fumarate (MS-209) was devoid of side effects related to the primary toxicity of constituents(72). On the other hand, their ability to inhibit P-gp function has been markedly improved. However, since these P-gp modulators significantly interact with other ABC transporters such as MRP-1 and also are substrates for cytochrome P450 34A enzyme, their clinical uses have been faced with restriction (73, 74).

The recent-generation of inhibitors are designed specifically for low pharmacokinetic interaction and high transporter affinity. Blocking of cytochrome P450, which is main cause of many adverse effects related

to second-generation of inhibitors, has been removed in third-generation of inhibitors, consist of laniquidar (R101933), oc144-093 (ONT-093), zosuquidar (LY335979), elacridar (GF-120918)(75) and tariquidar (XR9576) (76). Several later-generation act on multiple ABC transporters. Among them, Biricodar (VX-710) and GF-120918, bind to Pgp as well as MRP1 and ABCG2, respectively (77).

Despite the promising above mentioned characteristics, the studies were abolished early due to toxicities of drugs (78). Phase III trials using third-generation of inhibitors will be crucial in determining efficiency of this therapeutic strategy.

Post-transcriptional regulation of P-gp

Autophagy is the catabolic process that involves lysosomal degradation and as the ubiquitin-proteasome pathway plays a same role in protein degradation (79, 80). Various membrane proteins, including plasma transporters and receptors, recycle back to the cell surface through endocytic recycling system.

Discarded proteins into early and late endosomes which sort the cell membrane can easily fused with lysosome.

Rab GTPases constitute the largest member of small GTPase superfamily and coordinate vesicular trafficking of different proteins involved in endocytosis. Recent studies have showed that Rab4 and Rab5 are key regulators of P-gp trafficking, recycling and control the P-gp lysis placed on the cell membrane (81). Cell membrane P-gp expression is attenuated while original type or active mutant of Rab4 overexpressed, whereas negative mutant did not alter P-gp expression on the cell surface (82). Hence, ubiquitin-proteasomal degradation pathway could be present as rapid proteolysis process, and endocytic recycling system acts as slow system in P-gp homeostasis.

The serine/threonine protein kinase Pim-1 was basically discovered as the proviral integration site in Moloney murine leukemia virus lymphomagenesis (83, 84). Pim-1 gene is highly expressed in various human malignancies such as myeloid and lymphoblastic leukemia (85). It stabilizes 150 kDa P-gp which is underglycosilated form, and encourages its glycosylation and subsequent translocation to the cell surface. These results reveal that Pim-1 protects the 150 kDa form of P-gp from degradation, hence regulate P-gp expression on the cell surface and partially established previous findings which claimed that the ubiquitin-proteasome pathway is important in the degradation of P-gp (86).

A number of studies have shown that specific serine residues in the P-gp are phosphorylated by protein kinase A and C (PKA and PKC) (87, 88).

Modulation of P-gp expression by MAPK signaling pathway

There are several distinct pathways in the regulation of P-gp expression and among them post-transcriptional modifications, such as phosphorylation, glycosylation and ubiquitination play a central role. In this regard, we have reviewed different inhibitors that affect P-gp expression in Pg-p-positive colorectal cancer cell line and found that MAPK/extracellular signal-regulated kinase (MEK) and a heat shock protein 90 (HSP90) inhibitors down-regulate p-gp expression (89).

Mitogen-activated protein kinase (MAPK) pathways are comprised of a three families: namely the extracellular signal-regulated kinase (ERK), the c-Jun N-terminal kinase (JNK) and p38 kinase. The ERK pathway is the well-studied of the human MAPK pathway, and its altered regulation has been clearly observed, particularly in cancer cells, whereas the other two pathways involve in stress-activated signaling (90, 91). This pathway triggers multistep activation of receptor tyrosine kinases, Ras, Raf, MEK and P90 ribosomal S6 kinase (P90RSK). Activated ERK regulates fundamental cellular processes, including proliferation, differentiation, and apoptosis, by the direct activation of downstream transcription factors or through p90RSK(90). HSP90 is an energy-dependent chaperone protein, regulating those cells exposed to extreme stresses and stabilizes wide variety of client proteins (92). The activity of Raf and MEK proteins are straighten by HSP90 and therefore allows their kinase functions. These findings indicate that inhibitors of HSP90 and MAPK block the ERK pathway, which lead to the downregulation of P-gp expression.

Several studies have reported the correlation of P-gp with p38 MAPK: MRP1 and MDR1 mRNA expression were reduced in 5-fluorouracil-resistant hepatocellular carcinoma cells, when p38 MAPK pathway switched on (93), whereas the MDR1 gene expression and activator protein-1 (AP-1) in vincristine-resistant gastric carcinoma cells were decreased in response to p38 inhibition (94).

Furthermore, the JNK pathway has been implicated in the regulation of the promoter of MDR1 gene. Seven-in absentia homologue 1 (SIAH1), an E3 ubiquitin ligase that triggers the ubiquitin-proteasomal degradation, is responsible for JNK activation and downregulation of MDR1 by increasing c-Jun binding to the AP-1 site in the MDR1 promoter (95).

A cyclosporine analog reduces the expression of MDR1 gene through inhibiting nuclear factor kappa B (NF-κB) and activating JNK/c-Jun/AP-1 (96).

Hence, the JNK/c-Jun/AP-1 pathway serves as a negative regulator of MDR1 gene expression. However, a number of studies have claimed that AP-1 is the main activator of MDR1 gene (94-97). Thus, MDR1 promoter is subjected to dual regulation in the AP-1 site.

Conversely, a bunch of researches have demonstrated that downstream transcriptional factors are responsible for high expression of MDR1 gene in the ERK pathway (98-100). Therefore, ERK pathway is considered as main regulator of MDR1 expression at both posttranscriptional and transcriptional levels (89). Targeting resistance genes is a recent therapeutic approach in order to tackle cancer cells. Although, the MDR1 levels in cells are often reflective of its gene amplification, the high expression of the protein can also be related to transcription step. Bartsevich and colleagues introduced transcriptional repressors that specifically bind to the MDR promoter, using combinatorial peptide libraries (Figure4).

Once repressor proteins are expressed in highly re-

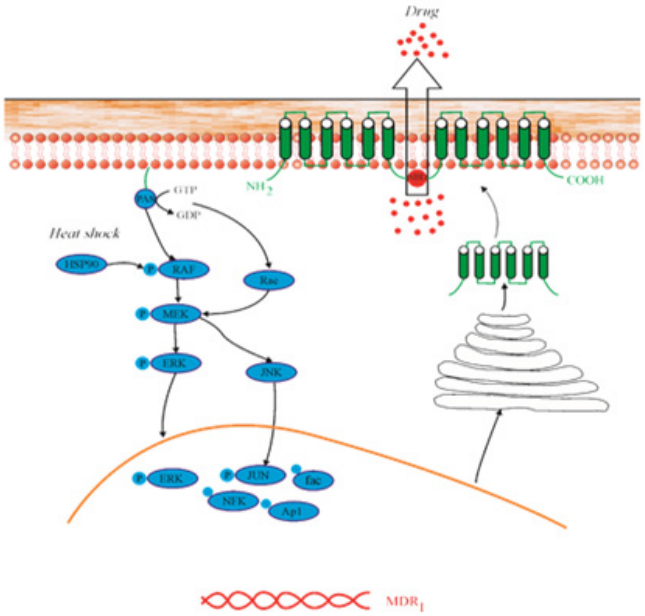

Figure4. Schematic model representing regulations of MDR1 gene. HSP90 provides MEK and Raf proteins kinase activities by straightening their folding. MEK and Raf activate the ERK pathway, resulting in the upregulation of P-gp expression but the expression of the MDR1 gene is downregulated by activating JNK/c-Jun/AP-1.

sistant cancer cells, P-gp levels selectively reduce and considerable increase in chemosensitivity is observed (101-103).

Targeting of MDR gene in cancers

A binding site for various numbers of transcription factors (including NF-κB, AP-1, FKHRL1/FOXO3a and FKHR/FOXO1) has been discovered in the MDR1 promoter region and has been proven to be involved in transcriptional activation of MDR1. Previous studies have demonstrated that NF-κB, FOXO3 and AP-1 directly bind and activate the MDR1 promoter (86-89, 103-105).

It was also shown that heat shock protein (HSP) 27 and heat shock factor (HSF) 1 attenuate MDR1 expression through blocking the NF-κB pathway in drug-resistant breast cancer cells (106). Therefore, NF-κB is one the crucial signaling systems in the regulation of MDR1 expression. Moreover, activation of PI3K-Akt signaling pathway is involved in the expression of MDR1 via NF-κB, a downstream target of Akt. The PI3K induces production of phosphatidylinositol-3,4,5-triphosphate (PIP3) which is essential for translocation of Akt to the cell membrane where it is phosphorylated by 3-phosphoinositide-dependent kinase 1 (PDK1) (107).

The MDR1 gene expression is also modulated with Wnt/catenin pathway. More recently, several microRNAs (miRs) have been reported to implicate in the regulation of MDR1 mRNA (108). As previously described, downregulation of MDR1 mRNA is correlated with the expression of subset of these microRNAs (MiR-137, miR-200c and miR-122) (109-112), whereas another groups of miRs (miR-19a/b, miR-221 and miR-222) are involved in upregulation of MDR1 transcription in various cancer cells. Although, miRs act as a double-edged sword, they are becoming a novel therapeutic target, particularly in chemoresistant cancer therapy (113).

There are wide varieties of mechanisms which enable the targeted regulation of aberrantly expressed genes, including small interfering RNA (si-RNA) and anti-sense oligonucleotide.

Currently, researchers exploit an indispensable bioinformatics tool to design specific siRNA for thousands of target mRNAs. These synthetic oligonucleotides employ similar mechanism to endogenous one to silence their interest gene (114).

At the moment, using RNAi as a robust therapeutic strategy has attracted considerable attention for treating several incurable diseases, especially cancer (115-119).

The siRNA offers numerous advantages compared to other gene silencing approaches. First, siRNA can be precisely designed to recognize and knockdown almost any gene. Second, siRNA can be readily packaged and expressed into vectors, despite the time-laboring process of negative mutant synthesis. Third, siRNA-based gene suppression is more selective and safer than the other nucleotide-based methods. Therefore, siRNA silencing strategy serves as a promising therapeutic method to knockdown defected genes, which are mainly responsible for malignant manner of tumors (120). Gene-silencing by siRNAs provide an alternative to conventional therapies for cancer which only alleviate the clinical signs without any effect on involving gene of cancer cells (121).

Development of RNA interference (RNAi) for cancer therapy could fundamentally change treatment of this uncontrollable disease (122). Despite the other diseases, cancer cells are faced with different challenges, such as choosing suitable targets, reducing toxicity and finding effective delivery system. Surprisingly, RNAi can also be utilized to switch resistance genes off and promote the effects of cancer chemotherapy (123). Thus, MDR1 can be one of the potential candidate targets for RNAi gene silencing (124).

RNAi strategy, dissimilar to chemical inhibitors, may represent a more precise method, which specifically suppresses the expression of protein targets, e.g. P-gp. Synthetic analog of RNAi, siRNA, was used to explore the therapeutic potential of this pathway in cancer patients.

The siRNAs are double strand RNA molecules present in the cell which first cleaved by dicer enzyme into short 21-25 nucleotide fragments. These effector RNAs then joined to the RNA induced silencing complex (RISC) where the duplex RNA unwound and produce a guide sequence strand to target the breakdown of homologous RNA (125).

The best two advantages of siRNA are lower toxicity on nontarget cells and higher specificity on interest gene, as compared to the traditional MDR1 inhibitors (126, 127).

Several reports have shown that an active siRNA normally contains 30-52% GC content. It was also found that the increased efficiency of siRNAs have been associated with the presence of A/U at the 5' end of the antisense strand (128, 129). Following the successful internalization and endosomal escape, siRNA has to detach from its packaging carrier in order to interact with the RISC complex. This process eventually leads to specific binding of siRNA to a certain mRNA and subsequent degradation of target mRNA. Targeted therapy with siRNA is expected to successfully downregulate

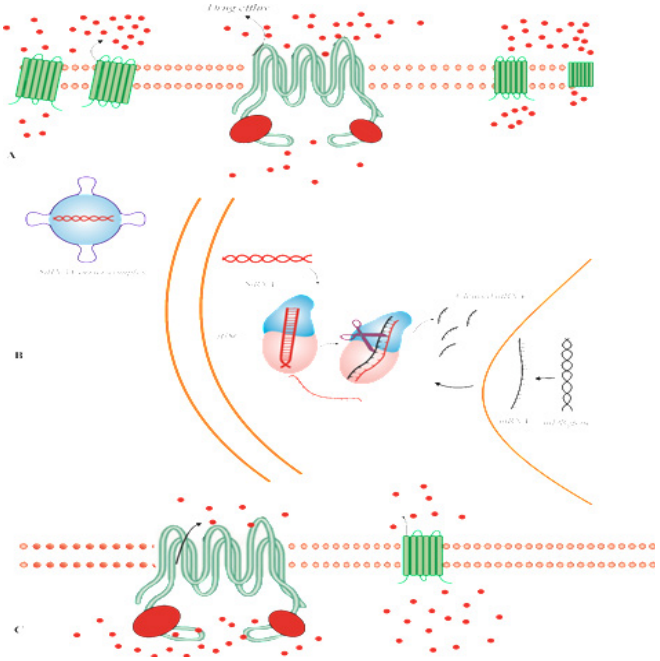

Figure 5. (A) P-Glycoprotein is involved in plasma membrane drug efflux **(B).** The RNA induced silencing complex (RISC) **(C)** successful siRNA delivery lead to decreased MDR1 expression.

the MDR1 gene and this could possibly lead to reduced number of P-gp transporter on plasma membrane, reduced pumping chemotherapeutic drugs out of the cells and improving accumulation of drugs in involved site, resulting in increased cellular cytotoxicity (Figure 5).

Chemical modifications to siRNA sequence are required, since 2'-OH end of molecule is not fully affected by siRNA-RISC complex. These changes, including 2'-fluoro and 2'-OMe, are predicted to improve siRNA stability and increase its half-life compared with wild-type siRNA (130).

Despite the promising findings in recent *in vitro* researches with siRNAs, there are many adverse effects associated with inflammatory nature of these molecules which make it inappropriate for human use. Therefore, it is highly important to rule out these potential concerns before starting any clinical trial using siRNA for MDR1 gene knockdown.

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