

Review Article

Regulations of P-Glycoprotein/ABCB1/MDR1 in Human Cancer Cells

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Multidrug resistance (MDR) in cancer cells is a phenotype whereby cells display reduced sensitivity to anticancer drugs, based on a variety of mechanisms, including an increase in drug efflux, the reduction of drug uptake, the activation of cell growth and survival signaling, the promotion of DNA repair, and the inhibition of apoptosis signaling. Increased expression of the plasma membrane drug efflux pumps, the ATP-binding cassette (ABC) transporters, is involved in MDR. P-Glycoprotein/ABCB1 is a member of the ABC transporter family, and facilitates the efflux of various anticancer drugs, including anthracyclines, *vinca* alkaloids, epipodophyllotoxins, taxanes, and kinase inhibitors, from cells. P-Glycoprotein is also expressed in normal tissues and cells, including the kidney, liver, colon, and adrenal gland, to transport and/or secrete substrates and at the blood-brain, blood-placenta, and blood-testis barriers to protect these tissues from toxic substances. To understand the mechanistic functions of P-glycoprotein and to overcome MDR, investigators have identified the substrates and competitive inhibitors of P-glycoprotein. Recently, we and other groups reported associations between cellular signaling pathways and the expression, stability, degradation, localization, and activity of P-glycoprotein. The present review summarizes the currently available information about the transcriptional and posttranslational regulation of P-glycoprotein expression and function.

1. Molecular Characterization of P-Glycoprotein in Cancer

Resistance to a broad spectrum of chemotherapeutic agents in cancer is called “multidrug resistance” (MDR) and is a major problem in chemotherapy. MDR often appears during cancer chemotherapy or during recurrence after chemotherapy [1]. It is caused by various mechanisms, including an increase in drug efflux, the reduction of drug uptake, the activation of growth signaling and DNA repair pathways, and the inhibition of the apoptosis signaling pathway by the induction of antiapoptotic molecules [2].

The ATP-binding cassette (ABC) transporters are key molecules in the drug uptake and efflux [3]. They are characterized by transmembrane domains (TMDs) and nucleotide-binding domains (NBDs) with the Walker A, Walker B, and Signature C motifs [3]. The human ABC transporters consist of 48 or 49 family members [3]. Among these, P-glycoprotein

(ABCB1), breast cancer resistance protein (BCRP/MXR/ABC-P/ABCG2), and multidrug resistance-associated proteins (MRP1/ABCC1 and MRP2/ABCC2) function as drug efflux pumps of anticancer drugs, and their expression confers an MDR phenotype on cancer cells [4–8].

Human P-glycoprotein is encoded by the *multidrug resistance 1* (*MDR1*) gene, located on chromosome 7q21, and is widely expressed on the plasma membrane, Golgi membrane, and intracellular canaliculus of normal human tissues, including the liver, kidney, colon, adrenal gland, intestine, placenta, hematopoietic precursor cells, and endothelial cells at the blood-brain, blood-placenta, and blood-testis barriers. P-Glycoprotein functions in the transport and/or secretion of its substrates and the protection of these tissues from physiologically active substances, cytotoxic agents, and xenobiotics [9–11]. P-Glycoprotein expression is significantly elevated in drug-resistant tumors and pumps out various anticancer drugs, such as anthracyclines, *vinca* alkaloids,

epipodophyllotoxins, and taxanes, and other drugs, including digoxin, HIV protease inhibitors, statins, and xenobiotics [12–17]. Thus, P-glycoprotein expression in cancer cells confers MDR to these anticancer drugs.

P-Glycoprotein consists of 1280 amino acids and is expressed as a 170–180 kDa plasma membrane glycoprotein that possesses two symmetrical NBDs and TMDs consisting of six α -helical membrane-spanning domains [4–6]. Twelve transmembrane segments in the TMDs of P-glycoprotein form an active pore, with both NBDs oriented in the cytoplasm, and drugs are exported through this pore. Transmembrane segments 1, 5, 6, 11, and 12 play important roles in the binding of substrates to P-glycoprotein, allowing it to give variety and specificity of substrates [18–21]. For instance, the substitution of phenylalanine with alanine at position 335 in TM6 reduces the protein's affinity for vinblastine and actinomycin D compared with that of the wild-type P-glycoprotein, whereas this substitution still confers resistance to colchicine and doxorubicin [19]. However, P-glycoprotein with a triple substitution in the TM12 (L975A/V981A/F983A) loses its transport activity for rhodamine 123 and daunorubicin, although it maintains the weak drug transport activity for bodipy-verapamil, calcein-AM, and bodipy-paclitaxel [21]. Thus, modulation of the drug-binding sites of P-glycoprotein results in unique transport patterns for each drug.

2. Posttranslational Regulations of P-Glycoprotein

2.1. Modulation of P-Glycoprotein Expression by the Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway. In our previous studies, estrogens downregulated P-glycoprotein expression in the estrogen receptor-positive P-glycoprotein-expressing breast cancer cell lines MCF-7/MDR and T-47D/MDR and downregulated BCRP expression in the estrogen receptor-positive BCRP-expressing MCF-7, MCF-7/BCRP, and T-47D/BCRP cells. However, they did not downregulate P-glycoprotein expression in the estrogen receptor-negative P-glycoprotein-expressing breast cancer cell line MDA-MB-231/MDR or the estrogen receptor-negative doxorubicin-resistant ovarian cancer cell line NCI/ADR-RES [22, 23]. The downregulation of BCRP was dependent on 17β -estradiol (E2) mediated posttranscriptional protein biosynthesis but not on the transcription of the *BCRP* gene or on protein degradation [22]. Another research group reported that phosphatidylinositol 3-OH kinase (PI3K) inhibitors downregulated BCRP expression on the cell surface of BCRP expressing LLC-PK1 cells [24]. These reports suggest that certain signaling pathways are involved in the regulation of P-glycoprotein expression, probably through posttranscriptional modifications, such as phosphorylation, glycosylation, or ubiquitination. To examine this hypothesis, we screened various inhibitors that alter P-glycoprotein expression in a P-glycoprotein-expressing human colorectal tumor cell line, HCT-15, and found that MAPK/extracellular signal-regulated kinase (MEK) inhibitors (U0126 and PD98059) and a heat shock protein 90 (HSP90) inhibitor (tanespimycin, 17-N-allylamino-17-demethoxygeldanamycin [17-AAG]) downregulated P-glycoprotein expression [25].

MAPK signaling includes three pathways: the extracellular signal-regulated kinase (ERK) pathway (also known as the classical MAPK pathway), the p38 MAPK pathway, and the c-Jun N-terminal kinase (JNK) pathway. The ERK pathway is one of the most important cell growth signaling pathways, particularly in cancer cells, whereas the p38 MAPK and JNK pathways involve stress-responsive signaling [26, 27]. The ERK pathway consists of the receptors of tyrosine kinase, Ras, Raf, MEK, ERK, and p90 ribosomal S6 kinase (p90RSK). Activated ERK regulates various cellular events, including cell growth, differentiation, development, and apoptosis, through p90RSK or by the direct activation of downstream transcription factors [26]. HSP90 is a chaperone protein, regulating cells exposed to environmental stresses, and controls the stability, folding, and activity of various HSP90-associated proteins [28]. HSP90 straightens the folding of Raf and MEK proteins and thus provides their kinase activities. The findings described above suggest that inhibitors of MEK and HSP90 inhibit the ERK pathway, resulting in the downregulation of P-glycoprotein expression.

The PI3K inhibitor LY294002, the p38 MAPK inhibitor SB203580, and the JNK inhibitor SP600125 were tested and did not affect P-glycoprotein expression [25]. Various reports have described the association between p38 MAPK and P-glycoprotein: the activation of p38 MAPK reduced the expression of *MDR1* and *MRP1* mRNAs in 5-fluorouracil-resistant BEL-7402/5-FU cells [29], whereas the inhibition of p38 MAPK reduced the activator protein-1 (AP-1) activity and *MDR1* gene expression in vincristine-resistant SGC7901/VCR cells [30]. SB203580 also reversed the P-glycoprotein-mediated MDR of L1210/VCR cells without affecting P-glycoprotein expression [31]. The JNK pathway is also reported to be involved in the regulation of the promoter of the *MDR1* gene. Seven-in absentia homologue 1 (SIAH1), an E3 ubiquitin ligase that regulates the ubiquitin-proteasomal degradation of a number of proteins, activated JNK and reduced *MDR1* mRNA expression by promoting c-Jun binding to the AP-1 site in the *MDR1* promoter [32]. A cyclosporine analog, PSC833, downregulated the expression of the *MDR1* gene by activating JNK/c-Jun/AP-1 and suppressing nuclear factor kappa B (NF- κ B) [33]. Thus, the JNK/c-Jun/AP-1 pathway is a negative regulator of *MDR1* gene expression. However, some research groups have demonstrated that AP-1 is the gene activator of *MDR1* [30, 34]. Therefore, dual regulation must occur at the AP-1 site in the *MDR1* promoter.

MEK inhibitors reduced not only the endogenous P-glycoprotein of HCT-15 and SW620-14 cells but also the exogenous P-glycoprotein of MCF-7/MDR and MDA-MB-231/MDR cells without affecting the *MDR1* mRNA levels. The knockdown of the *ERK* or *p90RSK* genes also reduced P-glycoprotein expression in all of the above cell lines [25]. These results are supported by a study in which arsenite increased P-glycoprotein expression with the upregulation of phosphorylated ERK1/2, which was cancelled by the pretreatment with U0126 [35]. In contrast, several groups have reported that *MDR1* gene expression is upregulated by downstream transcription factors in the ERK pathway [36–38]. Thus, the ERK pathway regulates P-glycoprotein/*MDR1*

expression at both the transcriptional and posttranscriptional levels.

Stimulation with the epidermal growth factor (EGF) or basic fibroblast growth factor after serum starvation activated the EGF receptor signaling pathway and increased P-glycoprotein expression [25]. The overexpression of H-Ras, c-Raf, MEK1/2, ERK1/2, or p90RSK1/2 also increased P-glycoprotein expression, whereas the overexpression of the p110 α subunit of PI3K, wild-type or mutant phosphatase and tensin homolog deleted from chromosome 10 (PTEN), or Akt1, all of which are involved in the PI3K-Akt signaling pathway [2, 39], did not affect P-glycoprotein expression [25]. Thus, the MAPK pathway, but not the PI3K-Akt pathway, controls P-glycoprotein expression.

Treatment of HCT-15 cells with U0126 reduced the [³⁵S]methionine/cysteine-labeled P-glycoprotein in a pulse-chase experiment, whereas the [³⁵S]methionine/cysteine-labeled P-glycoprotein levels in untreated HCT-15 cells were relatively stable. U0126 also synergistically activated paclitaxel-induced apoptosis signaling in P-glycoprotein-expressing cells [25]. These data suggest that the MAPK signaling pathway positively regulates P-glycoprotein expression and protects it from degradation. This pathway is therefore thought to be a good target for overcoming P-glycoprotein-mediated MDR.

2.2. Ubiquitin-Proteasomal Degradation of P-Glycoprotein.

Two protein degradation processes have been suggested to degrade BCRP: properly folded BCRP with N-linked glycosylation is transferred to the plasma membrane and then degraded *via* the autophagic pathway, or misfolded BCRP (e.g., caused by single-nucleotide polymorphisms in the *BCRP* gene) undergoes ubiquitin-proteasomal degradation [40, 41]. Misfolded Δ F508-CFTR/ABCC7, which is the most common mutation in a chlorine-selective anion channel and causes cystic fibrosis, is rapidly degraded by the endoplasmic reticulum-associated degradation [42–45]. We examined the degradation mechanism of P-glycoprotein and found that treatment of cells with proteasome inhibitors (MG132, lactacystin, or bortezomib) showed a rapid increase in the endogenous and exogenous levels of P-glycoprotein [46]. However, the inhibition of autophagic degradation by treating the cells with bafilomycin A1 did not affect the levels of P-glycoprotein. MG132 enhanced the ubiquitination of P-glycoprotein and delayed the disappearance of ubiquitinated P-glycoprotein in a cycloheximide chase experiment, suggesting that the ubiquitin-proteasome pathway regulates the degradation of P-glycoprotein.

The degradation of proteins is important for the maintenance of protein homeostasis in cells, and the ubiquitin-proteasome pathway plays a central role in this degradation [62–65]. Polyubiquitin chains act as markers on the target proteins, which are recognized by the proteasome. The conjugation of ubiquitin to a substrate is regulated by the sequential reaction of three enzymes, the ubiquitin-activating enzyme E1, the ubiquitin-conjugating enzyme E2 (UBE2), and the ubiquitin E3 ligase. The E3 ligases bind directly to the substrate proteins. Therefore, they are the most important components of the ubiquitin-conjugation process.

We searched for the binding partners of the C-terminal intracellular fragment of P-glycoprotein that are associated with its degradation using an immunoprecipitation-matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI/TOF MS) screening and identified F-box protein 15 (FBXO15, also known as FBX15) as a binding candidate [46]. We then confirmed FBXO15 as one of the binding partners of P-glycoprotein by demonstrating that wild-type P-glycoprotein coimmunoprecipitated with FBXO15. The ubiquitin E3 ligases are classified into three different groups, based on the presence of HECT, U-box, or RING-finger domains. In some cases, E3 ligase is composed of several proteins, such as the SCF complex, which consists of S-phase kinase associated protein 1 (Skp1), Cullin1 (Cul1), Rbx1, and a variable substrate-specific F-box protein [62–67]. The FBXO15 is categorized in the O family of F-box proteins and is reported to form the ubiquitin E3 ligase complex SCF^{FBX15} with Rbx1, Skp1, and Cul1 [66, 68]. However, the substrates of SCF^{FBX15} had not been identified until this study.

We screened 23 UBE2s to identify the proteins that associate with both FBXO15 and P-glycoprotein [46]. Among these, UBE2R1 (also known as CDC34 or UBCH3) was identified as the binding partner of both proteins, and SCF^{FBX15} is expected to be a mediator between UBE2R1 and P-glycoprotein in the ubiquitin-proteasomal degradation of P-glycoprotein. In the presence of MG132, the knockdown of *FBXO15* and/or *UBE2R1* reduced the level of ubiquitinated P-glycoprotein without affecting the level of total P-glycoprotein. In contrast, in the absence of MG132, the knockdown of *FBXO15* or *UBE2R1* increased P-glycoprotein levels without affecting *MDR1* mRNA levels. Consistent with these findings, *FBXO15*-depleted cells displayed resistance to vincristine and reduced levels of intracellular rhodamine 123, a fluorescent substrate of P-glycoprotein, compared with those of the control cells transfected with nonsilencing scrambled small-interfering RNA (siRNA). These results indicate that the ubiquitin-proteasomal degradation pathway, including UBE2R1 and the SCF^{FBX15} complex, regulates the degradation of P-glycoprotein. However, the crosstalk between the ubiquitin-proteasomal degradation of P-glycoprotein and MEK inhibition has not yet been clarified.

2.3. Trafficking and Recycling of P-Glycoprotein.

Autophagy is the lysosomal degradation pathway through endocytosis and is of the same importance as the ubiquitin-proteasome pathway in protein degradation and homeostasis [69, 70]. Several membrane proteins, including receptors and transporters, recycle to the plasma membrane through recycling endosomal system. Some cargo proteins sort the cell membrane including discarded proteins into internal luminal vesicles of multivesicular bodies (early endosomes), and mature multivesicular bodies (late endosomes) can fuse with the lysosome. The fusion vesicles are called as autolysosomes, and the vesicles and cargo proteins are proteolyzed by the lysosomal enzymes.

Rab GTPases belong to the largest families of small GTPase and regulate vesicular transport of many proteins in endocytosis, exocytosis, and recycling. One of the Rab

GTPase family, Rab11a, was reported to undergo trafficking wild-type and mutant ($\Delta F508$) CFTRs to the apical recycling in human airway epithelia cells [71]. Recently, Rab4 and Rab5 are reviewed to regulate P-glycoprotein trafficking and recycling and control the degradation of P-glycoprotein localized on the cell-surface membrane [72]. Overexpression of wild-type or constitutively active mutant of Rab4 decreased cell-surface P-glycoprotein expression, whereas the dominant-negative mutant did not affect P-glycoprotein expression on the plasma membrane [73]. This phenomenon is caused by Rab4-mediated regulation of P-glycoprotein exocytosis in early endosome. In contrast, Rab5 is suggested to regulate both exocytosis and endocytosis of P-glycoprotein [74, 75]. Overexpression of dominant-negative mutant of Rab5 accumulated intracellular P-glycoprotein expression, suggesting that Rab5 regulates P-glycoprotein exocytosis [74]. Another group demonstrated that Rab5 also regulates the endocytosis of P-glycoprotein [75]. Overexpression of wild-type Rab5 resulted in recycling P-glycoprotein from the plasma membrane into intracellular fractions. Thus, some small GTPases, including Rab4 and Rab5, control P-glycoprotein expression on the plasma membrane through its trafficking and recycling.

Bafilomycin A1 is an inhibitor of fusion of endosome with lysosome. As mentioned above, we treated cells with bafilomycin A1 for 6 h, but the drug did not affect P-glycoprotein expression [46]. This result suggests that trafficking and recycling of P-glycoprotein are predicted to need considerable time. Actually, some groups reported half-life of the disappearance of P-glycoprotein from the plasma membrane that range between 15–72 h on several cell lines and under different experimental conditions [76–79]. Therefore, the ubiquitin-proteasomal degradation pathway may exist as rapid proteolysis system, and the endosomal trafficking and recycling pathway does as slow system in P-glycoprotein homeostasis.

2.4. P-Glycoprotein Stability and Glycosylation Regulated by Pim-1 Kinase. The serine/threonine protein kinase Pim-1 was originally identified as the proviral integration site in Moloney murine leukemia virus lymphomagenesis [80, 81]. Overexpression of Pim-1 is often observed in diverse human malignancies, including acute myeloid leukemia, acute lymphoblastic leukemia, prostate cancer, and gastric cancer [82]. Pim-1 promotes tumor cell growth by promoting cell cycle progression, cell migration, and protein translation and by the suppression of apoptosis. Two protein isoforms of Pim-1, 33 kDa Pim-1S and 44 kDa Pim-1L, have been reported to be alternative translation products and display different subcellular localization: Pim-1S in both the cytoplasm and nucleus and Pim-1L primarily on the plasma membrane.

P-Glycoprotein contains a Pim-1 phosphorylation consensus sequence, QDRKLS, located between amino acids 678 and 683, and the 44 kDa Pim-1L, but not 33 kDa Pim-1S, has been reported to mediate the phosphorylation of Ser683 in P-glycoprotein [83]. This Ser683 phosphorylation protects P-glycoprotein from degradation and allows its glycosylation and cell-surface expression. Knockdown of

Pim-1 by the introduction of a short hairpin RNA reduced the phosphorylation and stability of P-glycoprotein, and an *in vitro* kinase assay showed the Pim-1L-mediated phosphorylation of P-glycoprotein. HL60/VCR cells are vincristine-resistant human promyelocytic leukemia cells that express P-glycoprotein, and their treatment with a Pim-1 inhibitor, SGI-1776, promoted the rapid proteolytic degradation of P-glycoprotein with a half-life of 1 h, whereas the half-life in untreated cells was 9 h. Cotreatment of the cells with SGI-1776 and MG132 retarded the disappearance of P-glycoprotein.

Pim-1 stabilized the 150 kDa P-glycoprotein, which is an underglycosylated form of P-glycoprotein, and promoted its glycosylation and subsequent translocation to the cell surface. Pim-1 inhibition also sensitized P-glycoprotein-overexpressing cells to doxorubicin in a colony formation assay. These results indicate that Pim-1 regulates P-glycoprotein expression on the cell surface by protecting the 150 kDa form of P-glycoprotein from proteasomal degradation and partially support our finding that the ubiquitin-proteasome pathway is important in the degradation of P-glycoprotein [46].

Pim-1L is also reported to phosphorylate Thr362 in BCRP and promotes its dimerization in human prostate cancer cells [84]. Pim-1L was identified as a BCRP-binding protein in a yeast two-hybrid screening. The binding of Pim-1L to BCRP was confirmed *in vivo* and *in vitro*, and their colocalization was confirmed in cultured cells. The substitution of threonine with alanine at position of 362 (T362A) in BCRP abolished the cell-surface expression, dimerization, and transport activity of BCRP, whereas the substitution of this threonine with aspartic acid (T362D) increased the cellular resistance to mitoxantrone and topotecan, despite the knockdown of *Pim-1*. Thus, Pim-1L moderates MDR in cancer cells by regulating not only P-glycoprotein expression but also BCRP expression, although the regulatory mechanisms are quite different for both proteins.

2.5. Phosphorylation of P-Glycoprotein by Protein Kinase C (PKC) and Protein Kinase A (PKA). In the 1990s, PKC and PKA were shown to phosphorylate serine residues in the linker region of P-glycoprotein [85, 86]. Several groups reported that PKC and PKA are essential for the translocation and function of P-glycoprotein, whereas others have refuted this, although all groups agreed about the PKC- or PKA-mediated phosphorylation of P-glycoprotein. Chambers et al. identified three PKC-mediated phosphorylation residues (Ser661, Ser667, and Ser671) and three PKA-mediated phosphorylation residues (Ser667, Ser671, and Ser683) in P-glycoprotein using a synthetic P-glycoprotein peptide [85]. All of them lie on the intracellular linker region between the first NBD and TM7.

The PKA-associated translocation of P-glycoprotein was reported by two different groups [87, 88]. Mammalian cells express two isoforms of PKA, type I and type II, which are characterized by different regulatory subunits [89, 90]. The PKA type I complex is called “PKA-RI,” and the PKA type II complex is “PKA-RII.” PKA-RI localizes primarily in the cytoplasm and displays higher sensitivity to cAMP

than PKA-RII. In contrast, most PKA-RIIs are anchored at specific organelles and cellular structures by binding to A-kinase anchoring proteins (AKAPs). This provides an important level of control, ensuring the specificity of cAMP-mediated signal transduction. The PKA regulatory subunit, PKA-RII, is reported to regulate the efficient trafficking of P-glycoprotein to the apical canalicular plasma membrane [87]. Treatment of HepG2 cells with a glucosylceramide synthase inhibitor, D,L-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, inhibited the appearance of P-glycoprotein on newly synthesized apical surfaces. Hence, glucosylceramide synthesis is an important factor in the efficient Golgi-to-apical-surface transport of P-glycoprotein. AKAP350, which is one of the anchoring proteins of PKA-RII, is also implicated in the polarized expression of P-glycoprotein on the apical canalicular membrane [88].

In contrast, other research groups have refuted the functional association between P-glycoprotein and PKC or PKA. Germann et al. expressed wild-type and phosphorylation-defective mutant P-glycoprotein in mammalian cells and compared their expression levels and sensitivity to vinblastine, colchicine, and doxorubicin [86]. However, two types of cells displayed no differences in their P-glycoprotein expression or drug sensitivity, suggesting that the phosphorylation of P-glycoprotein is not essential for its expression and function. Cvijic and Chin also reported that PKA-RI activity was not correlated with P-glycoprotein expression [91]. Several P-glycoprotein-expressing cells with diverse PKA-RI activities displayed the equal levels of P-glycoprotein and no differences in their sensitivity to doxorubicin, paclitaxel, or colchicine were observed.

Thus, several groups have demonstrated conflicting results regarding the association between P-glycoprotein and PKC or PKA. PKA, including AKAP350, and PKC are recently thought to regulate the translocation of P-glycoprotein and its function *via* its phosphorylation, but the evidence is not yet conclusive.

3. Transcriptional Regulations of *MDR1* mRNA and P-Glycoprotein Expression

3.1. Transcriptional Regulation of *MDR1* Expression. Many transcription factor-binding sequences, such as those for AP-1, NF- κ B, forkhead transcription factors O1 (FOXO1, also known as FKHR) and O3a (FOXO3a, also known as FKHR1), and T-cell factor/lymphoid enhancer factor (TCF/LEF), have been identified in the promoter region of the *MDR1* gene, and the transcription activities of the *MDR1* promoter in response to these transcription factors have been demonstrated. As mentioned above, the transcription factors AP-1 and NF- κ B bind directly to the promoter region of the *MDR1* gene [30–34, 92, 93]. AP-1 has been reported to induce transcription from the *MDR1* promoter in vincristine-resistant human gastric cancer SGC7901/VCR cells and vinblastine-resistant Caco-2 cells [30, 34], whereas the expression of AP-1 has been shown to suppress this

transcription in various cell lines, including H460/MDR, SKOV3/MDR, A498, and SK-MES-1/DX1000 [32, 33]. These discrepant results are thought to be determined by coregulators, such as c-Jun, c-Fos, and ATF2. The activation of NF- κ B by vinblastine in Caco-2 cells, by insulin in rat hepatoma cells, and by reactive oxygen species and cadmium in kidney proximal tubule cells upregulated the expression of *MDR1* mRNA to defend tissues and cells from these substances [33, 34, 92–94]. Heat shock factor 1 and heat shock protein 27 have also been shown to suppress *MDR1* mRNA expression through the inactivation of NF- κ B in doxorubicin-resistant human breast cancer cells [94]. Thus, NF- κ B signaling is one of the important pathways involved in the transcriptional regulation of *MDR1*.

PI3K-Akt signaling is involved in the transcription of the *MDR1* gene. The PI3K-mediated production of the second messenger phosphatidylinositol-3,4,5-triphosphate (PIP3) triggers the 3-phosphoinositide-dependent kinase 1 (PDK1) mediated activation of Akt [2]. The Ras-related C3 botulinum toxin substrate 1 (Rac1) is a small G-protein of the Rho family and one of the activators of Akt through PI3K activation [95]. The activation of PI3K and Rac1 by the hepatocarcinogen 2-acetylaminofluorene induces *MDR1* mRNA through Akt activation in human hepatocarcinoma HepG2 cells and human embryonic kidney (HEK) 293 cells [96]. However, recent studies have shown different results, in which FOXO1 and FOXO3a, which are negatively regulated by the Akt-dependent inhibition of nuclear translocation [97], upregulated the *MDR1* promoter activity in doxorubicin-resistant breast cancer MCF-7/ADR cells and human leukemia K562 cells [98–100]. Silent information two ortholog 1 (SIRT1) also enhanced *MDR1* promoter activity *via* FOXO1 activation in MCF-7/ADR cells [99]. These differences are attributable to unknown factors, but a huge variety of epigenetic factors acting in the *MDR1* promoter region in each cell line are considered to be involved.

Wnt/ β -catenin signaling promotes the expression of *MDR1* mRNA. The activation of Wnt/ β -catenin signaling by cadmium, lithium chloride, glycogen synthase kinase-3 (GSK-3) inhibition, or cDNA transfection promoted the nuclear translocation of β -catenin and activation of the transcription factor TCF/LEF. Activated TCF/LEF upregulated the expression of its target genes, including *c-myc*, *cyclin D1*, and *MDR1*, to increase the proliferation, evasion of apoptosis, and resistance to metal toxicity of renal carcinoma cells, brain endothelial cells, and chronic myeloid leukemia cells [101–103]. GSK-3 is a downstream target of Akt and is negatively regulated by it [2]. Therefore, the activation of Akt enhances the transcription of the *MDR1* gene through the inactivation of GSK-3 and the activation of Wnt/ β -catenin signaling.

3.2. Association of MicroRNAs with *MDR1* mRNA and P-Glycoprotein Expression. A number of microRNAs (miRs) have been reported to modulate the expression of *MDR1* mRNA and P-glycoprotein (Table 1) [104]. Some of these,

TABLE 1: MicroRNAs regulate *MDR1* mRNA expression.

miRNA	Direct regulation (binding to the <i>MDR1</i> 3'-UTR)	Indirect regulation (target gene)	<i>MDR1</i> mRNA	Cell line	Reference
miR-451	✓		↑	2780DX5, KB-V1, MCF-7, Cancer stem cells	[47–49]
miR-27a	✓		↑	2780DX5, KB-V1, K562/DOX, NB4/R2, HL-60/DOX	[47, 50]
		✓ (HIPK2)		BEL/5-FU, A2780/Taxol	[51, 52]
miR-508-5p	✓		↑	SGC7901/VCR	[53]
miR-331-5p	✓		↑	K562/DOX, NB4/R2, HL-60/DOX	[50]
miR-298	✓		↑	MDA-MB-231-R	[54]
miR-145	✓		↑	HEK293, Caco-2	[55]
miR-137		✓ (YB-1)	↑	MCF-7/ADR	[56]
miR-200c		✓ (ZEB1/2?)	↑	MCF-7/ADM, MDA-MB-231	[57]
miR-122		✓ (Unknown)	↑	Hep3B, HepG2, Huh7, PLC/PRF/5	[58]
miR-138		✓ (Unknown)	↑	HL-60/VCR	[59]
miR-19a/b		✓ (PTEN)	↑	SKOV3/CIS	[60]
miR-130a		✓ (PTEN)	↓	SGC7901/VCR, SGC7901/ADR	[61]

including miR-451, miR-27a, miR-508-5p, miR-331-5p, miR-298, and miR-145, are directly associated with the 3'-untranslated region (UTR) of the *MDR1* mRNA and suppress P-glycoprotein expression [47–50, 53–55]. The expression of miR-27a and miR-451 reduced *MDR1* mRNA and P-glycoprotein expression and increased the sensitivity to anti-cancer drugs of the MDR cancer cell lines A2780DX5, KB-V1, and MCF-7/DOX and colorectal cancer stem cells [47–49]. The overexpression of miR-508-5p sufficiently reversed the resistance of gastric cancer cells to multiple chemotherapeutics agents by suppressing P-glycoprotein [53]. The expression of miR-331-5p has been reported to correlate inversely with the expression of P-glycoprotein in K562/DOX cells, and the overexpression of miR-331-5p in doxorubicin-resistant leukemia cell lines K562/DOX and HL60/DOX increased their sensitivity to doxorubicin [50]. Other microRNAs, miR-298 and miR-145, suppressed the transcription of the *MDR1* gene in doxorubicin-resistant MDA-MB-231 and HEK293 cells, respectively [54, 55].

MiR-137, miR-200c, miR-122, and miR-138 have been reported to reduce the expression of *MDR1* mRNA through other targets [56–59]. The low level of miR-137 expression has been found in multidrug-resistant MCF-7/ADM cells, and the expression of miR-137 correlates inversely with Y-box binding protein-1 (YB-1) and P-glycoprotein levels in breast cancer cells. An experimental study showed that miR-137 is involved in MDR in cancer cells through the modulation of P-glycoprotein by targeting YB-1 [56]. MiR-200c is downregulated in MCF-7/ADM cells compared with its expression in the parental MCF-7 cells, and miR-200c mimics enhanced the chemosensitivity of cells to epirubicin by reducing *MDR1* mRNA and P-glycoprotein expression [57]. A liver-specific microRNA, miR-122, is frequently downregulated in

hepatocellular carcinoma (HCC). The overexpression of miR-122 in HCC modulates the cellular sensitivity to doxorubicin and vincristine by lowering *MDR1* mRNA expression [58]. The vincristine-resistant leukemia cell line HL-60/VCR showed miR-138 upregulation. The upregulation of miR-138 reverses resistance to anticancer agents with reducing the expression of *MDR1* mRNA and P-glycoprotein [59]. MiR-27a indirectly modulates *MDR1* mRNA and P-glycoprotein expression by targeting homeodomain-interacting protein kinase-2 (HIPK2) in human ovarian cancer cells or the frizzled homolog/ β -catenin pathway in HCC [51, 52].

In contrast, several microRNAs are involved in the upregulation of *MDR1* transcription. The expression of miR-19a/b confers resistance to doxorubicin on gastric cancer cells by increasing their *MDR1* mRNA and P-glycoprotein expression and reducing the expression of antiapoptotic factors Bcl-2 and Bax [61]. The downregulation of miR-130a overcomes the cisplatin resistance in cisplatin-resistant ovarian cancer SKOV3/CIS cells by inhibiting *MDR1* mRNA and P-glycoprotein expression by targeting PTEN [60]. Thus, recent studies have suggested that many microRNAs suppress the transcription of the *MDR1* gene, whereas several microRNAs enhance it.

4. Perspective

Many studies have suggested that P-glycoprotein expression is associated with several cellular signaling pathways. Protein kinases, chaperons, ubiquitin-related enzymes, and transcription factors have been shown to regulate the sensitivity of cells to anticancer drugs by controlling the expression and function of P-glycoprotein (Figure 1). Several microRNAs have also been shown to modulate the transcription of *MDR1*

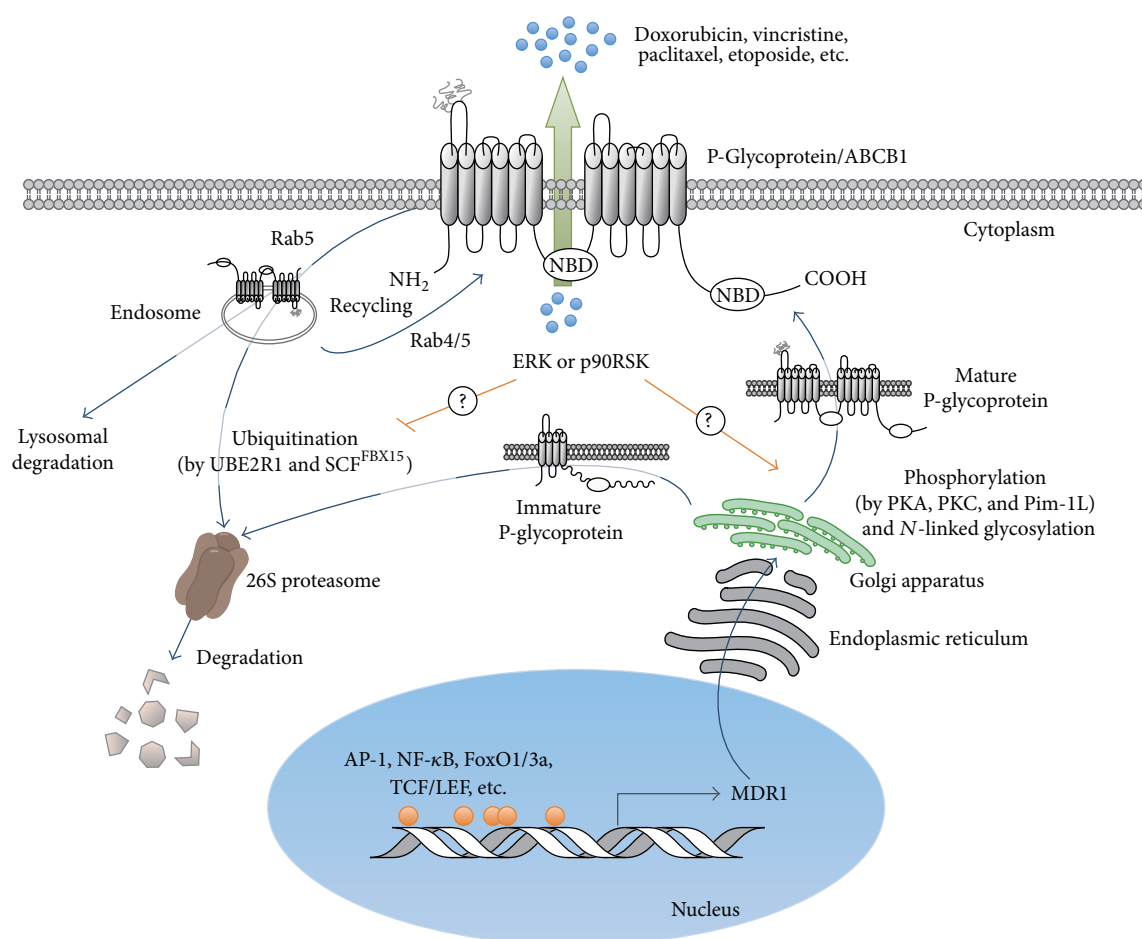


FIGURE 1: Schematic model in regulations of *MDR1* and P-glycoprotein.

mRNA and the expression of P-glycoprotein. Molecular analyses of P-glycoprotein are ongoing in many laboratories in an effort to understand its biological control and regulation and to establish new strategies to increase the clinical efficacy of anticancer agents.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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