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Molecular Pathways: Regulation and Therapeutic Implications of Multidrug Resistance

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Abstract

Multidrug transporters constitute major mechanisms of multidrug resistance (MDR) in human cancers. The *ABCB1* (*MDR1*) gene encodes a well-characterized transmembrane transporter, termed P-glycoprotein (P-gp), which is expressed in many normal human tissues and cancers. P-gp plays a major role in the distribution and excretion of drugs, and is involved in intrinsic and acquired drug resistance of cancers. The regulation of *ABCB1* expression is complex, and has not been well studied in a clinical setting. In this review, we elucidate molecular signaling and epigenetic interactions that govern *ABCB1* expression and the development of MDR in cancer. We focus on acquired expression of *ABCB1* that is associated with genomic instability of cancer cells, including mutational events that alter chromatin structures, gene rearrangements, and mutations in tumor suppressor proteins (e.g., mutant p53) that guard the integrity of genome. In addition, epigenetic modifications of the *ABCB1* proximal and far upstream promoters by either demethylation of DNA or acetylation of histone H3 play a pivotal role in inducing *ABCB1* expression. We describe a molecular network that coordinates genetic and epigenetic events leading to the activation of *ABCB1*. These mechanistic insights provide additional translational targets and potential strategies to deal with clinical MDR.

Keywords

Multidrug resistance; *ABCB1*; *MDR1*; transcription; epigenetic

BACKGROUND

The expression, function, and modulation of the multidrug resistance (MDR) transporter gene *ABCB1* is one of the most extensively studied mechanisms of drug resistance in human cancers (1–5). The role of P-glycoprotein (P-gp) is well established in hepatic drug excretion, limitation of gastrointestinal absorption of substrate drugs, and as a key component of the blood-brain, blood-testicular, and blood-placental barriers (3, 6–9). *ABCB1* transfection and its inhibition by gene knockouts, RNA silencing and small molecule transport inhibitors have proven its function as a determinant of resistance to various anticancer drugs, including anthracyclines, taxanes, vincas and others, including a large fraction of new drugs in development (1, 8, 9). Among many clinical trials with various inhibitors of P-gp in attempts to reverse drug resistance, the large majority have been negative, and only a few show some evidence of clinical benefit (2).

Despite the significant advances in understanding the functional aspects of P-gp, however, the regulation of the *ABCB1* gene and its impacts on clinical trials are not well-elucidated. In this review, we mainly focus on our recent understanding of genetic and epigenetic aspects of *ABCB1* expression, and their cooperation with transcription factors (TFs) on the *ABCB1* gene. We shed light on how molecular signaling coordinated with epigenetic mechanisms controls *ABCB1* expression. These regulatory insights derived from the *ABCB1* model may also be useful for deciphering non-*ABCB1*-mediated drug resistance and for prevention and molecular intervention of MDR in clinic.

REGULATION OF THE *ABCB1* GENE

Genomic instability and *ABCB1* activation

Mechanisms underlying the intrinsic genomic instability of cancers have been an important area of research. Using Luria-Delbruck fluctuation analysis in the human uterine sarcoma cell line MES-SA, we showed that spontaneous mutational events underlie acquired MDR in these cells, and activation of *ABCB1* is a frequent mechanism for cellular resistance to doxorubicin, paclitaxel, and vinblastine (10–13), but not to etoposide (14). Structurally, Fojo and co-workers proposed that gene rearrangements serve as an important mechanism to control *ABCB1* expression in many cases by juxtaposition of the native *ABCB1* promoter to transcriptionally active promoters of many unrelated genes (15). This mechanism was also found in some cancer patient samples from both leukemias and lymphomas, suggesting its potential clinical importance (16). Several oncogenes including *Ras*, *c-Raf*, and *c-Raf* kinase have been implicated in *ABCB1* regulation (17–19). These studies suggest that oncogenes play a role in regulating the expression of *ABCB1*, possibly *via* malignant transformation processes.

During cellular transformation and tumor progression, one of the most commonly observed alterations is mutation in the *TP53* tumor suppressor gene. There is substantial evidence that p53 is involved in regulation of *ABCB1*. The general observation is that the wild-type p53 represses and mutant p53s enhance *ABCB1* promoter activity (5, 17, 20, 21). Moreover, the interacting site of p53 on the *ABCB1* promoter has been identified (22). Hence, alterations or interactions in the p53 system have linked MDR to the p53-mediated signal pathway. Suppression of MDM2, a potent inhibitor of p53, by Nutlin-3 inhibits the functions of P-gp and *ABCC1* (*MRP1*) in drug-resistant neuroblastoma and rhabdomyosarcoma cell lines (23). These studies suggest that the p53 system is involved in the regulation of *ABCB1*, possibly by direct interaction with the *ABCB1* promoter. Thus, restoration or mimicry of wild-type p53 function may be a strategy to reduce or prevent MDR and sensitize cancer cells to anticancer drugs.

Epigenetic mechanisms that control *ABCB1* expression

ABCB1 expression was also shown to be associated with demethylation of the *ABCB1* promoter in the promyelotic leukemia (PML) cell line HL-60 after drug selection (24), human T-cell leukemia cells treated with the demethylating agent 5'-azadeoxycytidine (25), AML clinical samples (26), adult acute lymphocytic leukemias (ALLs) (27), and bladder cancers (28). These data suggest that demethylation of the *ABCB1* promoter activates *ABCB1* expression in cancer cell lines and also in clinical cancer patients.

Jin and Scotto further showed that both histone acetyltransferases (HATs) and histone deacetylases (HDACs) regulate *ABCB1* mRNA levels in SW620 (human colon carcinoma) cells when the cells were transiently treated with the HDAC inhibitor (HDACi) trichostatin A (TSA) (29). We have demonstrated that a 20-fold increase in acetylated H3 (acetyl-H3) in the nucleosomes within the 968-bp region of the *MDR1* far upstream promoter (P2), but not within the native *ABCB1* promoter (P1), in doxorubicin single-step selected mutants of

MES-SA cells (13). Reciprocally, a 35-fold increase in acetyl-H3 was found in the *ABCB1* P1, but not in the P2 promoter, in step-wise selected MES-SA/Dx5 cells (13). The detailed molecular events that are responsible for this differential utilization of the *ABCB1* promoters are unclear. However, this is the first epigenetic evidence demonstrating that different cytotoxic regimens could result in distinct routes that lead to differential *ABCB1* activation. The importance of acetyl-H3 in the regulation of *ABCB1* was also confirmed by an independent study, in which Baker et al. demonstrated that upregulation of *ABCB1* by anticancer drugs was associated with a dramatically induced acetyl-H3, but not acetyl-H4, on the *MDR1* P1 promoter in CEM-Bcl2 and in SW620 cells by 24-hour drug induction (30). Similarly, a repressive histone marker, trimethylated H3-Lys9 was found to be associated with the expression of another multidrug transporter *ABCG2* (31). These studies indicate that the acetylation status of H3 plays an important role in the regulation of some ABC transporters.

Transcriptional regulation of the *MDR1* promoter

A wide-ranging evaluation of the roles of TFs in the regulation of *ABCB1* was provided by Scotto (5). Here, we focus on several TFs that are associated with either chromatin-remodeling or epigenetic interaction with the *ABCB1* promoter. In general, a chromatin-remodeling process is believed to be the initial step to induce gene expression. We demonstrated, by transient electroporation of C/EBP β expression plasmids into the cells, that C/EBP β (NF-IL6), a member of the CCAAT/enhancer binding protein family, can induce expression of the silent *ABCB1* gene in MCF-7 breast cancer cells (32). C/EBP β has been reported to up-regulate *ABCB1* promoter activity *via* a sequence motif resembling the C/EBP β DNA consensus sequence (33). However, we revealed a novel mode of action for this protein in the regulation of *ABCB1* expression by demonstrating that C/EBP β interacts with the *ABCB1* chromatin that contains an inverted CCAAT-box (Y-box) in a cell-type-dependent manner (32). The behavior of C/EBP β on the *ABCB1* promoter is consistent with the capacity of its N-terminal transactivation domain to recruit the human SWI/SNF chromatin-remodeling complex (34). Our study suggests that some TFs such as C/EBP β are directly involved in the development of acquired MDR by transactivating endogenous *ABCB1* (32).

Moreover, the human Brahma protein (hBrm), a component of the human SWI/SNF complex, is also implicated in the upregulation of *ABCB1* promoter activity, possibly via cooperation with C/EBP β on the Y-box in MCF-7 cells (32). Whether the hBrm-C/EBP β complex represents a positive or negative regulator for the chromatin-embedded *MDR1* gene was not determined in MCF-7 cells in the previous study (32). The association of the SWI/SNF complex (containing hBrm) on the *ABCB1* promoter was confirmed by El-Osta and Colleagues (35). In their study, hBrm appears to be complexed with the methyl-CpG binding protein (MeCP2) to repress the endogenous *ABCB1* promoter in CEM-CCRF cells. In general, we believe that the regulation of *ABCB1* expression either by C/EBP β or hBrm alone or by the C/EBP β -hBrm complex is both cellular-context and chromatin-status dependent. The regulatory complex may be controlled indirectly by autocrine production of interleukin 6 (IL-6), which was shown to associate with *ABCB1* activation *via* a C/EBP β pathway in breast cancer cells (36).

Cooperation among epigenetic components, TFs, and chromatin-remodeling factors

The interactions among DNA methylation, histone modification, chromosomal remodeling, and TF positioning are highly regulated in order to achieve a desired phenotype. In the case of *ABCB1*, a hypermethylated promoter with deacetylated H3 represents a doubly repressive status of the *ABCB1* promoter, that needs to be released prior to achieving *ABCB1* expression. Indeed, in P-gp negative MES-SA and MCF-7 cells, the *ABCB1*

chromatin was deacetylated (13, 37). The occupancy of both MeCP2 and the corepressor MBD2 would reinforce the repressive status of the *ABCB1* chromatin (37). Hence, release of both MeCP2 and HDAC1 from the methylated *ABCB1* promoter modulates *ABCB1* expression (38). Such a derepression mechanism facilitates acetylation of a preferred histone species (e.g., H3) at a specific residue (e.g., Lys9), thus enabling the opening of compact chromatin and positioning of general TFs as well as RNA polymerase II at the *ABCB1* promoter (Figure 1).

The Y-box of the *ABCB1* promoter is likely a central docking site to recruit TFs such as p300/CREB binding protein-associated factor (p/CAF), that has HAT activity, and to recruit C/EBP β that has chromatin-remodeling ability (29, 32). HAT complexes were shown to stabilize the SWI/SNF complex binding to promoter nucleosomes with specificity (39). Thus, these studies illustrate a structural and functional link among the Y-box, Y-box binding proteins, C/EBP β , HATs, HDACs, MeCP2, and the SWI/SNF complex on the *ABCB1* promoter. They also suggest a complex interaction pattern that is crucial for the initiation of a cluster of genes, including *ABCB1* that contribute to the intractability of cancer cells to various therapies (Figure 1). Y-box binding proteins thus represent important markers for MDR (40, 41). Disruption of the YB-1 also caused suppression of EGFR and HER-2/neu, reinforcing the putative association among the YB-1-Her2-*ABCB1* network (Figure 1).

CLINICAL-TRANSLATIONAL ADVANCES

Clinical relevance of *ABCB1*

The clinical relevance of *ABCB1* expression was predominantly focused on the *ABCB1*-encoded P-gp, which has been used as a therapeutic target to enhance chemotherapies by inhibiting the transporter function. However, the clinical significance of P-gp inhibition as a therapeutic strategy has proven to be quite difficult to establish. A major confounding issue in clinical trials of MDR modulation has been the altered pharmacokinetics of chemotherapeutic drugs such as doxorubicin, daunorubicin, etoposide, and taxanes, both as a result of P-gp inhibition and as a result of inhibition of other drug transporters and CYP 3A4 (6, 42–46). In some cases, such as the use of the cyclosporine analogue valspodar (PSC-833) in AML, substantially increased toxicity was observed, resulting in an increased toxic death rate (47).

The most positive data for MDR modulation was the SWOG 9126 trial using high dose cyclosporine infusion in relapsed and high risk AML (48). However, other Phase III studies in AML using cyclosporine or valspodar have been negative (47, 49). The discrepancies in results between these studies may be due to different patient populations, with SWOG 9021 containing a large proportion of secondary AML expressing high levels of P-gp. Patient selection on the basis of screening for functional P-gp expression may be important. Baer et al. reported a trend towards benefit in a subset of AML patients with PSC-modulatable efflux, who had an increase from 5 to 14 month median survival when valspodar was added to induction chemotherapy ($P = 0.07$) (47). Recently, a phase III trial of the potent and specific P-gp inhibitor zosuquidar was reported as negative in older patients with AML (50). In addition to studying an unselected AML population, the schedule of zosuquidar in this ECOG study was suboptimal, given the short half life of the drug (51, 52).

Incomplete inhibition and alternative MDR mechanisms in clinical trials

Incomplete P-gp inhibition, drug interactions leading to excessive toxicities, and suboptimal clinical trials designs are all reasons for the lack of success of P-gp inhibition as a therapeutic approach. However, the most significant obstacle may be the expression of multiple, redundant mechanisms of resistance in human cancers. In this regard, relatively

non-specific drugs such as cyclosporine may offer an advantage by inhibiting several drug transporters, although this advantage is counterbalanced by an increased potential for drug interactions and toxicities (53). Co-expression or selection for other drug transporters as alternative MDR mechanisms has been reported in the setting of inhibition of P-gp function in AML. These other transporters include *ABCC1* (*MRP1*), *ABCC2* (*MRP2*), and *ABCG2* (*BCRP/MXR*) (54–57). Co-expression of P-gp and other transporters has also been reported in normal and leukemic stem cells (58, 59). These data imply that the coexistence of P-gp with other transporters such as *ABCG2* is a default stem-cell program in AML, and perhaps in other cancers. Coexpression of *ABCB1* and *ABCG2* is obviously associated with the acetylation of histones, particularly H3 (13, 30, 31, 60). Thus, the role of epigenetic-based therapies on drug resistance should be further investigated using HAT (rather than HDAC) inhibitors, targeting acetyl-H3.

Epigenetic therapies and clinical MDR

The use of HDAC inhibitors in treating human cancer patients may result in expression of *ABCB1* or *ABCG2* or both. Thus, induction of *ABCB1* expression by FK228, a depsipeptide that specifically inhibits class I HDACs, was reported in normal peripheral blood mononuclear cells and in circulating tumor cells, acute promyelocytic leukemia (APL) cells, and osteosarcoma (16, 61–63). Coadministration of all-trans retinoic acid (ATRA) with FK228 increased both acetyl-H4 and acetyl-H3-Lys9 at the *ABCB1* promoter in acute APL cells (62). Hence, upregulation of *ABCB1* by HDAC inhibitors such as FK228 could reduce its antitumor efficacy through the classical MDR mechanisms.

Pathway intervention and prevention of MDR

Many survival signals that are associated with *ABCB1* activation have been endogenously active in AML, including PI3K/Akt, Raf, and PKC. These factors can also contribute to ABC transporter-independent drug resistance mechanisms. Thus, coexistence of other non-*ABCB1* resistance mechanisms in AMLs elucidates the limitations of P-gp inhibitors as a treatment strategy, and underlies the need both for a deeper understanding of the inter-relatedness of drug resistance mechanisms and for novel strategies to deal with resistance. Such strategies might include identification of shared regulators of multiple resistance pathways, and concurrent targeting of such pathways. Pharmacological reactivation of p53 in human cancer with a mutant p53 status is one such example. In murine tumor models, restoration of functional p53 induces a senescence program (in liver carcinomas and in sarcomas) and apoptosis (in lymphoma), which consequently leads to tumor clearance or regression (64, 65). These genetic experiments in mice have proved the principle of p53-based therapy. Restoration of functional p53 might also increase therapeutic efficacy of some anticancer drugs through the suppression of *ABCB1*. Acetylation of p53 results in destabilization of the p53-MDM2 interaction loop, thus activating p53-mediated stress response (66). The SNP309 G/G genotype that influences MDM2 transcription levels is considered an independent risk factor in B-CLL (67) and is associated with poor survival among early-stage NSCLC patients with squamous cell histology (68). Inhibitors that target MDM2-p53 interactions might also concomitantly target MDR mechanisms.

CONCLUSIONS

Multiple genetic and epigenetic regulatory mechanisms constitute a tightly coordinated network that determines predominant drug-resistant phenotypes including classical P-gp-related MDR. We elucidate the complexities of MDR in human malignancies. The role of P-gp expression in clinical drug resistance and drug disposition is well established, and transport by P-gp is an important consideration in the development of new anticancer drugs. However, P-gp targeting to sensitize cancers to therapies has not been successful in clinical

trials. The negative clinical trials are linked to limited inhibition of P-gp, excess toxicities from inhibition of drug disposition, alternative MDR and other drug resistance mechanisms, and suboptimal clinical trials designs. Targeting the regulation of *ABCB1* and related resistance mechanisms by new therapeutic approaches, including epigenetic modulators, will be subject to some of the same constraints as P-gp inhibitors. These include the complexity and redundancy of drug resistance mechanisms, effects on cytotoxic drug distribution and excretion, as well as the fundamental importance of these MDR mechanisms in stem cell biology and survival.

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Figure 1. Cooperation and coordination of signaling networks of multidrug resistance

Drug-resistance activating signals produce pleiotropic effects that lead to transactivation and coordination of various drug resistance programs. The drug-resistance activating system includes the p53 networks, anti-apoptotic pathways, JAK/STAT pathways, Ras-based MAPK pathways, classical MDR genes such as *ABCB1*, *ABCG2*, and *ABCC1*, and other alternative forms of drug resistance mechanisms. Transcriptional activator complexes for the *ABCB1* gene are likely shared by a large set of transcriptional factors that are involved in conferring drug resistance and cell survival. Transcription repressors that prevent the assembly of activator complexes are dissociated from promoters of the target genes prior to *ABCB1* activation. These derepression mechanisms are modulated by the genomic instability of cancers, epigenetic modifications of histones, and tissue-specific factors. The yellow arrows indicate activation or positive regulation, whereas the red line indicators

denote suppression or negative regulation. Question marks indicate hypothetical or undefined factors or pathways.

Abbreviations: *ABCB1* P1, the *ABCB1* proximal promoter; Ac, acetylated histone; AKT, v-akt murine thymoma viral oncogene homolog; AP-1, the activator protein 1 (a transcription factor); c-Jun, a transcriptional factor encoded by the Jun proto-oncogene; ERK, extracellular signal-regulated kinase; gp130, glycoprotein 130; GPCR, G protein-coupled receptor; HAT, histone acetyltransferase; HDAC, histone deacetylases; Her2, human epidermal growth factor receptor 2; IGF-1, insulin-like growth factor 1; IL-6, interleukin-6; IL-6R, Interleukin-6 receptor; JAK, Janus kinase; JNK1, c-Jun N-terminal kinase; MDM2, Mdm2 p53 binding protein homolog (mouse); MDR, multidrug resistance; MeCP2, methyl CpG binding protein 2; MEK, mitogen-activated protein kinase kinase; mTOR, the mammalian target of rapamycin; p53, the tumor suppressor protein p53; pCAF, p300/CBP-associated factor; PM, plasma membrane; Pol II, RNA polymerase II; Raf, a proto-oncogene serine/threonine-protein kinase; Ras, a protein subfamily of small GTPases; STAT, signal transducer and activator of transcription; SWI/SNF, a nucleosome remodeling complex.