The role of nuclear Y-box binding protein 1 as a global marker in drug resistance

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Abstract
Gene expression can be regulated by nuclear factors at the transcriptional level. Many such factors regulate MDR1 gene expression, but what are the sequence elements and transcription factors that control the basal and inducible expression of this gene? The general principles through which transcription factors participate in drug resistance are now beginning to be understood. Here, we review the factors involved in the transcriptional regulation of the MDR1 gene. In particular, we focus on the transcription factor Y-box binding protein 1 and discuss the possible links between Y-box binding protein 1 expression and drug resistance in cancer, which are mediated by the transmembrane P-glycoprotein or non-P-glycoprotein. [Mol Cancer Ther 2004;3(11):1485–92]

Introduction
Drug export from cells is mediated through a group of proteins belonging to the ATP binding cassette family of transporters. The 170-kDa transmembrane protein P-glycoprotein (PGP), which is encoded by the multidrug resistance 1 (MDR1) gene, is a representative example of an ATP binding cassette transporter. PGP consists of two membrane-spanning domains and two nucleotide binding domains and has been reported to affect the pharmacokinetics of drugs by limiting the rate at which they are absorbed (1–5). Various molecules are targeted by drug treatments for cancer; however, PGP expression is responsible for resistance to the widest range of anticancer drugs (6, 7).

The expression of MDR1/PGP in human malignant cancers is expected to play a critical role in limiting their sensitivity to anticancer agents. Therefore, the determination of MDR1 gene expression levels, along with studies of the regulatory mechanisms of this gene, will be useful in developing tailor-made therapeutic strategies for cancer patients.

The partial sequence of the human MDR1 gene was first reported in the 1980s (8), and its complete sequence, including clustered CpG sites that are not associated with a TATA box, is now known (9). Within the MDR1 promoter sequence, a GC box forming a Sp1 site and an inverted CCAAT (ATTGG) site for Y-box binding protein 1 (YB-1) or nuclear factor Y (NF-Y) binding both play key roles in MDR1 gene expression (10).

MDR1 gene expression is often observed in recurrent cancers and appears after the chemotherapeutic treatment of various human malignancies. In cultured human cancer cells, the MDR1 promoter was activated by both PGP targeting drugs (vincristine and doxorubicin) and non-PGP-targeting drugs (5-fluorouracil and etoposide; ref. 11). In addition, treatment with retinoic acids and other differentiating agents resulted in enhanced expression of the MDR1 gene product PGP (12). Expression of the MDR1 gene was also up-regulated by heat shock, arsenate, and serum starvation in cultured human cancer cells (13–16). Consistent with these findings, MDR1 gene expression was markedly induced by anticancer agents (17); the gene promoter was also activated in response to both anticancer agents and UV light (18, 19). These results show that MDR1 gene expression is highly susceptible to various environmental stimuli (Table 1) and might therefore be stress responsive (11).

This review focuses on the molecular mechanism of the transcriptional regulation of human MDR1/PGP and the role of YB-1 expression in the acquisition of drug resistance.

Transcriptional Regulation of the Human MDR1 Gene
Many studies have shown the involvement of various cis-acting elements in MDR1 gene expression, suggesting...
pleiotropic mechanisms (10). As shown in Table 1, several transcription factors are expected to play critical roles in the basal expression of the \textit{MDR1} promoter in addition to stimulus-induced activation.

\textbf{Y-Box Binding Protein 1}

Many reports on the factors associated with drug resistance have shown a plausible association of YB-1 with drug resistance both in cultured cancer cells and in numerous clinical human tumor samples.

YB-1 is a member of the cold shock domain (CSD) protein family, which is found in the cytoplasm and nucleus of mammalian cells. It has pleiotropic functions in the regulation of gene transcription and translation, DNA repair, drug resistance, and cellular responses to environmental stimuli (20–22). The structures of YB-1 and two other members of the CSD family, hdbpA (23) and Conthin/hdbpC (24), are presented in Fig. 1A. The YB-1 gene, which is located on chromosome 1p34 (25, 26), contains eight exons spanning 19 kb of genomic DNA (Fig. 1B). The 1.5-kb mRNA encodes a 43-kDa protein comprising three domains: a variable NH\textsubscript{2}-terminal tail domain (A/P domain), a highly conserved nucleic acid binding CSD, and a COOH-terminal tail domain (B/A repeat; refs. 27–29). The A/P domain (amino acids 1–51) seems to be involved in transcriptional regulation, whereas the CSD domain and part of the B/A repeat (amino acids 51–205) function in binding the Y-box (inverted CCAAT box) or double-stranded DNA. Most of the COOH-terminal region of the B/A repeat domain (amino acids 129–324) is thought to bind ssDNA or RNA, and part of this region (amino acids 129–205) is involved in dimerization.

We identified YB-1 as a transcription factor that binds to the inverted CCAAT box of the \textit{MDR1} promoter (30). Decreased expression of YB-1, resulting from the introduction of YB-1 antisense expression constructs into cancer cells, markedly reduced the activation of the \textit{MDR1} gene by DNA-damaging agents (31).

YB-1 is normally present in the cytoplasm, although it is translocated to the nucleus when cells are exposed to anticancer agents, hyperthermia, or UV light irradiation (19, 32, 33). YB-1 is often overexpressed in malignant cells and its expression is regulated by both the proto-oncogene product c-Myc and the tumor suppression gene product p73 (25, 34). The COOH-terminal tail domain seems to play a key role in the localization of YB-1 to either the cytoplasm or the nucleus (32). Studies have shown that cell cycle–specific nuclear translocation is mediated by cooperation of the CSD and COOH-terminal tail domain (35) and that the nuclear translocation of YB-1 requires wild-type p53 (36). The introduction of antisense RNA into human cancer cell lines (37) and the targeted disruption of one Y-box allele in chicken DT40 cells (37) both inhibited growth. By contrast, the targeted disruption of one allele of the YB-1 gene in mouse ES-1 cells had no effect on the growth rate (38).

\textbf{Nuclear Factor Y}

The CCAAT box is among the most ubiquitous DNA elements in both forward and reverse orientation. NF-Y is the major transcription factor recognizing the CCAAT box (39). This heteromeric protein is composed of three subunits, NF-YA, NF-YB, and NF-YC, all of which are necessary for DNA binding (Fig. 1A). Mutation and/or deletion of the CCAAT box have been shown to result in a significant loss of \textit{MDR1} promoter activity (40). It has been reported that both the inverted CCAAT box and the GC box are required for activation of the \textit{MDR1} promoter by UV light, and NF-Y, not YB-1, is thought to be the factor regulating the \textit{MDR1} gene (41). However, these findings are not consistent with the results discussed above. The YB-1 protein is abundant and localized in the cytoplasm; however, when the effect of YB-1 overexpression on \textit{MDR1} promoter activity was evaluated in human cancer KB cells, it was unclear whether the nuclear YB-1 content was increased. As YB-1 is known to repress translation, increased levels of cytoplasmic YB-1 might inhibit the translation of luciferase mRNA. Further studies are required to resolve this issue. Treatment with a histone deacetylase inhibitor (trichostatin) induced a marked increase in the amount of \textit{MDR1} mRNA, although this drug-induced increase was inhibited in dominant-negative NF-Y mutants (42). NF-Y therefore seems to regulate \textit{MDR1} gene expression through an interaction with p300/CBP-associated factor, which shows histone acetylation activity. NF-Y might also be responsible for the sodium butyrate–induced \textit{MDR1} gene up-regulation in colon cancer cells (43). This transcription factor therefore plays a pivotal role in \textit{MDR1} gene expression. Recently, the antitumor agent HMN-I76, which interacts with NF-YB, has been shown to inhibit \textit{MDR1} gene expression and to restore chemosensitivity to MDR cells (44).

\begin{table*}[h]
\centering
\caption{Transcriptional regulation of the \textit{MDR1} gene in human cell lines}
\begin{tabular}{lll}
\hline
Transcription factor & Inducers & References \\
\hline
NF-Y & None & (40) \\
& Sodium butylate & (43) \\
& Trichostatin A & (42) \\
Sp1 & None & (45) \\
YB-1 & UV light & (30, 32) \\
& Anticancer agents & (31) \\
Nuclear factor-interleukin-6 & Phorbol ester & (52, 78) \\
EGR1 & Phorbol ester & (45, 46) \\
HSF1 & Heat shock & (15, 55) \\
20-kDa protein & Serum starvation & (16) \\
& Anticancer agents & (31) \\
Transcription factor & None & (56) \\
Human T-cell lymphotrophic & Virus infection & (79) \\
virus-1 Tax & & \\
SXR & Digoxin & (80) \\
MDR1 promoter-enhancing & None & (59, 60) \\
factor 1/RNA helicase A & & \\
Nuclear factor-kB & Daunomycin & (58) \\
p53 & None & (49–51) \\
\hline
\end{tabular}
\end{table*}

\begin{footnotesize}
\begin{enumerate}
\item K. Kohno and M. Kuwano, unpublished data.
\end{enumerate}
\end{footnotesize}
expression of both manner (45). Treatment with phorbol ester induced the mutually influence MDR1 with the Sp1 binding sites, it is conceivable that they response element 1 (EGR1) binding motif partially overlaps differentiation, and neoplastic changes. As the early growth various cellular functions including proliferation, apoptosis, of many eukaryotic genes. The Sp1 family is involved in SV40 promoter. A GC box is found in the promoter region transcription factor specifically bound to the GC box of the Sp1 (40, 45). Sp1 was first cloned and identified as a decreased its activity as a result of the transcription factor mutation of the oncosuppressor gene WT1 alone did not enhance MDR1 promoter activity. Coexpression of the oncosuppressor gene WT1 resulted in the inhibition of MDR1 promoter activation by EGR1 or phorbol ester (47). Therefore, the direct binding of WT1 to the GC box might compete with Sp1 to down-regulate the MDR1 gene. These findings suggest that interactions between EGR1 and WT1 might play a key role in MDR1 promoter activation.

p53

Mutant p53 has been shown to enhance MDR1 promoter activity in mouse cells; this was reversed by wild-type p53 (14, 48). By contrast, stimulation of the MDR1 promoter by wild-type, but not mutant, p53 was shown in several human p53-null cancer cell lines. The MDR1 promoter region −39 to +53 is responsible for this p53-mediated activation (49), whereas the region −189 to +133 is thought to be responsible for negative regulation by wild-type p53 (50). In addition, p53 has been reported recently to bind directly to a novel binding element (−72 to −40) within the MDR1 core promoter and to repress its promoter activity (51).

Nuclear Factor-Interleukin-6

The treatment of human monocytic cells with phorbol ester enhanced MDR1 promoter activity through interaction with nuclear factor-interleukin-6, which is a CCAAT/enhancer binding protein family member. This study also revealed that the mitogen-activated protein kinase pathway activates nuclear factor-interleukin-6 (52). In addition, CCAAT/enhancer binding protein β has been shown recently to transactivate the MDR1 promoter by interaction with the Y-box (53).

Heat Shock Factor

MDR1 promoter activation in response to arsenate or heat shock seems to be mediated through a heat shock element in the −178 to −165 region. An additional region at −136 to −76 has also been proposed as a critical heat shock element for the heat shock response (15, 54), although no direct binding of heat shock factor to this region has been shown. Recently, Vilaboa et al. (55) reported that infection with adenovirus carrying heat shock transcription factor 1 cDNA increased the levels of MDR1 mRNA and PGP.

Transcription Factor 4/β-Catenin

Transcriptional profiles produced using cDNA microarrays in human colon cancer cell lines identified the MDR1 gene as the target of transcription factor 4/β-catenin. Seven transcription factor 4/β-catenin binding sites were in the promoter region between −2,030 and +31 (56).

Figure 1. A, protein structure and functional domains of hdbpB/YB-1, hdbpA, hdbpC, NF-YA, NF-YB, and NF-YC. A/P, alanine and proline domain, residues 1-82, 1-50, and 1-85 in hdbpA, hdbpB/YB-1, and Contrin/hdbpC, respectively. CSD, residues 83-161, 51-129, and 86-164. B/A repeat, basic and acidic amino acid, residues 162-372, 130-324, and 165-364. The CSD domains of the three genes are highly homologous. Of the three subunits of NF-Y, NF-YB and NF-YC contain histone folding motifs homologous to the yeast transcription factors HAP3 and HAP5, respectively, NF-YA contains a domain homologous to HAP2, which interacts with NF-YB and NF-YC, and the heterotrimer of NF-Y binds to DNA. Both NF-YA and NF-YC contain glutamine-rich domains and activate transcription. B, general structure of the genomic DNA, mRNA, and protein product of YB-1. The gene is mapped on chromosome 1p34 and has eight exons (E1, E2, E3, E4, E5, E6, E7, and E8). The YB-1 protein consists of 324 amino acids. B, basic amino acid clusters; A, acidic amino acid clusters.

Sp1 and Early Growth Response Element 1

The introduction of mutations in the GC-rich region −59 to −45 (G region) of the MDR1 promoter markedly decreased its activity as a result of the transcription factor Sp1 (40, 45). Sp1 was first cloned and identified as a transcription factor specifically bound to the GC box of the SV40 promoter. A GC box is found in the promoter region of many eukaryotic genes. The Sp1 family is involved in various cellular functions including proliferation, apoptosis, differentiation, and neoplastic changes. As the early growth response element 1 (EGR1) binding motif partially overlaps with the Sp1 binding sites, it is conceivable that they mutually influence MDR1 gene expression in a competitive manner (45). Treatment with phorbol ester induced the expression of both EGR1 and MDR1 genes in human leukemia cells (46). However, the expression of EGR1 alone did not enhance MDR1 promoter activity. Coexpression of the oncosuppressor gene WT1 resulted in the inhibition of MDR1 promoter activation by EGR1 or phorbol ester (47). Therefore, the direct binding of WT1 to the GC box might compete with Sp1 to down-regulate the MDR1 gene. These findings suggest that interactions between EGR1 and WT1 might play a key role in MDR1 promoter activation.
Nuclear Factor-κB

The hepatocarcinogen 2-acetylaminofluorene was shown to activate the MDRI gene in human hepatoma cells and the induction of MDRI by 2-acetylaminofluorene was mediated by a nuclear factor-κB binding site located around –6 kb (57). Another group showed that the inhibition of nuclear factor-κB reduced levels of MDRI mRNA and PGP expression and that nuclear factor-κB transactivated the MDRI promoter in human colon cancer HCT15 cells (58). This study identified a nuclear factor-κB binding site in the first intron.

MDR1 Promoter-Enhancing Factor 1/RNA Helicase A

MDRI promoter-enhancing factor 1 has been shown to bind to the CCAAT sequence causing up-regulation of the MDRI gene (59). RNA helicase A has also been reported to bind to the CCAAT box as a member of the MDRI promoter-enhancing factor 1 complex (60). Overexpression of RNA helicase A enhanced the expression of both the MDRI promoter-reporter construct and endogenous PGP.

Clinical Implications of PGP Expression and Nuclear Translocation of YB-1

PGP triggers resistance to a wide range of anticancer agents including Vinca alkaloids, anthracyclines, epipodophyllotoxins, and taxols (7). In addition, YB-1 plays a role in limiting the drug sensitivity of cancer cells by increasing the expression of PGP and other proteins. Immunohistochemical studies of YB-1 expression in the nuclei of untreated primary breast cancers showed an almost complete association between nuclear YB-1 and PGP expression in 9 of 27 cases (Table 2; ref. 61). Studies of clinical specimens have also shown an association between YB-1 and PGP in osteosarcoma (62), synovial sarcoma (63), breast cancer (64, 65), ovarian cancer (66–68), and prostate cancer (Table 2; ref. 69). Figure 2 shows examples of the presence and absence of YB-1 and PGP in clinical samples of osteosarcoma and synovial sarcoma based on the results of immunohistochemical analyses with anti-YB-1 and anti-PGP antibodies.

There was a significant correlation between the nuclear expression of YB-1 and the presence of PGP in 69 cases of osteosarcomas (62). A recent study confirmed that YB-1 expression was specifically associated with the overexpression of PGP rather than with three other ATP binding cassette transporters: MRP1, MRP2, and MRP3 (63). By contrast, no association was observed between YB-1 and PGP expression in colon cancers (70). It remains unclear whether YB-1 is directly involved in the transcriptional regulation of PGP in human malignancies. Nevertheless, measurements of the expression of YB-1 and PGP could suggest treatment modalities for individual cancer patients. Recently, we showed that coexpression of YB-1 and PGP correlated with poor prognosis in epithelial ovarian cancer (67). The expression of MDRI is augmented in cancerous areas in breast cancer and other tumors, resulting in drug resistance. Furthermore, the presence of YB-1 in the nuclei of cancer cells is closely associated with the clinical outcome. YB-1 could therefore be a useful indicator of malignancy as well as a promising target for cancer therapy. We recommend the use of non-PGP-targeting drugs against malignant tumors with nuclear YB-1 expression.

Clinical Implications of Nuclear Localization of YB-1: Drug Resistance to non-PGP-Targeting Drugs

As described above, YB-1 is translocated to the nucleus in response to various environmental stresses including UV light, anticancer agents, heat, and infection in cultures of cancer cells (21). YB-1 was shown to be overexpressed in cisplatin-resistant cell lines, and antisense YB-1 RNA triggered the augmentation of sensitivity to cisplatin, mitomycin C, UV light, and hydrogen peroxide (30, 38). YB-1 associates with p53 (71) and proliferating cell nuclear antigen (72), both of which modulate DNA repair, cell cycle, transcription, and drug sensitivity. Moreover, wild-type p53 is required for the nuclear translocation of YB-1, which in turn inhibits p53-induced cell death (36). However, it remains unclear how reduced YB-1 expression increases resistance to non-PGP-targeting DNA-damaging agents such as cisplatin and mitomycin C. Potential mechanisms might include a reduction in the YB-1 interaction with proliferating cell nuclear antigen, which is necessary for nucleotide excision repair, or in the interaction with p53. However, pleiotropic drug resistance to DNA-interacting drugs (e.g., aphidicolin, hydroxyurea, cytarabine, etoposide, doxorubicin, and mafosfamide) is associated with the increased expression of YB-1 and 19 other genes that are involved in DNA replication, repair, and stress responses (73).

YB-1 could therefore be a useful indicator of malignancy as well as a promising target for cancer therapy. We recommend the use of non-PGP-targeting drugs against malignant tumors with nuclear YB-1 expression.

Table 2. The association of nuclear expression of YB-1 with PGP-mediated and/or non-PGP-mediated drug resistance in human malignancies

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Malignant characteristics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovarian cancer</td>
<td>PGP*</td>
<td>(66)</td>
</tr>
<tr>
<td></td>
<td>PGP*</td>
<td>(67)</td>
</tr>
<tr>
<td></td>
<td>Cisplatin resistance</td>
<td>(68)</td>
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<tr>
<td>Breast cancer</td>
<td>PGP*</td>
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<td>PGP*</td>
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<td></td>
<td>Drug resistance</td>
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<tr>
<td>Osteosarcoma</td>
<td>PGP*</td>
<td>(62)</td>
</tr>
<tr>
<td>Synovial sarcoma</td>
<td>PGP*</td>
<td>(63)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>PGP*</td>
<td>(69)</td>
</tr>
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</table>

*These studies also reported a significant correlation between nuclear YB-1 expression and disease progression or prognosis.
adenocarcinomas, and 1 of 4 mucinous adenocarcinomas (Table 2). There was also a positive correlation between the nuclear expression of YB-1 and poor prognosis in synovial sarcoma (63).

Analysis of the clinical relevance of YB-1 expression in the cytoplasm or nucleus in 83 cases of breast cancer, after a median follow-up of 61 months, revealed that the 5-year relapse rate was 66% in patients with high YB-1 expression who received postoperative chemotherapy (65). By contrast, none of the patients with low YB-1 expression experienced relapse. Taken together, these findings indicate that the overexpression and nuclear expression of YB-1 have a predictive value in some human malignancies, both with and without postoperative chemotherapy.

An investigation of 588 genes associated with mouse lung tumor progression revealed that 19 were differentially expressed between lung adenoma and adenocarcinoma; YB-1 was one of these candidate lung tumor progression genes (74). Overexpression of YB-1 was observed in >90% of anaplastic thyroid carcinomas, whereas it was absent in normal follicles and other pathologic tumor types. These findings suggested the involvement of YB-1 in the anaplastic transformation of thyroid carcinoma (75). YB-1 expression induced a strong cellular resistance to malignant transformation through the phosphatidylinositol 3-kinase pathway possibly through the inhibition of protein synthesis that is required for the phosphatidylinositol 3-kinase– or Akt-induced oncogenic transformation (76).

**Conclusion**

The ancestral protein YB-1 modulates cell growth, apoptosis, drug resistance, DNA repair, transcription, and translation as a pleiotropic regulator. YB-1 overexpression or nuclear YB-1 expression might play a key role not only in the acquisition of PGP-mediated drug resistance but also in sensitivity to non-PGP-targeting chemotherapeutic agents. YB-1 in the nucleus modulates drug resistance to PGP-targeting and non-PGP-targeting drugs in cancer cells.
that are exposed to anticancer and other cytotoxic DNA-damaging agents (Fig. 3). In one response pathway to environmental stimuli, YB-1 is translocated to the nucleus and up-regulates MDR1 gene expression through binding to the Y-box on the promoter. Alternatively, YB-1 might operate its DNA repair pathway through interactions with p53 (71), proliferating cell nuclear antigen (72), and other molecules (77) when DNA is damaged (Fig. 3). Further research is needed to fully understand the role of YB-1 in cancer and drug resistance.

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References


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