Hyperthermia-induced Nuclear Translocation of Transcription Factor YB-1 Leads to Enhanced Expression of Multidrug Resistance-related ABC Transporters*

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Genotoxic stress leads to nuclear translocation of the Y-box transcription factor YB-1 and enhanced expression of the multidrug resistance gene MDR1. Because hyperthermia is used for the treatment of colon cancer in combination with chemoradiotherapy, we investigated the influence of hyperthermia on YB-1 activity and the expression of multidrug resistance-related genes. Here we report that hyperthermia causes YB-1 translocation from the cytoplasm into the nucleus of human colon carcinoma cells HCT15 and HCT116. Nuclear translocation of YB-1 was associated with increased MDR1 and MRP1 gene activity, which is reflected in strong efflux pump activity. However, a combination of hyperthermia and drug treatment effectively reduced cell survival of the HCT15 and HCT116 cells. These results demonstrate that activation of MDR1 and MRP1 gene expression and increased efflux pump activity after hyperthermia were insufficient to cause an increase in drug resistance in colon cancer cell lines. The ability of hyperthermia to abrogate drug resistance in the presence of an increase in functional MDR proteins may provide an explanation for the efficacious results seen in the clinic in colon cancer patients treated with a combination of hyperthermia and chemotherapy.

Multidrug resistance (MDR) of human malignant tumors represents a major cause of cancer chemotherapy failure. The development of the MDR phenotype is often associated with increased expression of certain ATP-binding cassette transporters (ABC transporters) such as P-glycoprotein and MRP1. The multidrug resistance gene (MDR1) encodes P-glycoprotein, which causes classical MDR. In contrast, the MRP1 gene encodes the multidrug resistance-related protein (MRP1), which is associated with an atypical non-P-glycoprotein-dependent MDR phenotype (1). Although both transporter proteins function as drug-efflux pumps, they share only 15% amino acid homology, transport a non-identical spectrum of anticancer substrates, and show a different distribution in human normal and cancer tissues (2, 3).

Colorectal tumors are one of the most prevalent human malignancies and are highly drug resistant because of an MDR phenotype. Intrinsic expression of the ABC transporters P-glycoprotein and MRP1 in colon cancer has been reported (4–6). To improve the response of colon cancer to chemotherapy, other treatment modalities such as radiation and hyperthermia when used in combination with chemotherapy may be able to increase the efficacy of cancer chemotherapy (7). However, environmental stresses such as drugs, radiation, and hyperthermia harbor the potential to induce or to enhance the MDR phenotype. Induction of MDR1 gene expression by exposure to drugs (8–10) or radiation (11) was described earlier. In contrast, the influence of hyperthermia on the expression of MDR genes has not been clearly established. Two groups reported a heat-induced elevation of MDR1 gene expression, either observed following a single treatment or after repeated treatments with heat (12, 13). Induction of MRP1 expression by drugs/chemicals has also been reported (3); however, the effects of radiation and hyperthermia on MRP1 gene expression have not been explored.

Signal transduction pathways that respond to external stimuli have been extensively investigated within drug-resistant cells. Involvement of YB-1 transcription factor (14) in mediating the effects of different external stimuli has been described for a variety of chemicals and drugs such as cisplatin, mitomycin C, etoposide and also for UV light (15–17). YB-1 belongs to the family of Y-box transcription factors, which were identified by their interaction with inverted CCAAT-boxes (Y-box; 14). Translocation of YB-1 from the cytoplasm into the nucleus was observed after treatment of cell cultures with DNA-damaging agents (15) as well as by UV irradiation (17). Nuclear expression of YB-1 has been correlated with intrinsic MDR1 expression for breast carcinomas (18) and for osteosarcomas (19). For colorectal carcinomas, an enhanced coexpression of YB-1 and topoisoamerase IIs have recently been described when compared with mucosa (20).

In the present study, we examined the subcellular distribution of YB-1 in colon carcinoma cells before and after treatment with hyperthermia. Our results show that hyperthermia causes nuclear accumulation of YB-1 and concomitant increase...
in MDR1 and MRP1 gene expression resulting in efflux pump activity. We further show that the increase in MDR1 and MRP1 gene expression and efflux pump activity after hyperthermia were unable to reduce the amount of drug in the cells to prehyperthermia levels. Even though treatment with hyperthermia resulted in an increase in MDR1 and MRP1 expression and function in HCT116 and HCT15 cells, paradoxically an enhancement of drug resistance was not observed. These observations are in accord with the clinical experience that demonstrates hyperthermia in combination with chemotherapy is an effective treatment for colon cancer.

**EXPERIMENTAL PROCEDURES**

**Cell Lines and Hyperthermia**—The human colorectal carcinoma cell lines HCT116 and HCT15, which possess different sensitivities to MDR-related drugs (highly resistant HCT15, moderately resistant HCT116; Ref. 21), were cultured as described previously (22). Hyperthermia was performed at 40 °C and 43 °C for 15, 30, 60, and 120 min. The human breast epithelial cell line HBL-100 was cultured in RPMI 1640 supplemented with 10% fetal calf serum and l-glutamine (200 

**Immunofluorescence Analysis**—Cells were grown on slides, fixed with acetone/methanol, and preincubated with phosphate-buffered saline containing 1.5% horse serum (30 min; Vector Laboratories, Burlingame, CA). Cells were incubated with the polyclonal anti-YB-1 antibody (1:200, 30 min; Ref. 18) and with an anti-rabbit IgG-fluorescein F(ab')2 fragment (1:200, 30 min; Roche Diagnostics, Mannheim, Germany). Specificity of the polyclonal anti-YB-1 antibody was proved by peptide competition assay and by Western blot using whole cell lysates. For staining of nuclei, 4,6-diamidino-2-phenylindole (DAPI; Roth, Karlsruhe, Germany) was added in the last incubation step. Staining was evaluated using a fluorescence microscope (Leica, Bensheim, Germany).

**Confocal Laser Scanning Microscopy**—The cells were double-labeled with the polyclonal antibodies against YB-1 (1:200) and monoclonal antibodies against lamin A/C (1:50; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) for 60 min and were incubated with an anti-rabbit IgG-fluorescein F(ab')2 fragment (1:200, 30 min) and an anti-mouse IgG-rhodamine F(ab')2 fragment (1:100; Roche) for 30 min. Analysis of YB-1 subcellular distribution was performed by confocal laser scanning microscopy using the LSM 410 (Zeiss, Jena, Germany; software version 3.80) at 1000-fold magnification.

**Electrophoretic Mobility Shift Assay (EMSA)**—Nuclear extracts were prepared as described (23). Briefly, 5.0 μg of each nuclear extract was incubated for 30 min at 30 °C with the 32P-endabeled single or double-stranded oligonucleotide. The following oligonucleotides were used: 5′-TGAGGCCTGTCTGCTGCA-3′, derived from the human MDR1 promoter sequence (24) to −83 from −67 X-box; 5′-CGCTCTGCAGTTGAC-3′, derived from the human HLA-DRα promoter (GenBank™ accession number M23101, nucleotides 31–50); and the nonspecific competitor oligonucleotide 5′-CCCTGCTACTTGCCGCCG-3′ derived from the human cyclin E promoter (GenBank™ accession number L48996, nucleotides 987–1006). The DNA-protein complexes were resolved on native 4% polyacrylamide gel electrophoresis. For autoradiography, X-Omat AR film (Kodak) was exposed overnight.

**Generation of HBL-100 Transfectants with Stable Expression of a V5-tagged YB-1 cDNA**—YB-1 cDNA (25, 26) was removed from pUC19 using the EcoRI restriction endonuclease and inserted into the EcoRI site of the vector pcDNA6 (Invitrogen) thereby ensuring infrase positioning with the V5-histidine tag of the pcDNA6 plasmid (pcDNA6/YB-1). Transfection of HBL-100 cells with pcDNA6/YB-1 were grown with blasticidine (Invitrogen, Groningen; 5 μg/ml) and incubated for 1640 supplemented with 10% fetal calf serum and L-glutamine (200 

**RNA Isolation and Real Time RT-PCR**—HCT116 and HCT15 cells were harvested prior to 0, 1, 2, 3, and 4 h posthyperthermia (43 °C for 2 h). The nuclei were isolated as described above (25). Total RNA was performed with the high pure RNA isolation kit (Roche Molecular Biochemicals) including a DNase incubation step. RNA concentrations were measured by using the RiboGreen RNA quantitation kit (Molecular Probes) on a BioRad model M4700 (BioRad, München, Germany). The amount of CAT protein was normalized to the protein content of the respective lystate.

**Microscopy**—Cells were grown in culture dishes, fixed with acetone/methanol, and preincubated with phosphate-buffered saline containing 1.5% horse serum (30 min; Vector Laboratories, Burlingame, CA). Cells were incubated with the polyclonal anti-YB-1 antibody (1:200, 30 min; Ref. 18) and with an anti-rabbit IgG-fluorescein F(ab')2 fragment (1:200, 30 min; Roche Diagnostics, Mannheim, Germany). Specificity of the polyclonal anti-YB-1 antibody was proved by peptide competition assay and by Western blot using whole cell lysates. For staining of nuclei, 4,6-diamidino-2-phenylindole (DAPI; Roth, Karlsruhe, Germany) was added in the last incubation step. Staining was evaluated using a fluorescence microscope (Leica, Bensheim, Germany).

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from the 4 °C values and the 37 °C values.

Cytotoxicity Assay—For the MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, Sigma, Deisenhofen, Germany, 5 mg/ml) colorimetric cytotoxicity assay, $8 \times 10^3$ cells were plated into each well of 96-well microtiter plates and grown for 24 h. Hyperthermia was performed at 40 °C or 43 °C for 2 h, and cells were kept at 37 °C. After 3 days, cells were treated with adriamycin (500, 1000, 1500, 2000 ng/ml; Amersham Pharmacia Biotech, Freiburg, Germany) for another 3 days. Non-treated cells, hyperthermia-treated cells, and drug-treated cells served as controls.

Statistical Analysis—For flow cytometry studies (immunoflow cytometry, adriamycin, and rhodamine assays) levels of statistical signif-
Hyperthermia-induced YB-1 Translocation and MDR

RESULTS

Hyperthermia-induced Translocation of YB-1 Transcription Factor—The localization of YB-1 was investigated in non-stressed and hyperthermia-treated (40 °C or 43 °C for 15, 30, 60, or 120 min, respectively) HCT116 and HCT15 cells. By means of immunofluorescence microscopy, YB-1 was detectable in the cytoplasm of the HCT116 (Fig. 1A) and HCT15 cells (Fig. 1E) under stress-free conditions. YB-1 was also observed in the nuclei of untreated HCT15 cells, which corresponded to the higher intrinsic drug resistance and to the higher intrinsic expression of MDR1 and MRP1 in the HCT15 cells compared with the HCT116 cells. Incubation of both cell lines at 43 °C led to an accumulation of YB-1 in the nuclei in a time-dependent manner with highest nuclear YB-1 accumulation observed immediately after the 2-h hyperthermia treatment (Fig. 1, C and G). Treatment of cells at 40 °C also resulted in a nuclear accumulation of YB-1 but at reduced levels than observed at 43 °C (data not shown). Translocation of YB-1 from the cytoplasm into the nucleus was more pronounced in HCT116 than in HCT15 cells (Fig. 1, C and G), which might indicate that hyperthermia-induced effects were cell type-specific and dependent on the intrinsic resistance status.

Using confocal laser scanning microscopy, YB-1 was mainly detectable in the cytoplasm of non-stressed HCT15 and HCT116 cells and additionally to a low extent within the nuclei of HCT15 cells, which confirmed the observations made with immunofluorescence (Fig. 1, I and J). Highest rates of hyperthermia-induced YB-1 translocation were observed immediately posthyperthermia in both cell lines (Fig. 1, K and L). These results were verified by Western blot using cytoplasmic and nuclear fractions of non-treated and hyperthermia-treated cells. Furthermore, a block of de novo protein synthesis using cycloheximide prior to hyperthermia did not influence the heat-induced nuclear accumulation of YB-1 (data not shown). Based on these observations, we concluded that YB-1 will be activated under hyperthermic conditions resulting in a translocation of YB-1 into the nucleus.

Hyperthermia-induced Binding of YB-1 to MDR1 Promoter Sequences—To examine whether nuclear translocation of YB-1 resulted in elevated binding activity to the human MDR1 promoter, nuclear extracts of non-treated and hyperthermia-treated HCT116 and HCT15 cells were analyzed by EMSA. Because it is known that YB-1 binds preferentially to single-stranded oligonucleotides (14), a radiolabeled single-stranded oligonucleotide containing the Y-box region from the MDR1 promoter sequence −86 to −67 was employed (Fig. 2, lanes 1–10). Retarded DNA-protein complexes were observed in the parental and in the hyperthermia-treated cells of both lines (lanes 1–4). To ensure that YB-1 interacts with the MDR1 promoter, supershift assays were performed. The preincubation of the nuclear extracts with increasing amounts of anti-YB-1 antibodies abolished protein-DNA binding, demonstrating that YB-1 is an integral part of this complex (data not shown).

In general, the signals for the YB-1-specific major DNA-protein complex were found to be higher in the non-treated and in the hyperthermia-treated HCT15 cells (lanes 3, 4) compared with the HCT116 cells (lanes 1, 2). The higher levels of YB-1 specific major DNA-protein complex in the HCT15 cells correspond to the higher intrinsic expression of MDR1 in the HCT15 cells. In both cell lines, hyperthermia resulted in an increase of the YB-1-specific DNA-protein complex (lanes 2, 4) demonstrating that translocation of YB-1 into the nucleus led to an increased binding to the MDR1 gene promoter.

Sequence specificity of binding to the MDR1 promoter Y-box was demonstrated by competition experiments. Competitor oligonucleotides containing the Y-box region of the MDR1 promoter prevented formation of a retarded YB-1-DNA complex (lanes 5 and 6). Identical results were obtained with a Y-box from the HLA-DRα promoter (lanes 7 and 8). However, a control oligonucleotide from the cyclin E promoter did not affect YB-1 complexes (lanes 9 and 10).

Because the transcription factor NF-Y has similar binding sites as YB-1, the role of NF-Y was investigated under hyperthermic conditions. A radiolabeled double-stranded oligonucleotide was employed, which also originated from the Y-box region of the MDR1 promoter sequence (same sequence as the single-stranded oligo; Fig. 2, lanes 11–15). The figure shows that NF-Y activity was not affected by hyperthermia because...
no differences were found between either non-treated cell lines (lanes 12 and 14) or between the non-treated and hyperthermia-treated cells (lanes 12–15). Based on these observations, it is unlikely that NF-Y is involved in the hyperthermia-caused modulation of MDR1 expression in the analyzed cell lines.

**MDR1 and MRP1 Promoter Activity in YB-1-transfected Cells**—Because translocation, nuclear accumulation, and binding of YB-1 to MDR1 promoter sequences were observed to be hyperthermia inducible, the effect of YB-1 on MDR1 and MRP1 promoter-driven CAT expression was examined in a breast epithelial cell line, which was engineered to overexpress YB-1 (HBL-100/YB-1). To investigate the function of YB-1 in MDR1 and MRP1 gene control, parental and YB-1-overexpressing HBL-100 cells were transfected with the MDR1 promoter-harboring CAT construct pCAT-MDR and with CAT constructs containing MRP1 promoter variants, pCAT-MRP/A, pCAT-MRP/I, and pCAT-MRP/J.

Transfection with pCAT-MDR led to a 30-fold increase of CAT protein expression in the parental and in the YB-1-overexpressing HBL-100 cells, when compared with the pCAT-Control-transfected parental or YB-1-overexpressing HBL-100 cells, respectively (Fig. 3). However, the MDR1 promoter-driven CAT protein expression was almost 3-fold higher in the YB-1-overexpressing cells when compared with the parental HBL-100 cells. Thus, MDR1 promoter-driven CAT expression is directly dependent on the amount of available YB-1 within the cells.

Transfection with the MRP1 promoter-harboring constructs also resulted in an elevated CAT expression in YB-1-overexpressing HBL-100 cells compared with the parental cells (about 6-fold), which was rather similar for all three sublines transfected with the MRP1 promoter CAT constructs pCAT-MRP/A, -I, and -J. However, the amount of CAT protein in HBL-100/YB-1 cells was much lower in the MRP promoter transfectants (about one-tenth) compared with those of the pCAT-MDR transfectants.

The data obtained with the CAT reporter constructs indicate that both MDR1- and MRP1-driven CAT expression is dependent on the availability of YB-1, albeit to a somewhat different extent. Because the Y-box was identified within the MDR1 promoter but not within the MRP1 promoter, the MRP1 promoter may harbor additional sequences that bind YB-1. To prove this assumption, we used three overlapping single-stranded oligonucleotides representing the MRP1 promoter fragment J as competitors in EMSA with a Y-box from the MDR1 promoter. The common feature of these oligonucleotides is a GC content higher than 80%. Each of these MRP1 promoter oligonucleotides prevented formation of YB-1-containing retarded complexes, indicating that YB-1 specifically binds to the MRP1 promoter (data not shown). Thus, YB-1 is involved in regulating expression of both MDR1 and MRP1 genes.

**Expression of ABC Transporters MDR1/P-glycoprotein and MRP1—Hyperthermia-induced effects on the expression of MDR1 mRNA/P-glycoprotein and MRP1 mRNA/MPR1 were**
evaluated by real time RT-PCR and immunoflow cytometry. At the RNA level, hyperthermia-induced (43 °C for 2 h) increases in expression of the MDR1 and MRP1 genes have been observed in both colon carcinoma cell lines (Fig. 4). MDR1 mRNA expression increased by 4.5-fold (Fig. 4A), and MRP1 mRNA expression increased by 2-fold (Fig. 4B). Transcript levels of both genes were strongly increased immediately or 1 h posthyperthermia.

Significant induction of P-glycoprotein was observed for HCT116 and HCT15 cells after treatment with hyperthermia at 43 °C for 2 h (Fig. 4, C and E). In HCT116 cells, P-glycoprotein expression was induced up to 5.5-fold when measured with MRK16 (p = 0.00216) and up to 9.5-fold when detected with C219 (p = 0.000583). In HCT15 cells, expression of P-glycoprotein was enhanced about 1.5-fold when determined with MRK16, or 3-fold when measured with C219 (p = 0.0286).

MRP1 expression was also found to be inducible by hyperthermia with the highest induction rates after treatment at 43 °C for 2 h (Fig. 4, D and F). In HCT116 cells, MRP1 expression was 6.5-fold (p = 0.00216) or 4.5-fold (p = 0.00216) increased when determined with MRPr1 or MRPm6, respectively. A 2.5-fold (MRPr1, p = 0.0286) or 1.7-fold (MRPm6, p = 0.0571) elevation of MRP1 was detected in HCT15 cells.

When hyperthermia was carried out at 40 °C or for shorter times at 43 °C, either little or no enhancement of P-glycoprotein and MRP1 expression was observed (data not shown). Thus, the hyperthermia-induced increase in ABC transporter expression occurred in a temperature- and time-dependent manner.

**Functional Activity of ABC Transporters—**To evaluate whether the hyperthermia-induced expression levels of P-glycoprotein and MRP1 correspond with an increase in efflux pump function, adriamycin (Fig. 5) and rhodamine (Fig. 5, A and C) were performed. Unexpectedly, accumulation of adriamycin was elevated within both cell lines at 120 h posthyperthermia (Fig. 5A): 1.4-fold in HCT116 (p = 0.0571) and 1.7-fold in HCT15 (p = 0.0286) when compared with the respective parental cell line, although an enhanced hyperthermia-induced expression of both ABC transporters was shown at these time points.

To further analyze whether drug accumulation and efflux is increased under hyperthermic conditions, the rhodamine assay (15-min incubation in rhodamine, 1-h incubation in rhodamine-free medium) was performed. Although the overall accumulation of rhodamine was increased in the hyperthermia-treated cells, the net efflux was also increased in both cell lines. In HCT116 cells, a 4.5-fold elevation (p = 0.0794) and in HCT15 cells, a 2.3-fold increase (p = 0.0286) in the net efflux was determined, reflecting the functional activity of the hyperthermia-induced P-glycoprotein. However, hyperthermia-induced P-glycoprotein expression was apparently not sufficient to prevent drug accumulation in HCT15 and HCT116 colon carcinoma cells.

**Chemosensitivity of Hyperthermia-treated Cells—**Next, chemosensitivity toward the MDR-associated drug adriamycin was analyzed in untreated and hyperthermia-treated HCT15 and HCT116 cells 3 days posthyperthermia (43 °C for 2 h; Fig. 6, A and B). Hyperthermia by itself did not significantly affect cell survival of HCT15 cells and led to 20% reduced cell survival of HCT116 cells. However, a dose-dependent decrease in cell survival was observed in the presence of adriamycin; for HCT15 within the concentration range of 1000–2000 ng/ml and for HCT116 within 500 and 2000 ng/ml. After treatment with 2000 ng/ml adriamycin, survival was 68% for HCT15 and 52% for HCT116 cells when compared with untreated cells. These values reflect the intrinsic resistance of the highly multidrug-resistant HCT15 cells and of the moderately multidrug-resistant HCT116 cells. Cell survival decreased significantly to 16% in HCT 15 cells when treated with the combination of hyperthermia and adriamycin when compared with adriamycin alone (p = 0.0007). The same observation was made for HCT116 cells by comparison of hyperthermia- and drug-treated cells versus exclusively drug-treated cells, showing a
Hyperthermia-treated human colon carcinoma cells. B and the MDR-associated drug adriamycin of the HCT15 (A), and drug-treated cells (without hyperthermia) with respect to non-treated cells (100%) was calculated survival of hyperthermia- and drug-treated cells (ng/ml) for another 72 h. The MTT assay was carried out, and cell with adriamycin at different concentrations (500, 1000, 1500, 2000 (43 °C for 2 h) cells were kept at 37 °C. After 72 h, cells were treated combination of both reached the ID/IC50 within the same drug alone (concentration range up to 2000 ng/ml), whereas the treatment of either hyperthermia (43 °C for 2 h) or adriamycin (inhibitory dose, with respect to hyperthermia) or the IC 50 decrease in cell survival by 35% (5). Thus, the ID50 (inhibitory dose, with respect to hyperthermia) or the IC50 (with respect to drug treatment) was not reached with the sole treatment of either hyperthermia (43 °C for 2 h) or adriamycin alone (concentration range up to 2000 ng/ml), whereas the combination of both reached the ID/IC50 within the same drug concentration range for both cell lines. Therefore, pretreatment with hyperthermia did significantly increase the cytotoxic effects of adriamycin in both colon carcinoma cell lines, confirming the data obtained with the functional assays. By contrast, mild hyperthermia (40 °C for 2 h) did not lead to any significant modulation of cell survival when used alone (compared with untreated cells) or in combination with adriamycin (compared with adriamycin-treated cells; data not shown).

**DISCUSSION**

Hyperthermia-induced translocation of the transcription factor YB-1 from the cytoplasm into the nucleus was observed in HCT15 and HCT116 cells and was found to be time- and temperature-dependent with highest translocation rates observed at 43 °C compared with 40 °C. The mechanism of YB-1 translocation as a reaction to external stress stimuli has also been reported after treatment of cell cultures with DNA-damaging agents (15) or UV irradiation (17). Thus, translocation caused by environmental stress factors is a general activation mechanism of YB-1. The activities of a variety of other transcription factors such as NF-κB and STAT1 are also regulated by intracellular redistribution. Moreover, the nuclear translocation of DNA-binding proteins as a response to hyperthermia has also been reported for the heat shock transcription factors HSF1 and HSF3 (31).

It was previously shown that YB-1 is involved in the regulation of P-glycoprotein expression in human breast carcinomas and osteosarcomas (18, 19). The promoter of the MDR1 gene contains a Y-box, which is responsible for basal MDR1 expression (24). In this study, the direct hyperthermia-induced binding of YB-1 to the Y-box of the MDR1 gene promoter was demonstrated in HCT15 and HCT116 cells. Previous reports discussed the DNA double strand-binding protein NF-Y as a potential regulator of MDR1 expression acting via the CCAAT-box (32). NF-Y was also shown to be involved in mediating effects of several stimuli such as sodium butyrate or UV irradiation (33, 34). In this study, however, hyperthermia did not alter the binding of NF-Y to the Y-box of the MDR1 promoter, suggesting that NF-Y is not involved in hyperthermia-regulated transcriptional control of the MDR1 gene.

The crucial role of YB-1 in MDR1 regulation was demonstrated by using stably YB-1-transfected HBL-100 cells. In HBL-100/YB-1 cells MDR1 promoter-driven CAT gene expression was YB-1-dependent. However, YB-1 overexpression resulted in enhanced MRP1 promoter-driven CAT expression levels as well, although no Y-box motif was identified in the human MRP1 promoter (27). This suggested that YB-1 interacts with unknown MRP1 promoter elements. EMSA revealed that YB-1 binds to oligonucleotides derived from the MRP1 promoter fragment J. These oligonucleotides are all very GC-rich (about 80%). The interaction of recombinant YB-1 with similar GC-rich oligonucleotides was described recently (35). Therefore we conclude that YB-1 may interact with the MRP1 promoter directly and that the hyperthermia-induced translocation of YB-1 into the nucleus may result in an YB-1-dependent activation of the MRP1 promoter.

Our data show that after hyperthermia, P-glycoprotein and MRP1 protein levels were strongly up-regulated. It has been previously reported that heat shock activates MDR1 gene expression (12, 13). Our data provide a mechanism that shows how YB-1 links hyperthermia to gene activation. Furthermore, this is the first report to show that YB-1 is involved in regulating transcription of the MRP1 gene. Although several cis elements have been identified in the promoter of the MDR1 gene, which respond to environmental stress (36–38), no such elements were detected in the MRP1 promoter (27). Thus, the observed YB-1 interaction with the MRP1 promoter provides a potential mechanism how environmental stress leads to MRP1 gene activation.

Increased transcription of MDR1 and MRP1 in response to hyperthermia was associated with elevated levels of the corresponding proteins and strongly increased efflux pump activity. Despite this result, we observed accumulation of anticancer drugs in hyperthermia-treated colon cancer cells. In this case, however, increased pump activity did not lead to an enhanced MDR phenotype which has been described for chemoresistance mechanisms (39).

Furthermore, mechanisms other than the induction of the...
ABC transporters might possibly be involved in the development/regulation of the drug resistance; e.g., the hyperthermia-induced phosphorylation of P-glycoprotein (40) or heat-dependent factors regulating the membrane topology of a P-glycoprotein sequence (41). In addition, other protection mechanisms against environmental stress may be present in the colon carcinoma cells (6, 42). Moreover, general effects of thermal stress such as the induction of epithelial permeability and induction of apoptosis (43) may also affect survival.

In summary, we demonstrate here that hyperthermia induces YB-1 translocation from the cytoplasm into the nucleus leading to an enhanced YB-1 binding to the MDR1 promoter sequence, followed by an increase in the expression levels of MDR1/P-glycoprotein and MRPI/MRP1 expression. However, the hyperthermia-induced increases in these drug resistance genes and functional proteins did not result in drug resistance following treatment with adriamycin and hyperthermia. In contrast, combination of hyperthermia and drug treatment resulted in a significant reduction in cell survival of both colon carcinoma cell lines. Further studies using patient samples are required to evaluate the effect of hyperthermia and chemotherapy on MDR genes and proteins in a clinically relevant situation to assess the risk of inducing MDR proteins, which may potentially complicate subsequent chemotherapy (44). Moreover, analyses of patient samples would provide information necessary for determining the appropriate regimen of chemotherapy with respect to potential hyperthermia-induced resistance gene expression.

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