Increased Survival after Treatments with Anticancer Agents of Chinese Hamster Cells Expressing the Human M, 27,000 Heat Shock Protein

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ABSTRACT

A family of 10 thermoresistant cell lines cloned from Chinese hamster cells transfected with a plasmid containing the structural gene for the small human M, 27,000 heat shock protein (HSP27) was used to assess the putative role of this heat shock protein in chemoresistance. These cells express varying amounts of human HSP27 in addition to the normal level of endogenous hamster HSP27. As previously observed in the case of thermoresistance, a significant positive linear correlation (P < 0.05) was found between cell survival in response to doxorubicin and the total amount of HSP27 expressed. Some clones were also examined for resistance to other drugs and chemicals. A statistically significant increase in survival relative to the parental cells was observed following treatment with daunorubicin (three clones studied), colchicine, vincristine, actinomycin D, hydrogen peroxide, and sodium arsenite (one clone studied). However, the clone which expressed the highest level of HSP27 was as sensitive as control cells to the cytotoxic action of bis-chloronitrosourea and 5-fluorouracil. The relationship between HSP27 overexpression and increased resistance to cytotoxic agents was also evaluated in three independent pooled cell populations stably transformed with both the human HSP27 and the xanthine-guanine phosphoribosyltransferase gene and selected on the basis of resistance to mycophenolic acid and aminopterin. The results indicated that these cells survived significantly better than the control cells transfected with the marker gene only when exposed to doxorubicin. HSP27-mediated cellular protection was not associated either with decreased drug accumulation or with overexpression of P-glycoprotein. It is suggested that HSP27 might be involved in some form of chemoresistance and could participate in the development of clinical resistance to antineoplastic drugs.

INTRODUCTION

The development of drug resistance is one of the major problems found in cancer chemotherapy. Two different but frequently associated forms of resistance characterize the pharmacological treatment of cancer. The first is due to alterations in the pharmacokinetic properties of anticancer drug in treated patients, e.g., increased inactivation or excretion of drugs by liver or kidneys. The second is cellular resistance, which can be innate or acquired. Innate resistance identifies cells or tumors which are already resistant to chemotherapy at the time of their malignant transformation. The acquired cellular resistance is characterized by a state of decreased responsiveness or lack of responsiveness of the cells or tumors to the drug administered. In many instances, such as resistance to antimitabolites, acquired drug resistance is associated with changes in the level or activities of metabolic or target enzymes (1, 2). In general, these mechanisms of drug resistance are not effective against agents from different classes of drugs. In other instances, the acquired resistance is characterized by a state of cross-resistance to a number of structurally unrelated antineoplastic drugs (3, 4). This is called pleiotropic drug resistance or multiple drug resistance.

Various biochemical and cytogenetic alterations have been associated with the multidrug resistance phenotype. The most frequently reported alteration is the overexpression of a plasma membrane glycoprotein of M, 170,000 designated Pgp (5-8). Overexpression of Pgp has been observed in cell cultures made resistant to antineoplastic agents (9, 10) and also in cells obtained from patients who were clinically resistant to chemotherapy (11-13). Pgp increases the efflux of drugs out of the cells, thus decreasing their accumulation and the amount available to react with their cellular targets (14). Multiple drug resistance mechanisms unrelated to the overexpression of Pgp ("atypical" multiple drug resistance) have also been described. A surface membrane protein distinct from Pgp but which would also contribute to decreased cellular accumulation of drugs by resistant cells has been reported (15). A multidrug-resistant phenotype may also be acquired by genetic modifications that alter the concentration of topoisomerase II or its ability to form the cleavable complex in the presence of drugs. Such alterations have been suggested for etoposide-resistant CHO cells that were cross-resistant to amsacrine, mitoxantrone, and DOX (16), for P-388 cells resistant to amsacrine (17), and for 9-hydroxyellipticine-resistant Chinese hamster lung cells which are cross-resistant to putative topoisomerase II inhibitors (18, 19). Alterations in glutathione redox cycle may also be associated with some forms of multidrug resistance (20). It has recently been reported that transfection of the c-myc gene into NIH-3T3 but not into MCF-7 cells can confer resistance to DOX (21, 22). Other mechanisms involved in multidrug resistance are described in recent reviews (2, 3, 23, 24).

Heat shock and several cytotoxic chemicals have the property of inducing in cells the synthesis and accumulation of a small set of proteins known as heat shock proteins. Induction of HSP generally correlates with the development of thermotolerance and also a transient state of cross-tolerance to several cytotoxic agents (25-29). Furthermore, it has recently been reported that heat shock and arsenite produced cross-resistance to vinblastine and also increased the expression of a Pgp gene in a human renal carcinoma cell line (30). Some of the HSP inducers were also found to induce resistance to commonly used therapeutic drugs such as DOX and VM-26 (28, 31), and conversely, drugs such as VM-26 and bleomycin were found to induce the transcription of certain heat shock genes (31-33). Furthermore, several Ca++ active agents and inhibitors of protein glycosylation induce a transient state of resistance to DOX, epipodophyllotoxins, actinomycin D, and vincristine. Intriguingly, all of these agents also induce synthesis and accumulation of a HSP-related family of proteins known as glucose-regulated proteins and the phosphorylation of a M, 27,000 HSP (HSP27).
cultures. The human HSP27 protein expressed in the transfected cells was obtained and found to express constitutively varying amounts of the human HSP27 protein. In these cells, a positive correlation was found between the amount of total HSP27 protein expressed and the level of thermoresistance. The availability of these cell lines provided a unique opportunity to directly test the hypothesis that HSP27 might also participate in cell resistance to anticancer agents. We report here that the HSP27-transfected cells are protected against the acute toxicity of DOX and that the level of cellular protection correlates positively with the level of expression of HSP27. HSP27-mediated protection is also suggested in the case of a variety of commonly used chemotherapeutic agents and chemicals.

MATERIALS AND METHODS

Cells and Culture Media

O23 cells are a tumorigenic subclone of the Chinese hamster lung fibroblast cell line CCL39 (40). Parental O23 cells and their transfec-
tants were cultivated in DMEM (GIBCO) containing NaHCO₃ (2.2 g/ liter) and glucose (4.5 g/liter) and supplemented with 10% fetal bovine serum. CH²C5 and parental AUXB1 cells were cultivated in α-minimal essential medium containing 10% fetal bovine serum and added nucleosides (41). These cells were kindly provided by Dr. Victor Ling from the Ontario Cancer Institute of Canada. The cultures were maintained at 37°C in a humidified atmosphere containing 5% CO₂.

Two groups of HSP27-transfected cell lines were used in the present study. The first group comprised 10 distinct clonal isolates which were designated 1.1 to 1.5, 1.7, 1.8, and 2.2 to 2.4 and were characterized in a previous study (39). Briefly, the cells were derived from O23 cells transfected with the pHs2711 plasmid, which contains the entire sequence of the human HSP27 gene (42). Clonal isolates of O23 cells were isolated from a pooled population of colonies that emerged after treating the transfected cells with three 44°C heat shocks of 3.5-4 h at 5, 18, and 48 days after transfection. No cells survived when an identical treatment was applied to untransfected cultures. The human HSP27 protein expressed in the transfected cells was found to be indistinguishable from authentic human HSP27 found in human WI-38 or HeLa cells based on a number of criteria including immunogenicity, migration on 21% gels, and ability to be phosphorylated upon heat shock. A correlation was found in 15 of these clones between the levels of expression of the human HSP27 protein and cell thermoresistance.

The second group of HSP27-transfected cells were obtained for the present study by cotransfecting O23 cells with plasmid pHs2711 and a GPT gene, used as a dominant selectable marker gene, followed by selection in DMEM lacking guanine and containing 5% dialyzed fetal bovine serum, xanthine (250 µg/ml), hypoxanthine (15 mg/ml), thymidine (10 µg/ml), aminopterin (2 µg/ml), and mycophenolic acid (25 µg/ml) (43). The primary colonies surviving the selection in three distinct cotransfection experiments (10, 32, and 55 colonies, respectively) were pooled in three independent stable transfected cell lines designated gptHS1, gptHS2, and gptHS3. These cell lines expressed and the level of thermoresistance. The availability of these cell lines provided a unique opportunity to directly test the hypothesis that HSP27 might also participate in cell resistance to anticancer agents. We report here that the HSP27-transfected cells are protected against the acute toxicity of DOX and that the level of cellular protection correlates positively with the level of expression of HSP27. HSP27-mediated protection is also suggested in the case of a variety of commonly used chemotherapeutic agents and chemicals.

HSP27 AND DRUG RESISTANCE

Treatment with Cytotoxic Agents

Monolayers of cells in their exponential phase of proliferation were treated for 1 h with various concentrations of the following agents: DOX (1-25 µg/ml); DNR (0.1-5 µg/ml); the epipodophyllotoxins VMP-26 (0.1-5 µg/ml) and VP-16 (0.1-50 µg/ml); actinomycin D (0.1-12.5 µg/ml); colchicine (1-25 µg/ml); vincristine (1-60 µg/ml); BCNU (5-20 µg/ml); 5-FU (0.1-1000 µg/ml); hydrogen peroxide (H₂O₂, 0.1-1.0 mM), and sodium arsenite (32.5-325 µg/ml). After incubation with the drugs, the cells were tested for survival. DOX was a generous gift from Adria Laboratories of Canada, Ltd.

Survival

Immediately after each treatment, the cells were washed twice, trypsinized, and plated at appropriate dilutions in triplicate Petri dishes in drug-free medium at 37°C. Relative survival was calculated from the number of single cells which formed colonies of more than 50 cells within 7-10 days. The survival data were corrected for the plating efficiency of the appropriate control.

Cellular Transport of Daunorubicin

The cellular transport of DNR was measured following established methods (44, 45).

Uptake. Experiments were done on log phase cultures of parental O23 cells and HSP27-transfected clonal isolates 1.5, 2.2, and 2.3. They were plated at a concentration of 350,000 cells/well in 6-well dishes and incubated for various periods of time at 37°C in 1 ml of DMEM containing 10% fetal calf serum and 0.5 µCi/ml [³H(G)]DNR (3.2 Ci/ mmol; NEN, Mississauga, Ontario, Canada) plus 2 µg/ml of unlabeled DNR. After incubation, the medium was removed, and the cells were washed three times with 1 ml of cold PBS (pH 7.2). One ml of 0.1 N NaOH was added to the cell monolayers, and aliquots were used for liquid scintillation counting. Zero time samples were obtained by adding the radiolabeled medium at 0°C. For all other time points, the incubation medium containing the drug was preincubated at 37°C before being added to the cells. The zero time values were subtracted from each subsequent determination for both the uptake and efflux studies.

Efflux. The experiments were carried out on cells loaded for 1 h with 0.5 µCi/ml of [³H(G)]DNR and 2 µg/ml unlabeled DNR as described above. After loading, the cell monolayers were washed three times with 1 ml of cold PBS and then incubated at 37°C for various periods of time in drug-free DMEM containing 10% fetal bovine serum. The efflux of DNR was evaluated by measuring the amount of radioactivity released in the media. Drug retention was determined by subtracting the amount of radioactivity released in the medium from the amount found in the 60-min loaded cells. Results are expressed as pmol of drug taken up or released by 10⁶ cells.

Protein Determination

Hu27Ab and Ha27Ab are two rabbit antisera recognizing specifically human and hamster HSP27, respectively (39). HSP27 was immunode-
were incubated for 1 h with 2 μg/ml DNR in DMEM medium containing 10% fetal bovine serum at 37°C. To minimize inward and outward fluorescence property of DNR upon excitation at 488 nm. The cells used to measure the DNR content per single cell, making use of the Flow Cytometric Analysis were prepared by using the procedure described by Haber et al. (47). As mouse ascites fluid by Centocor (Malvern, PA). The membranes

Statistical Analysis

Depending on the experiments, the results were analyzed using t test, rho coefficient, and two-way analysis of variance. A P value less than 0.05 was considered statistically significant.

RESULTS

Effects of Cytotoxic Agents on the Survival of Chinese Hamster Cells Transfected with the Human HSP27 Gene. We recently reported that an elevated constitutive expression of HSP27 was sufficient for conferring a thermoresistant phenotype (38, 39). This conclusion was mainly based on a gene transfection study in which Chinese hamster and mouse cell lines transfected with the human HSP27 gene were shown to have a level of thermoresistance that correlated with expression of the human protein. Because the induction of HSP has often been associated with an increased cellular resistance not only to heat shock but also to several cytotoxic agents, it appeared reasonable that HSP27 might also be involved in cellular protection against chemicals. In order to test this hypothesis, we first compared the response to DOX of parental O23 cells to the response of clone 2.2, the thermoresistant HSP27-transfected clonal cell line which expressed the highest level of human HSP27. The effect of increasing concentrations of DOX applied for 1 h on the survival of 2.2 and O23 cells is shown in Fig. 1. The results indicated that the cell survival of transfected cells was higher than that of the parental cells for all concentrations of DOX. The survival of clone 2.2 was 10 times higher than that of the parental cells at the highest concentration of DOX used. Two-way analysis of variance showed that the two curves were statistically different (P < 0.02). At 10 μg/ml of DOX, data from nine different experiments, done during a 1-year-period, showed that the cell survival was higher (P < 0.01; t test) in transfected cells (114 ± 27, 3 × 10⁻⁵) than in parental cells (24 ± 3 × 10⁻⁵). In comparison, the relative survival of the classical multidrug-resistant CH⁴C5 cells after a similar treatment was 0.89.

We further ascertained the relationship between expression of HSP27 and chemoprotection by studying the effects of a single concentration of DOX (8 μg/ml for 1 h) on the survival of a total of 10 clonal isolates of heat-selected HSP27-transfected O23 cells expressing various amounts of human HSP27 (Fig. 2). Total level of HSP27 (hamster plus human) was evaluated in all of these clones by Western blot analysis using Hu27Ab and Ha27Ab, two specific antibodies against human and hamster HSP27, respectively (39). Densitometric quantification of the blots indicated that the clones contained variable amounts of human HSP27, from 1.2 to 12.0 ng/μg (Fig. 2, inset), in addition to a constant level of 2.0 ng of hamster HSP27/μg protein (data not shown). Survival studies from...
The relationship between HSP27 expression and cellular protection against chemicals was further evaluated in experiments in which drugs other than DOX were tested. To this purpose, we first used, as prototype, the clonal isolate 2.2, which had the highest level of HSP27 and which was the most resistant to DOX. Clone 2.2 contains the basal amount of 2 ng of hamster HSP27/µg protein in addition to the normal level of endogenous hamster HSP27. A statistically significant increase in survival in response to DOX was causally related to the presence of the additional amount of human HSP27 in the cells.

The mean cell survivals ± SE measured in three to four different experiments were 52.9 ± 16 x 10^-4; 21.1 ± 2.3 x 10^-4; 67.8 ± 15.9 x 10^-4; and 7.6 ± 2.6 x 10^-4 for parental cells after treatment with DNR, vincristine, VM-26, VP-16, or sodium arsenite, survival of gptHS3 treated with actinomycin D and colchicine, treated with DNR, vincristine, VM-26, VP-16, or sodium arsenite, survival of gptHS2 treated with colchicine or actinomycin D, and survival of gptHS3 treated with actinomycin D and colchicine. These data strongly suggested that overexpression of human HSP27 was responsible for the resistant phenotype of the gptHS cells to heat and doxorubicin. The survival of the gptHS transfected cells relative to their respective controls was also evaluated after treatments with other agents. A statistically significant increase in survival was obtained for gptHS1 treated with actinomycin D and colchicine and for gptHS3 treated with colchicine. Survival of gptHS1 treated with DNR, vincristine, VM-26, VP-16, or sodium arsenite, survival of gptHS2 treated with colchicine or actinomycin D, and survival of gptHS3 treated with actinomycin D were also consistently higher than the survival of their respective controls (data not shown). However, statistical significance was not reached in these latter cases. An easier evaluation would require analysis in clonal isolates of the gptHS cell lines which would express higher levels of human HSP27 and presumably a higher level of resistance.

![Graph](cancerres.aacrjournals.org/article/5248/fig4.png)

**Fig. 4.** Survival of parental O23 cells (C) and clonal isolates 2.2 (■) after treatment for 1 h to increasing concentrations of (A) sodium arsenite and (B) H2O2. In both cases, the survival of 2.2 cells is significantly higher than that of parental cells (P < 0.02 by two-way analysis of variance).
HSP27 AND DRUG RESISTANCE

Figure 5. Effect of HSP27 overexpression on cell survival after heat shock or DOX exposures. A, HSP27 transfectants gptHS1, gptHS2, gptHS3, gptHS4, and 2.2 cells and their respective control cell lines were treated for 3 h at 44°C and then plated at 37°C for colony formation. B, colonies formed by gptHS and control gptC cells after treatment for 1 h in the presence of DOX (10 µg/ml). The colonies emerged after plating 10⁴ cells. The values were normalized for the plating efficiency of the various cell lines (50–70%). Bars, SE of three observations. * and **, statistical significance at the level of P < 0.05 and P < 0.01, respectively (unpaired t test). gptHS4 cells did not express any detectable amount of human HSP27 and were used as negative controls. Open columns, controls; shaded columns, HSP27 transfectants.

Cellular Transport of Daunorubicin. Multidrug resistance is frequently associated with increased efflux and decreased retention of drug. We therefore investigated whether modification of the cellular transport of drugs could explain the increased drug resistance of the HSP27-transfected cells as compared to parental O23 cells. DNR was used as a prototype of drugs to which the transfected cells were more resistant. DNR transport was measured in parental O23 cells, in clones 1.5, 2.2, and 2.3, and for reference purposes, in CHRC5 cells and their wild-type AUXB1 cells. The results presented in Fig. 6A showed that the drug uptake and efflux were approximately the same in AUXB1, O23, and the HSP27-transfected cells. As expected, it was markedly reduced in the multidrug-resistant CH⁵C5 cells. Under the conditions used, the accumulation of DNR by CH⁵C5 cells was about 4% (3.2 pmol/10⁶ cells) that of the other cell lines studied after 60 min. Values of accumulation for the 2.2, 1.5, 2.3, and O23 cells were 90.1, 104.2, 110, and 94.6 pmol/10⁶ cells, respectively. Nevertheless, as shown in Fig. 6B, the survival of the transfected clones following exposure to DNR under the same condition (2 µg/ml for 1 h) was significantly higher (P < 0.01) than that of the parental O23 cells. From these results, it was concluded that a decreased drug accumulation was unlikely to be responsible for the increased survival of the HSP27 transfectants to DNR exposure. This was further confirmed by measuring the levels of Pgp in the parental cells and the HSP27 transfectants. As shown in Fig. 7, only low level expression of Pgp could be detected with the C219 monoclonal antibody either in the parental O23 cells or in the HSP27-transfected 2.2 and gptHS1 cells. The expression of Pgp in both parental and HSP27 transfectants was comparable and was negligible in comparison to that of the CH⁵C5 cells used as positive control. Membranes from AUXB1 cells also contained very low amounts of Pgp. It thus appeared unlikely that overexpression of Pgp was involved in the protection observed against chemicals in the Chinese hamster cells transfected with the human HSP27 gene. Drug resistance in the transfected cells could, however, be the result of a small subpopulation of cells overexpressing Pgp. This possibility was investigated by measuring the level of DNR accumulated after 1 h of exposure in individual cells using flow cytometry. An identical profile of DNR content per cell was found for both the 2.2 and O23 cells. Furthermore, no small subpopulation of cells with low amount of DNR was detected in the transfected cells, as it would have been expected if some Pgp-overexpressing cells were present (data not shown).

DISCUSSION

The role of the small Mr 27,000 heat shock protein, HSP27, in cell protection against doxorubicin and other cytotoxic agents was investigated in Chinese hamster O23 cells overexpressing constitutively human HSP27 as a result of transfection with pHs2711, a recombinant plasmid containing the entire human HSP27 gene (39, 42). Constitutive elevated expression of the human HSP27 protein was previously shown to be sufficient to confer a permanent thermoresistant phenotype (39). In the present study, two groups of HSP27-transfected cells were used as experimental models. The first group of transfectedants was composed of 10 clonal isolates expressing various amounts of HSP27. In these cells, selected on the basis of their thermoresistance, a significant positive correlation was found between the HSP27 content and the survival in the presence of DOX. The second group comprised three independently derived cell lines obtained after cotransfeting the human HSP27 gene and the selectable marker gene GPT. A statistically significant increased survival after DOX treatment was found in the three cell lines overexpressing HSP27 in comparison to the survival of their respective controls. The protection against DOX was thus independent of the mode of selection of the HSP27-overexpressing cells. In addition, clonal variability or a second mutation was not responsible for the effects observed, since more than 100 distinct clones were studied either individually or grouped in pool. Hence, it is concluded that there exists a causal relationship between HSP27 expression and resistance to DOX. Since heat shock induces accumulation of HSP27, these results might explain, at least in part, the reported protective effect of prior heat shock on subsequent exposure to DOX and other anticancer agents (28, 31).

The possibility that elevated expression of HSP27 provides protection against chemicals other than DOX was also evaluated. Except for the drugs 5-FU and BCNU, for which the transfected and control cells were equally sensitive (data not shown), an increased survival was found in all of the HSP27-overexpressing cell lines which were tested against the toxicity of DNR, actinomycin D, colchicine, vincristine, hydrogen peroxide, and sodium arsenite. Whether a dose-response relationship exists between the levels of HSP27 expression and resist-
Fig. 6. A, kinetics of uptake and efflux of DNR by the HSP27-transfected clonal isolates 1.5, 2.2, 2.3, and their parental O23 cells, and by the multidrug-resistant CHC5 cells and their parental AUXB1 cells. For uptake measurements, monolayers of exponentially growing cells were incubated for various periods of time in complete DMEM containing 0.5 μCi/ml of [3H]DNR and 2 μg/ml of unlabeled DNR. At the indicated times, the cells were washed with cold PBS and lysed in NaOH (0.1 N). The radioactivity was counted in the lysates. Each value is the mean of duplicate samples. At 60 min (arrow), the cells were washed and exposed to drug-free medium. Cellular retention of drug was measured at various times thereafter by subtracting the amount of radioactivity counted in the medium from the amount accumulated in the cells after 60 min. B, survival of parental O23 cells and clonal isolates, 1.5, 2.2, and 2.3 after treatment for 1 h in the presence of DNR at a concentration of 2 μg/ml. Exposure of the cells to DNR was performed in conditions identical to those used to measure DNR accumulation at 60 min. The results [means ± SE (bar)] are from three to four different experiments. The survival of the HSP27-transfected cells is, in all cases, significantly higher than that of the parental O23 cells (P < 0.01 by paired t test).

ance to these agents, as observed in the case of DOX (Fig. 2) and hyperthermia (39), remains to be evaluated. Work is in progress to address this question using cells stably transfected with a plasmid containing the HSP27 sequences under the control of an inducible promoter. Nevertheless, the results suggest that overexpression of HSP27 also confers resistance to a variety of drugs and chemicals in addition to heat and DOX.

Data are very limited concerning the physiological functions and biochemical properties of HSP27. Therefore, it is difficult to ascertain the mechanisms of its protective action against chemicals. Cross-resistance to structurally unrelated antineoplastic agents has been observed before and has been described as multidrug resistance. In most of these cases, cellular resistance was found to be associated with a reduced accumulation and retention of the drugs in the resistant cells as compared to the parental sensitive cells, due to an increased drug efflux caused by the overexpression of the membrane Pgp (6, 49). In contrast to what is observed with typical multidrug resistance, the HSP27-associated increase in cell survival was not characterized by a decreased drug accumulation or by an increased expression of Pgp. In addition, flow cytometric analysis showed that the profile of DNR content per individual cells in the transfected clones was superposable to the profile obtained with the sensitive parental O23 cells. In no case could a small subpopulation of HSP27-transfected cells with negligible DNR content be detected, as it would have been expected if some cells were overexpressing Pgp. These observations ruled out the possibility that an increased synthesis of Pgp might have resulted from the selection procedures with heat shock or with aminopterin and mycophenolic acid and also that only a minor subpopulation of Pgp-overexpressing cells contributed to the general resistant phenotype. Because the levels of protection reported in the present study were relatively small as compared to those observed in Pgp-overexpressing cells, it remains possible that a very low and undetectable increased amount of Pgp or an interaction between HSP27 and Pgp might contribute to the resistant phenotype of the HSP27 transfectants. Nevertheless, a more likely explanation is that drug resistance in HSP27-transfected cells, as in several other cell lines, results from a mechanism other than alterations in cellular transport (15, 47, 50).

The finding that 2.2 cells are resistant to two known inducers of HSP synthesis, hydrogen peroxide and sodium arsenite, is consistent with the observations of a cross-resistance between heat shock and H2O2 (26) and between heat shock and sodium arsenite (27). This may have some significance in the understanding of the mechanisms underlying the HSP27-mediated protective effect against drugs. H2O2 has a central role in cellular function in producing oxygen free radicals. It has been suggested that the mechanism of anthracycline cytotoxicity, especially DOX toxicity, might be related to its enzymatic reductive activation to a semiquinone free radical metabolite with subsequent generation of toxic reactive oxygen species including superoxide anion, hydrogen peroxide, and finally, hydroxyl radical (51, 52). These activated forms of oxygen have been implicated in the cytotoxicity of DOX in various in vitro systems (53, 54). Mimnaugh et al. (55) showed that MCF-7 cells resistant to DOX are also resistant to superoxide and H2O2, especially through elevation of glutathione peroxidase, glutathione-S-aryltransferase, and to a lesser extent, superoxide dismutase and mitochondrial NADH-dehydrogenase. It is possible that the protection afforded by HSP27 gene transfection against H2O2 and DOX has a common mechanism involving the participation of HSP27 in the cellular enzymatic system which protects the cells against oxyradical toxicity. This is consistent with the observation that thiol oxidation by diamide...
induces both thermoresistance and synthesis of HSP (27, 56).

HSP27 exists in a native conformation mostly as soluble cytosolic aggregates of about M, 500,000. Upon heat shock or exposure of the cells to various cytotoxic agents, HSP27 is phosphorylated, further aggregates, and becomes Triton insoluble (36, 39, 57, 58). All of these changes in properties occur within a few minutes, suggesting that HSP27 might act at an early step of the stress response. This was supported by the observations that cycloheximide, A23187, and ethyleneglycolbis(β-aminopropyl ether)-N,N′,N′,N′-tetraacetic acid induce rapid phosphorylation of HSP27 and an immediate state of thermoresistance (36). Similarly, various agents that are known to modify the cell response to chemicals were recently shown to induce phosphorylation of HSP27. For example, the glucose-regulated protein inducers A23187, 2-deoxyglucose, tunicamycin, β-mercaptoethanol, and glucosamine and glucose starvation all induce HSP27 kinase activities and protect against DOX and VM-26 (34, 35, 37, 59). HSP27 is also rapidly phosphorylated following treatment of cells with tumor necrosis factor or activators of protein kinase C such as phorbol esters (62-64). Alternatively, Arrigo et al. (57) have provided some evidence suggesting that HSP27 is normally in or associated with the Golgi apparatus. This might have some significance to explain the protection mechanism since recent studies have shown that drugs such as DNR are taken up by the Golgi apparatus, from which they are transferred to the lysosome or to the mitochondria or out of the cells (65-67). It is possible that HSP27 might participate at some vesicle level in the trapping of drugs. This may result in keeping some drug away from the intracellular drug target, thus explaining the increased cell resistance.

HSP27 expression is under hormonal regulation in certain tissues and tumors whose normal growth and development are strongly hormonally controlled (68, 69). Moreover, HSP27 has been shown to be identical to a protein previously referred to as a M, 24,000 protein and whose presence in breast and endometrial adenocarcinomas was correlated with the expression of estrogen receptors (68, 70, 71). Furthermore, it has recently been reported that HSP27 content varied widely in primary breast tumors and that the determination of its level of expression might have some prognostic value in this disease (72-74). Considering that overexpression of HSP27 can participate in the acquisition of a low level of drug resistance in vitro, there is a strong rationale to closely examine the role of HSP27 as a potential factor determining chemoresistance heterogeneity found in clinic.

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