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Regulation of HSP60 mRNA expression in a human ovarian carcinoma cell line

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Abstract. The expression of the 60-kDa heat-shock protein (HSP60) varies markedly among patients with ovarian carcinoma, and high-level expression predicts poor survival in such patients treated with cisplatin (DDP)-containing chemotherapy programs. We investigated the expression of HSP60 in human ovarian carcinoma 2008 cells and an 11-fold DDP-resistant subline 2008/C13*5.25. Heating for 2 h at 44°C produced a 2.7±0.16-fold increase (mean ± SD) that was maximal at 4 h after the start of heat exposure. Exposure to an IC_{50} concentration of DDP for 1 h induced a 1.8±0.03-fold increase in *hsp60* expression. The opposite was true for cadmium and zinc, both of which induced increases in metallothionein II_A but not in the *hsp60* message. 2008/C13*5.25 cells constitutively overexpressed *hsp60* mRNA by 1.7±0.16 orders of magnitude and contained a 3.8±0.45-fold higher level of HSP60 as detected by immunocytochemical staining. 2008/C13*5.25 cells showed 1.2-fold cross-resistance to thermal killing. Expression of *hsp60* was markedly reduced in 2008 xenografts as compared with 2008 cells growing in vitro; however, neither serum starvation nor refeeding altered the message level. Exposure to a variety of growth factors and drug treatments known to alter the DDP sensitivity of 2008 cells, including epidermal growth factor, 12-*O*-tetradecanoylphorbol-13-acetate, buthionine sulfoximine, ouabain, and forskolin, did not alter *hsp60* expression. These results suggest a role for HSP60 in mediating resistance to both DDP and hyperthermia but indicate that the *hsp60* mRNA levels are not regulated by the factors listed above.

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Abbreviations: DDP, cisplatin; EGF, epidermal growth factor; IC_{50}, concentration producing 50% cell kill in a clonogenic assay; $MTII_A$, metallothionein II_A; PBS, phosphate-buffered saline; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; BSO, buthionine sulfoximine; AMCA, 7-amino-4-methylcoumarine-3-acetic acid; $CdCl_2$, cadmium chloride; $ZnCl_2$, zinc chloride

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Introduction

Heat-shock proteins (HSP) are a unique set of highly conserved proteins induced by exposure to heat and a variety of other types of stress [33]. It is commonly accepted that the function of the HSP is either to protect cells against subsequent heat stress or to enhance the ability of cells to recover from the toxic effects of heat or other types of stress.

Transcriptional activation of heat-shock genes is mediated by the binding of heat-shock factor to a conserved nucleotide sequence (the heat-shock element) present in the promoter of heat-inducible genes [33, 48]. The heat-shock element sequence for eukaryotes is rotationally symmetric (C–GAA–TTC–G) and is generally located on the coding strand of DNA upstream from the TATA box [33, 43, 48]. However, not all heat-shock genes induced by one type of stress are necessarily induced by other forms of stress, and the pattern of HSP response to various agents can be quite different and complex [7].

The majority of studies on the regulation of the *hsp* genes have focused on the *hsp70* gene and on *hsp* genes other than *hsp60*, and relatively little is known about *hsp60* regulation beyond the fact that it participates in the heat-shock response and is inducible by exposure to the calcium ionophore A23 187 and to the proline analog L-azetidine-2-carboxylic acid [40]. For example, although many agents, including inhibitors of respiration, sulfhydryl reagents, and hormones [30], are known to induce other HSPs, the effect

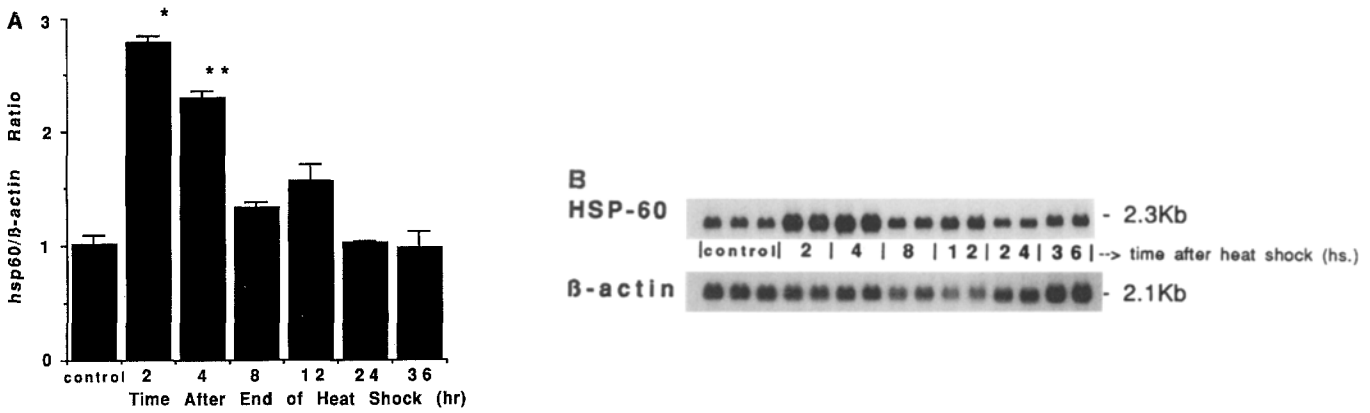

Fig. 1. A Ratio of HSP-60/β-actin mRNA hybridization intensity observed in 2008 cells as a function of time after heat shock. Cells were treated for 2 h at 44°C and incubated at 37°C for the time increments shown. Data represent mean values ± SD. * $P = 0.03$; ** $P = 0.04$. **B** Representative Northern blots illustrating the changes in the level of *hsp60* mRNA expression. Control cells received no heat shock

of many of these substances on *hsp60* expression is unknown.

HSP60 is a homolog of GroEL and is a highly conserved intrinsic mitochondrial protein. It is also a member of the "chaperonin" family of proteins [17]. It acts, in combination with HSP70, to facilitate the posttranslational folding and assembly of oligomeric protein structures [8, 45]. Recently, the complete nucleotide sequence for human HSP60 cDNA was identified [23].

We have previously reported that there is marked heterogeneity in the expression of *hsp60* mRNA as detected by Northern-blot analysis both among normal tissues in the adult and fetus and between tumor samples from patients with testicular, colon, and ovarian cancers [26]. More recently, we found that the level of *hsp60* mRNA expression is a valuable prognostic factor for patients with epithelial ovarian cancer treated with platinum compounds [27]. Patients whose tumors overexpress *hsp60* mRNA have a much shorter survival than do those whose tumors express very low levels. Except for the observation that it can be induced by exposure to heat, A21 389, and L-azetidine-2-carboxylic acid, little is known about the regulation of HSP60 expression in comparison with the other major HSPs. Likewise, no other information is currently available that bears on the question as to whether overexpression of the *hsp60* gene confers DDP resistance.

We investigated the effect of factors known to alter the sensitivity of human ovarian carcinoma 2008 cells to DDP on the expression of *hsp60* mRNA. We report herein that in addition to being rapidly induced by exposure to heat, *hsp60* mRNA expression is induced by acute exposure to DDP. In addition, cells selected for DDP resistance by chronic in vitro exposure to DDP express both increased *hsp60* mRNA and HSP60 protein. However, despite the finding that *hsp60* expression is altered by environmental factors, both growth factors and activators of signal-transduction pathways known to alter DDP sensitivity in these cells [2–5, 9, 19, 35] failed to alter *hsp60* mRNA expression.

Materials and methods

Drugs and chemicals. Cisplatin (DDP) was obtained from Bristol-Myers Squibb (Syracuse, N. Y.). Ouabain octahydrate, forskolin, epidermal growth factor (EGF), cadmium chloride ($CdCl_2$), zinc chloride ($ZnCl_2$), and 12-*O*-tetradecanoylphorbol 13-acetate (TPA) were obtained from Sigma Chemical Co. (St. Louis, Mo.). Buthionine sulfoximine (BSO) was obtained from Chemical Dynamics Co. (South Plainfield, N. J.). Anti-rabbit IgG goat polyclonal antibody conjugated to 7-amino-4-methylcoumarin-3-acetic acid (AMCA) was purchased from Jackson ImmunoResearch Laboratories, Inc. (West Grove, Pa.). Anti-human HSP60 rabbit polyclonal antibody was a gift from Dr. R. S. Gupta (McMaster University, Health Science Center, Ontario, Canada).

Cells and culture conditions. The DDP-sensitive human ovarian carcinoma cell line 2008 was established from a patient with a serous cystadenocarcinoma of the ovary [12]. The 11-fold DDP-resistant subline 2008/C13*5.25 was derived by 13 monthly selections with 1 μ*M* DDP followed by chronic exposure to DDP concentrations increased stepwise to 5.25 μ*M* [1]. Cells were maintained in logarithmic growth in RPMI 1640 medium containing 5% heat-inactivated bovine calf serum and 2 m*M* L-glutamine without antibiotics. Xenografts were produced by inoculating 2008 cells subcutaneously into BALB/c nu/nu mice.

The 2008 cells in logarithmic phase were preincubated with 50 or 500 μ*M* BSO for 24 h, with 0.1 or 0.5 μ*M* ouabain for 1 h, with 37 μ*M* $ZnCl_2$ or 37 μ*M* $CdCl_2$ for 8 h, and with 0.3 μ*M* DDP for 5 days, respectively. After these preincubations, the media were removed from each flask. No additional incubation was performed prior to RNA preparation. The 2008 cells in logarithmic phase were also incubated with 50 μ*M* forskolin for 1 h, with 0.1 μ*M* TPA for 1 or 24 h, and with 10 n*M* EGF for 1 h followed by an additional 2-h incubation in RPMI 1640 media without chemicals prior to RNA preparation. Starvation was performed for 24 h in RPMI 1640 medium without bovine calf serum. After starvation, RNA was prepared from 2008 cells either immediately or following an additional 12- or 24-h incubation in RPMI 1640 supplemented with 10% heat-inactivated bovine calf serum. In other experiments, 2008 cells were incubated at 44°C for 2 h and were then allowed to recover from heat shock at 37°C for various intervals prior to harvesting for the preparation of RNA. After each treatment, the adherent cells were washed with PBS prior to RNA preparation. Thermal sensitivity was determined by incubating 2008 and 2008/C13*5.25 cells at 45°C for various periods and then determining cell survival by colony-formation assay as previously described [19].

Northern-blot analysis. Total RNA was prepared from log-phase cells using the guanidine isothiocyanate-cesium chloride technique [11]. Aliquots of 10 μg total RNA were electrophoresed in 0.66 *M* formaldehyde gels and transferred to nitrocellulose filter membranes (Micron

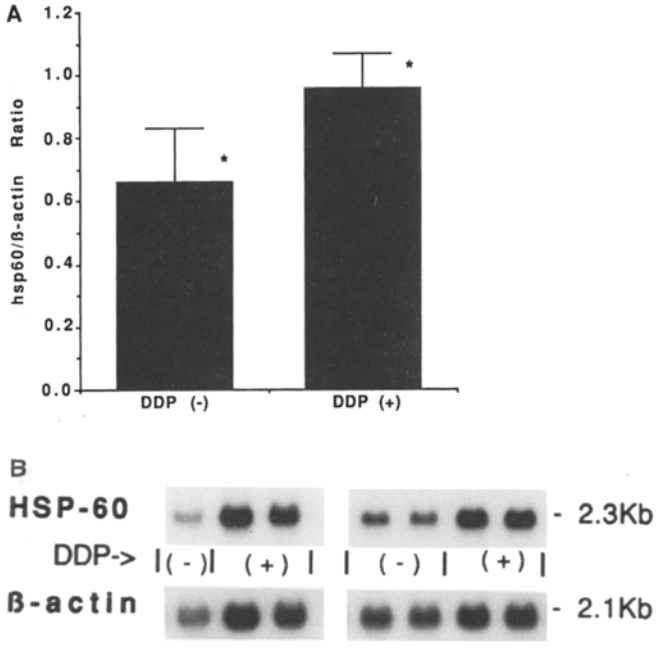

Fig. 2. A Ratio of HSP-60/β-actin mRNA hybridization intensity observed in 2008 cells incubated with and without DDP. Cells were cultured for 5 days in the presence or absence of 0.3 μ*M* DDP. Data represent mean values ± SD. **B** Representative Northern blots showing the induction of *hsp60* expression by DDP

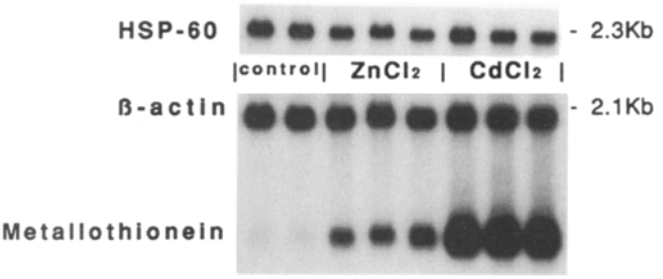

Fig. 3. Northern-blot analysis of RNA prepared from 2008 cells treated with 37 μ*M* $ZnCl_2$ or 37 μ*M* $CdCl_2$ at 37° C for 8 h

Separation Inc., Westboro, Mass.). After baking for 2 h at 80° C under vacuum, filters were prehybridized in 1 *M* NaCl containing 50% formamid, 10% dextran sulfate, and 1.0% sodium dodecyl sulfate (SDS) for at least 1 h and were then hybridized at 42° C for 24 h in the same solution containing denatured salmon sperm DNA (200 μg/ml) and an *hsp60* probe labeled by random primer extension with [^{32}P]-deoxycytidine triphosphate ([^{32}P]-dCTP) [46] to a specific activity of 10^8 cpm/μg DNA. After hybridization, the filters were washed in 2 × SSPE containing 0.1% SDS at 42° C and then exposed to XAR-5 X-ray film. Following stripping, filters were rehybridized with β-actin or $MTII_A$, probes [16, 24]. Relative signal intensities were determined by densitometry and normalized to the expression of β-actin. The significance of differences was evaluated by Student's *t*-test; all *P* values are two-tailed.

Flow-cytometric analysis. Trypsinized cells were fixed in 100% cold methanol for 10 min, washed four times with PBS, and suspended in a rabbit anti-human HSP60 polyclonal antibody at room temperature for 2 h. After four washing steps in PBS, the cells were suspended in a goat anti-rabbit polyclonal antibody conjugated with AMCA and were incubated for 1 h. The cells were then washed, resuspended in PBS at a concentration of 10^6 cells/ml, and analyzed on an Ortho Diagnostics CytoFluorograph.

Statistical analysis. All comparisons were made using Student's *t*-test.

Results

Induction of hsp60 expression in 2008 cells by heat shock

We had previously identified *hsp60* as a gene that was overexpressed in 2008 cells selected for 2- to 3-fold DDP resistance on the basis of its isolation by subtractive hybridization of these low-level resistant cells against parental DDP-sensitive 2008 cells [13]. Using a probe for the carboxy-terminal portion of the cDNA sequence, we examined the effect of heat shock on *hsp60* expression in human ovarian carcinoma 2008 cells. A 2.3-kb message was readily detected in both control and heated cells. Figure 1 summarizes the results of densitometric analysis of Northern blots using total RNA and probing with β-actin to control for lane loading.

When 2008 cells were heated at 44° C for 2 h and allowed to recover at 37° C, *hsp60* mRNA levels reached a maximum at 4 h after the start of hyperthermia (2 h after the end of heat exposure) and then decreased gradually, reaching baseline values by 24 h (Fig. 1). The induction amounted to 2.7 ± 0.06 orders of magnitude ($P = 0.03$) at 2 h and to 2.3 ± 0.07 orders of magnitude ($P = 0.04$) at 4 h. This result is in agreement with data reported by other investigators [18], indicating that the magnitude of the induction of *hsp60* expression by heat shock is substantially lower than that observed for other members of the HSP family such as HSP70 or HSP90.

Induction of hsp60 mRNA expression by DDP in 2008 cells

Heavy metals have been reported to induce a variety of stress-related genes. A 1-h exposure of 2008 cells to an IC_{50} concentration of DDP (3.5 μ*M*) increased *hsp60* mRNA expression 1.8 ± 0.03-fold at 8 h ($P < 0.03$) and 1.3 ± 0.01-fold at 12 h ($p = 0.12$) after exposure. A 5-day exposure of 2008 cells to an IC_{50} concentration of DDP (0.3 μ*M*) caused a 1.5 ± 0.11-fold increase in *hsp60* expression ($P = 0.03$; Fig. 2), and the increased expression persisted for at least 8 h after the end of DDP exposure. On the other hand, such treatment had no effect on the $MTII_A$ mRNA level. Incubation of 2008 cells with IC_{50} concentrations of either $CdCl_2$ or $ZnCl_2$ for 8 h had no effect on *hsp60* mRNA levels, but under the same conditions of exposure, $CdCl_2$ and $ZnCl_2$ produced a 5.9-fold and an 11.6-fold induction of $MTIII_A$ mRNA, respectively (Fig. 3). Thus, *hsp60* expression was induced to a greater extent by DDP than by $ZnCl_2$ or $CdCl_2$. This result suggests that either the *hsp60* promoter does not contain the metal-responsive elements found in the $MTII_A$ promoter or the elements fail to activate transcription.

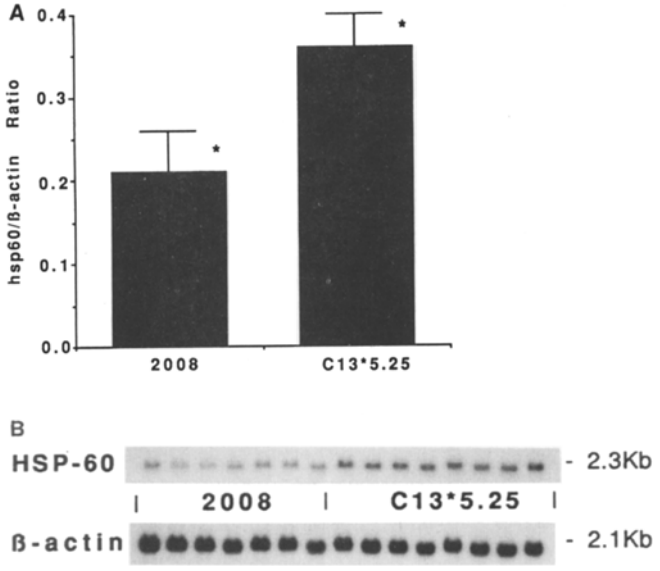

Fig. 4. A Ratio of HSP-60/β-actin mRNA hybridization intensity observed in DDP-sensitive (*2008*) and -resistant (*2008/C13*5.25*) human ovarian carcinoma cells. Data represent mean values ± SD. **B** Representative Northern blots showing the increased expression of *hsp60* in 2008/C13*5.25 cells. The *first seven lanes* show the expression in 2008 cells (three different samples, each loaded in duplicate and triplicate), and the *next eight lanes* show the expression in 2008/C13*5.25 cells (four different samples, each loaded in duplicate)

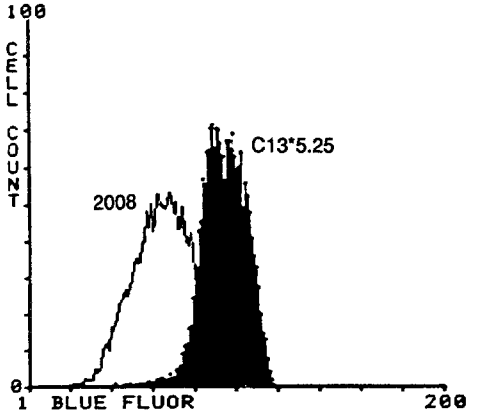

Fig. 5. Flow-cytometric quantitation of HSP60 protein in 2008 (*left curve*) and 2008/C13*5.25 cells (*right curve*)

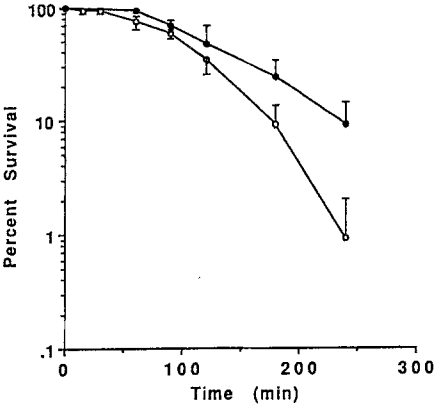

Fig. 6. Sensitivity of 2008 (*open circles*) and 2008/C13*5.25 (*filled circles*) cells to killing by hyperthermia (45° C). Each point represents the mean value for 3 experiments performed with triplicate cultures; vertical bars, ± SD

*Expression of hsp60 mRNA in DDP-resistant 2008/C13*5.25 cells*

We compared the expression of *hsp60* in parental 2008 cells and a subline, 2008/C13*5.25, selected in vitro for 11-fold resistance to DDP. Basal expression of *hsp60* was increased 1.7 ± 0.16-fold in 2008/C13*5.25 cells relative to 2008 cells ($P = 0.002$). Figure 4 shows a representative Northern blot comparing the *hsp60* mRNA levels in the two types of cells.

Despite the small increase observed in the steady-state mRNA level, Fig. 5 shows that there was a larger difference between the two types of cells in their expression of *hsp60* at the protein level. When cells were stained with a highly specific rabbit anti-HSP60 antibody and staining was quantitated by flow cytometry, 2008/C13*5.25 cells were found to express 3.8 ± 0.45-fold higher levels of the protein ($P = 0.03$, $n = 3$).

*Thermal sensitivity of 2008 versus 2008/C13*5.25 cells*

Dose-response curves describing the thermal sensitivity of 2008 and 2008/C13*5.25 cells were determined from experiments in which the cells were exposed to 45° C for increasing periods. Figure 6 shows that over the first 2 logs of cell kill, 2008/C13*5.25 cells were significantly more resistant than 2008 cells. The IC_{50} value for the 2008 cells was 101 ± 5 min, whereas it was 116 ± 4 min for the 2008/C13*5.25 cells ($P < 0.05$). Thus, in the latter cell line there was a significant degree of cross-resistance between DDP and hyperthermia.

Effect of environmental factors on hsp60 expression

When 2008 cells were grown as a xenograft, *hsp60* expression was only 0.36 ± 0.04-fold ($P < 0.01$) that observed when the same cells were growing in the log phase in vitro. This finding indicates that *hsp60* expression can be regulated by environmental factors. To determine whether these factors were present in serum or whether the difference in expression observed in vitro versus in vivo could be accounted for by differences in cell-doubling time, the level of expression was determined in 2008 cells after growth for 24 h in serum-free medium and again at 12 and 24 h after serum refeeding. Neither starvation nor refeeding had a discernable effect on *hsp60* expression (data not shown).

Effect of factors that modulate DDP sensitivity on the hsp60 mRNA content of 2008 cells

Taken together, the results presented above suggested a link between constitutive low-level overexpression of the

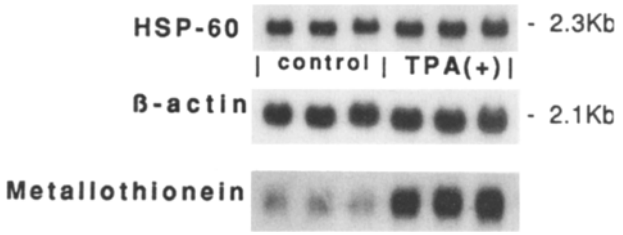

Fig. 7. Representative Northern-blot analysis of RNA prepared from 2008 cells treated with 0.1 μ*M* TPA at 37° C for 1 h. The blot shows the analysis of three different samples of untreated cells and three different samples of cells treated with TPA. Densitometric scanning indicated a β-actin ratio in treated versus untreated cells of 1.0 ± 0.1 (SD)

hsp60 gene and the DDP-resistant phenotype, and, in combination with evidence indicating that *hsp60* mRNA expression is an important determinant of survival in patients with ovarian carcinoma treated with DDP [27], prompted further investigation of the factors that regulate *hsp60* expression. If the expression of *hsp60* is a determinant of sensitivity to DDP, then one might expect things that are known to alter the DDP sensitivity of 2008 cells to regulate *hsp60* mRNA levels. Our studies have indicated that the DDP sensitivity of 2008 cells can be enhanced by depletion of glutathionine following treatment with BSO [2, 3], activation of protein kinase A with forskolin [35] or of protein kinase C with TPA [19], or exposure of cells to EGF [9]. In contrast, DDP sensitivity can be reduced by pretreating the cells with ouabain [4, 5].

Northern-blot analysis comparing the *hsp60* mRNA levels in 2008 cells treated with ouabain, forskolin, BSO, EGF, and TPA under conditions known to affect sensitivity to DDP revealed that none of these treatments altered *hsp60* mRNA expression (data not shown). Figure 7 shows a representative experiment with TPA in which the effect of TPA on $MTII_A$ expression was included as a control. TPA had no effect on *hsp60* expression but did markedly increase the expression of $MTII_A$. Thus, in the 2008 cells the *hsp60* message levels were not responsive to activators of signal-transduction pathways known to influence DDP sensitivity.

Discussion

The results of this study demonstrate that DDP can increase the cellular content of *hsp60* mRNA and that cells selected for 11-fold DDP resistance constitutively overexpress *hsp60* mRNA and HSP60 protein to a moderate degree. These results confirm our previous preliminary reports [13, 14, 26], but in both situations the magnitude of the increased expression was relatively small. However, unlike many of the other HSPs, even heat-shock itself produced only a modest increase in the *hsp60* message level (2.7-fold) in 2008 cells, a result that is consistent with reports by other investigators [18]. The peak *hsp60* mRNA level following a 1-h exposure to an IC_{50} concentration of DDP was 66% of that produced by a 2-h exposure to heat shock at 44° C, and the *hsp60* mRNA content following a 5-day exposure to an IC_{50} concentration of DDP was 55% of that caused by a 2-h exposure to 44° C. In addition, the effect of DDP selection appeared to be greater on the HSP60 protein level than on the message level. The biologic significance of this small increase is uncertain, but the discrepancy observed in the 2008/C13*5.25 cells between the magnitude of the increase in *hsp60* mRNA content versus HSP60 protein suggests that the extent of HSP60 protein induction by DDP may be greater than would be suggested by the small increase produced in mRNA content. No conclusion can be drawn as to whether the increase in *hsp60* mRNA content in either the DDP-treated 2008 cells or the constitutively expressing 2008/C13*5.25 cells resulted from increased transcription or from stabilization of the *hsp60* message.

Induction of the *hsp60* message showed some specificity for DDP and hyperthermia. In contrast to DDP, exposure to toxic levels of cadmium and zinc failed to induce *hsp60* expression, even under conditions where they produced marked increases in the $MTII_A$ mRNA content. Conversely, DDP failed to increase the $MTII_A$ message level at all. We can conclude from these results that metal-responsive elements either are not present in the promoter of the *hsp60* gene or are nonfunctional with respect to the activation of transcription. This is somewhat surprising, since heavy metals are capable of inducing the expression of other HSPs, particularly HSP-70 [36, 41, 42, 52]. It has been reported that expression of *hsp70* mRNA increases after treatment with $CdCl_2$ and $ZnCl_2$ and that this increase correlates well with enhanced HSP70 protein synthesis in fish cell lines [36]. Wu et al. [52] have reported that the *hsp70* gene can be regulated by at least two distinct domains, one that contains sequences responsive to heat shock and cadmium and a second domain necessary for transcription stimulated by serum. Furthermore, those authors have identified a sequence homologous to the core of the human $MTII_A$ metal-responsive element in the promotor of the cadmium-inducible human *hsp70* gene. Our data argue that in the case of the *hsp60* promoter, this metal-responsive element is missing or nonfunctional. The extent of and basis for the specificity of induction by DDP and hyperthermia remain to be defined.

The observation that there was a marked difference in *hsp60* expression in 2008 cells growing in vitro versus in vivo indicates that environmental factors can regulate this expression, but the finding that neither serum starvation nor refeeding altered *hsp60* expression indicates that the modulation is unlikely to be due to either changes in the proliferation rate or factors present in serum. This result obtained for *hsp60* expression stands in contrast to the situation for *hsp70*, where serum refeeding produces a large induction [51], and suggests either that the promoter does not contain AP1 sites responsive to the induction of *c-fos* or *c-jun* [47, 50] or that other factors repress the responsiveness of such sites.

The results obtained with factors capable of altering the DDP sensitivity of 2008 cells also give some insight into regulation of the *hsp60* gene. The promoter is not likely to contain functional TPA- or cyclic adenosine monophosphate (cAMP)-responsive elements or elements responsive to activation of the EGF receptor. This observation was also unexpected, since it has been reported that

growth factors including EGF induce *hsp89* mRNA expression in breast cancer cell lines [21] and that growth factors acting via tyrosine kinase receptors up-regulate *hsp90* mRNA levels in chicken hepatoma cell lines [22]. On the other hand, the expression of *hsp70* in human hepatoma cells was unaffected by EGF but was induced specifically by insulin [49]. Activation of the EGF receptor in 2008 cells with a 1-h exposure to 10 n*M* EGF increased the sensitivity to DDP by 3.1 orders of magnitude [9], but since this treatment did not alter *hsp60* expression, it is unlikely that the EGF effect is mediated via new synthesis of HSP60.

TPA also enhances sensitivity to DDP in 2008 cells, in this case by 2.5 orders of magnitude, and the effect is mediated through the stimulation of protein kinase C (PKC) and the subsequent phosphorylation of a variety of protein substrates [19]. HSP-60 expression in 2008 cells was not affected by TPA treatment, making it unlikely that the TPA effect requires new HSP60 synthesis or that the promoter contains a functional TPA-responsive element. The effect of TPA may nonetheless be mediated through phosphorylation of HSP60, and TPA has in fact been reported to stimulate the phosphorylation of the 28-kDa PKC substrate, which belongs to the family of low-molecular-weight HSPs [20].

Sensitization of 2008 cells to DDP by BSO-mediated depletion of glutathione (GSH) requires both a marked and a prolonged reduction in GSH content [2, 3]. However, even under sensitizing conditions, BSO did not affect HSP-60 expression in 2008 cells. These results agree with the observation of Freeman et al. [15] that GSH is not involved in either the synthesis of HSPs or the development of thermotolerance.

Ouabain, a specific Na^+, K^+-adenosine triphosphatase (ATPase) inhibitor, decreases DDP accumulation in 2008 cells. With a 1-h preincubation, the effect of ouabain on DDP accumulation was linear at ouabain concentrations of up to 100 n*M*, and the maximal inhibition of approximately 45% was achieved at 500 n*M* [5]. Forskolin, on the other hand, increases DDP accumulation and enhances the cytotoxicity of DDP in 2008 cells. This effect is presumed to be mediated by the protein kinase A (PKA) signal pathway, and 50 μ*M* forskolin does in fact increase cAMP levels 222-fold in 2008 cells [35]. Since *hsp60* expression was not affected by 100–500 n*M* ouabain or by 50 μ*M* forskolin, neither the ion gradients regulated by Na^+, K^+-ATPase nor an increase in intracellular cAMP levels appear to be involved in the regulation of *hsp60* expression.

The finding that DDP exposure can induce increases in *hsp60* mRNA content and that 2008/C13*5.25 cells constitutively overexpress *hsp60* at the mRNA and protein levels suggests that HSP60 may play an etiologic role in determining the DDP-resistant phenotype. HSP60 is a chaperonin that associates with microtubules, and an abnormality of microtubular function has been identified in 2008/C13*5.25 cells [10]. The observation that high-level expression of *hsp60* mRNA is accurately predictive of poor survival in patients with ovarian carcinoma treated with DDP and its analogs provides additional circumstantial evidence that *hsp60* may be a DDP-resistance gene [27]. However, this evidence is only circumstantial; increased expression may be part of a nonspecific cellular response to injury. To date it has not been possible to test this hypothesis directly by preparing cells that constitutively over- or underexpress this gene in the absence of DDP selection. Although some human tumors markedly overexpress *hsp60* in vivo [26], such overexpression may be lethal in cell lines, or regulatory mechanisms may serve to decrease expression from the endogenous *hsp60* gene when transcription is driven from an exogenously introduced copy of the gene. It has been reported that DDP can selectively induce the expression of mouse *hsp25*, which corresponds to human *hsp27* [42], although *hsp27* expression is also induced by $CdCl_2$ [34, 44], whereas that of *hsp60* is not.

Our finding of cross-resistance between DDP and hyperthermia in the 2008/C13*5.25 cells is of substantial interest. Although HSP70 has been implicated as mediating thermotolerance in several systems [31, 32, 38, 39], as has HSP27 [28, 29, 31], the relationship between HSP expression and sensitivity to thermal toxicity remains controversial [6, 7]. There is currently no information as to whether isolated overexpression of *hsp60* can cause resistance to thermal killing. The time course of the increase and subsequent decrease in *hsp60* mRNA following heat exposure is somewhat more rapid than has been reported for the emergence and disappearance of thermotolerance in a variety of other mammalian cells [25, 28, 29, 31, 32, 37, 38]; however, a strategy for producing isolated high-level expression of *hsp60* will be required to resolve this issue.

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