HSP27 as a Mediator of Confluence-dependent Resistance to Cell Death Induced by Anticancer Drugs

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ABSTRACT

Resistance of colorectal cancer cells to chemotherapeutic drugs increases as cells reach confluence. Here we show that the small stress protein HSP27, which has been described to block necrotic and apoptotic cell death, accumulates in confluent human colorectal cancer cell lines HT-29 and Caco2. Cell confluence also induces HSP27 phosphorylation and changes in its intracellular distribution. We also show that overexpression of human HSP27 by transfection of HT-29 cells increased the resistance of cells to doxorubicin or cisplatin and prevented drug-induced apoptosis. Interestingly, nonconfluent HSP27-transfected cells and confluent control cells in which HSP27 is expressed at the same level displayed a similar drug resistance. HSP27-transfected cells did not exhibit an enhanced resistance when they reached confluence, nor was there an increased accumulation of HSP27. We have previously shown that HSP27 expression blocks tumor necrosis factor-induced cell death as a result of decreasing intracellular reactive oxygen species (ROS). Here we show that HSP27 overexpression in HT-29 cells, obtained either by transfection or by growing the cells at high density, correlated with a significant ROS decrease. We conclude that cell confluent-dependent HSP27 accumulation, probably due to its ability to decrease ROS levels, is essential for the establishment of the resistance of colorectal cancer cells when reaching confluence.

INTRODUCTION

Cells respond to environmental stress by preferential synthesis and accumulation of a conserved family of proteins referred to as heat shock proteins or stress proteins and by the acquisition of a dramatically increased capacity to survive subsequent hyperthermic stresses (1). To this group of proteins belongs the sHSP family. This family is composed of different proteins varying in size from 15 to 30 kDa, depending on the species, that share sequence homology. Recent data have shown that elevated expression of sHSP efficiently protected mammalian cell death induced by heat (2), certain commonly used anticancer drugs (3–5), oxidative stresses (6, 7), or the antitumoral cytokine TNF (8). The biochemical function of the sHSP family that results in an enhanced cellular protection against different forms of stress is not yet clear, but it may be a consequence of the ability of these proteins to act in vitro as molecular chaperones enhancing the renaturation of denatured polypeptides in an ATP-independent manner (9). In this capacity, they have been implicated as regulators of actin cytoskeleton organization (10, 11). However, we have recently observed that sHSPs protect against TNF-α-induced cell death by modulating ROS via a glutathione-dependent pathway (12).

HSP27 shares biochemical properties with the other members of the sHSP family, such as oligomerization and phosphorylation. HSP27 exists as large, dynamic cytoplasmic aggregates, with molecular native molecular masses ranging from 100–800 kDa. Numerous stimuli (stress, treatment with TNF and arsenite) as well as changes in cell physiology (serum starvation) were found to alter the oligomerization pattern of HSP27 (13–16).

A key feature of HSP27 is its rapid and pronounced phosphorylation, which occurs in different cellular conditions. HSP27 phosphorylation is observed after exposure to a variety of stresses, differentiating agents, mitogens, inflammatory cytokines such as TNF-α and interleukin 1, hydrogen peroxide, and other oxidants (17). Although not studied in every case, HSP27 phosphorylation seems to occur at the same serine residue of HSP27 (18), suggesting the involvement of the same kinase (MAPKAP kinase 2) that may be activated by different signal transduction mechanisms. The role played by this modification in the structure/function of HSP27 is not yet clear. For example, depending on the stimuli, phosphorylation correlates with either a reduced (TNF-α and phorbol ester; Refs. 15 and 19) or an increased (serum stimulation; Ref. 14) oligomerization of this protein. Other studies using directed mutagenesis of the HSP27 phosphorylated serine residues have also led to perplexing observations (20, 21).

HSP27 cellular localization can also be modulated by several stimuli such as heat, TNF-α, or serum deprivation (16, 17). HSP27 is constitutively expressed at low levels in the cytosol of most human cells (22). Within minutes of heat treatment, HSP27 is phosphorylated, shifts from a nonionic detergent-soluble to -insoluble cellular compartment, and relocalizes from the cytoplasm to within or around the nucleus (10, 13, 23). It has been suggested that these modifications in HSP27 properties may be part of a mechanism that activates the (protective) function of the protein.

We have previously reported that for most anticancer agents, their efficacy against colon cancer cells decreases progressively as cells reach confluence (24–26). This occurrence, which we have called confluence-dependent resistance, was attributed to a decrease in intracellular drug accumulation and/or a decrease in the sensitivity of the cells to the different drugs in the confluent population. Interested in knowing whether HSP27 could participate in the confluence-dependent resistance, we have studied the regulation of HSP27 expression by cell culture density in HT-29 and Caco2 human colorectal cancer cells. We have found that HSP27 mRNA and protein accumulate as cell confluence increases. In contrast, no difference of expression between confluent cells and exponentially growing cells was found for the other major HSPs (HSP60, HSP70, and HSP89). Cell confluence also induced HSP27 phosphorylation and changes in its intracellular distribution. These modifications provoked by cell confluence were not due to the nonproliferative status of the cells in the high-density cultures (i.e., most cells were in the G0-G1 phase of the cellular cycle), because serum starvation had a very different effect in HSP27 properties. Finally, we show data concerning HSP27 protective activity and the ability to decrease ROS that suggest a determinant role for HSP27 in the establishment of the resistance of colorectal cancer cells when they reached confluence.

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3 The abbreviations used are: sHSP, small heat shock protein; HSP, heat shock protein; ROS, reactive oxygen species; TNF, tumor necrosis factor; ECL, enhanced chemiluminescence; CDDP, cisplatin; DXR, doxorubicin; HE, hydroethidine; EB, ethidium bromide.
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MATERIALS AND METHODS

Cells. HT-29 and Caco2 human colon carcinoma cells were provided by the American Type Culture Collection (Rockville, MD). We recently described stable transfectants of human HT-29 cells that carry the gene encoding human HSP27 (4). One of the characterized clones (S3) was used in this work. Another cell clone that carried the hygromycin resistance gene and plasmid vector (C1) was used as control. Cells were grown as monolayers in a controlled atmosphere (37°C, 5% CO₂) using Ham’s F-10 medium supplemented with 10% FCS. The effect of serum starvation was studied in cells grown during 48 h in the absence of serum. Nonconfluent HT-29 or Caco2 populations (exponentially growing cells) were obtained by seeding 5 × 10⁵ or 2.5 × 10⁶ cells, respectively, in the wells of 96-well microculture plates and by seeding 2 × 10⁶ or 1 × 10⁷ cells in a 50-ml Falcon flask (Falcon, Lincoln Park, NY). About 35% confluence was obtained after a 48-h culture. Confluent populations were obtained by seeding 10 × 10⁵ or 5 × 10⁶ cells/well in 96-well microculture plates and by seeding 10 × 10⁵ or 5 × 10⁶ cells in a 50-ml Falcon flask.

Northern Blot Analysis. RNA was extracted using the guanidium isothiocyanate-cesium chloride method. Twenty µg of total RNA were separated by electrophoresis through 1% agarose-formamide gels followed by capillary transfer to nitrocellulose filters. The HSP27 probe was generated by PCR and contained the cDNA fragment coding for the 98 COOH-terminal amino acids (27). The actin probe was a 0.5-kb PvuII-XbaI fragment from pHM4A-1 (28). The purified fragments were labeled to high specific activity (2 × 10⁶ dpm/µg) with a random primer labeling system (Amersham, Arlington Heights, IL). Hybridization was done by standard procedure. Labeled probes were removed from filters by incubating them in water at 85°C for 1 h. Before hybridization with a second probe, removal was assessed by autoradiography. Two criteria were used to control for the amount of RNA loaded on the gel: (a) the intensity of the stained EB 18S and 28S RNAs; and (b) the signal given by hybridization of the filter with an actin probe. Both signals did correlate well, indicating that the cellular level of cytoskeletal actin mRNA remained relatively constant during the course of the experiment. Each RNA analysis was repeated at least twice. Blots were quantitated and normalized for actin using an Image Store analyzer (UVP, London, United Kingdom).

Cell Fractionation: Detergent Lysis and Hypotonic Lysis. Cells growing in 50-ml Falcon flasks were washed with PBS, scraped from the flasks, and pelleted at 1,000 × g for 5 min. Lysis was performed at 4°C in a buffer containing 10 mM Tris (pH 7.4), 1 mM MgCl₂, 10 mM NaCl, and 0.1% Triton X-100. The lysates were centrifuged at 2,000 × g for 10 min (pellet P2). The resulting supernatants were centrifuged at 20,000 × g for 10 min (pellet P20 and supernatant S). Before loading into the gel, subcellular fractions were boiled in Laemmli sample buffer. For hypotonic lysis, we used the protocol described above, except that the lysis buffer was devoid of Triton X-100.

Gel Electrophoresis and Immunoblotting. For immunoblotting, whole-cell lysates were prepared by lysing the cells with 2% SDS in 137 mM NaCl, 2.7 mM KCl, 8 mM Na₂HPO₄, 1.5 mM KH₂PO₄, and NaCl/IP (pH 7.4) at 68°C for 5 min. Protein concentration was measured in the supernatant by using the micro BCA protein assay (Pierce, Asnieres, France). Equal amounts of protein (100 µg) were separated in an 11% SDS-polyacrylamide gel. Proteins were transferred to a nitrocellulose filter by electroblotting. Blots were blocked with 5% bovine albumin in NaCl/IP (pH 7.4) and 0.1% Tween 20 and probed with mouse monoclonal antibodies (1:1000) to human recombinant HSP27, HSP90, and HSP90 (StressGen, Victoria, Canada). After three 5-min washes in NaCl/IP, pH 7.4, 0.1% Tween 20 (PBST), the blots were incubated with peroxidase-conjugated antimouse IgG. The ECL Western blotting analysis system (Amersham, Les Ulis, France) was subsequently used for protein detection. Blots were repeated twice and quantified using the Image Store analyzer. Two-dimensional electrophoresis was performed as described previously (29). As a control in the same two-dimensional Western blot in which HSP27 isoforms from human colorectal cancer cells were determined, HSP27 isoforms from HeLa cells extracts were also analyzed. A Bioprofil system (Vilber Lourmat) was used to quantify the different HSP27 isoforms. The analysis was performed within the range of proportionality of the film. To compare different samples, the percentage of each isoform was determined by dividing the value of each isoform by the sum of the values of all isoforms.

Drug Cytotoxicity Assay. Cells were treated with increasing concentrations of CDDP or DXR (Roger Bellon, Neuilly, France) for 4 h. The culture medium was then replaced by drug-free medium for 6 additional days. This long posttreatment period was chosen to give the cells enough time to die and detach from the well. The number of surviving cells was measured with a methylene blue colorimetric test (24).

Cell Morphological Studies. The percentage of apoptotic cells was determined by fluorescence microscopy after staining for 10 min at 37°C with Hoechst 33342 at a final concentration of 5 µg/ml. Detached cells were recovered by centrifugation of cell culture supernatant and mixed with the adherent cells recovered by trypsinization. Cells were judged apoptotic on the basis of characteristic chromatin hypercondensation. Cell viability was determined by trypan blue exclusion. Three hundred cells were counted in triplicate, and the percentages of apoptotic and dead cells were evaluated.

In Vivo Fluorescent Measurement of Intracellular ROS. A fluorescent probe, HE (Molecular Probe-Interichim, Montluçon, France), the Ni²⁺-reduced form of EB, was used to measure the intracellular content of ROS in living cells (30). Cells were washed twice with PBS and incubated for 10 min with 40 µg/ml HE. The flow cytometry analysis was performed using a FACScan flow cytometer (Becton Dickinson, Le Pont de Claix, France) using 488 nm excitation. Emission filter was 610 nm bandpass for oxidized HE (EB) fluorescence.

Cell Cycle Analysis. Confluent cells were treated for 4 h with CDDP and left to recover for different periods of time in drug-free medium. Cells were harvested after trypsinization (2.5 mg/ml trypsin and 0.2 mg/ml EDTA in Hank’s medium without Ca²⁺ or Mg²⁺). Monoparametric cell cycle analysis using propidium iodide was performed using a Becton Dickinson fluorescence-activated cell analyzer after nuclear staining with the cell cycle DNA reagent kit (Becton Dickinson). Data were interpreted using CELLLFIT software and the SOBR mathematical model provided by the manufacturer. Results represent a minimum of 10⁵ cells assayed for each determination.

Intracellular Platinum Accumulation. Cells (2 × 10⁶) were seeded into 5-cm Petri dishes, incubated at 37°C for 48 h, and exposed to 5 µg/ml CDDP for 4 h. After three washes in ice-cold PBS, the cells were scraped off the dishes, suspended in 0.5 ml of PBS, and stored at -20°C. After sonication, the total cellular content of CDDP was determined by atomic absorption spectroscopy (Perkin-Elmer Corp.). Platinum levels were expressed as nanograms of platinum/milligrams of protein.

RESULTS

Cell Confluence Induces Changes in HSP27 Expression in Two Human Colorectal Cancer Cell Lines. HSP27 steady-state protein expression was examined by immunoblot analysis in HT-29 and Caco2 cells cultured at different cell densities. HSP27 accumulated as cultures reached confluence. For both cell lines, HSP27 content in cells of the stationary phase was 10–15 times higher (depending on the cells used) than that in exponentially growing cells (Fig. 1A). As a control, we examined in parallel the topoisomerase II content in the cultures. As described previously (26), its expression was higher in exponentially growing cells than it was in confluent cultures. In contrast, no significant differences in protein content were seen for the major heat shock proteins HSP60, HSP70, and HSP90 (Fig. 1, A and B). Increased HSP27 steady-state protein in confluent cells seemed to be transcriptionally mediated, because HSP27 mRNA levels were also higher in the confluent populations (Fig. 1C).

HSP27 Phosphoisoforms Accumulate in Confluent Cultures. HSP27 phosphoisoforms, identified by their isoelectric point (14, 16, 21, 30) using whole extracts from HeLa cells as a control, were quantified in cells cultured at high density and in exponentially growing cells. The two-dimensional immunoblots presented in Fig. 2 show that in exponentially growing Caco2 cells, HSP27 is recovered at the level of the basic unphosphorylated isoform (Fig. 2, a) and the monophosphorylated isoform (Fig. 2, b) at 69 and 31%, respectively. Interestingly, in confluent cultures, the unphosphorylated isoform strongly decreases (about 14%) on behalf of isoform b and c, detectable at 50 and 36%, respectively. Hence, high cell density induced HSP27 phosphorylation.
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HSP27 Subcellular Distribution Is Modified in Cells Cultured at Confluence. We next analyzed the intracellular distribution of HSP27 in high-density stationary phase and exponentially growing Caco2 and HT-29 cells. After lysis of exponentially growing cells, HSP27 is mainly recovered in the 20,000 × g supernatant fraction, whereas the particulate fractions P2 (2,000 × g) and P20 (20,000 × g) contained less than 5% of its total cellular content (Fig. 3A). A different distribution of HSP27 was observed in the cells lysed from the confluent culture. In this case, we found an accumulation of HSP27 in the particulate P2 fraction (up to 40%, Fig. 3A). For both cultures, no HSP27 was detected in the particulate fractions when the cellular fractionation was performed in the presence of 0.1% of the nonionic detergent Triton X-100 (Fig. 3B). To analyze whether the change of cellular distribution of HSP27 was specific, the intracellular distribution of HSP70, a known cytoplasmic and nuclear gene product, was analyzed under the same conditions. HSP70 was equally recovered in the three fractions in both exponentially growing cells and confluent cells. No changes were observed when fractionation was performed in the presence of Triton X-100 (Fig. 3, C and D). This suggests that HSP27 and HSP70 have specific cellular locales in human colorectal cancer cells and that only the localization of HSP27 is modified by cell culture density.

Effect of Growth Arrest by Serum Starvation on HSP27 Properties. To analyze whether the effect of cell confluence in HSP27 was due to the lack of actively dividing cells, we analyzed cell growth arrested by serum starvation. Confluent cells and serum-starved cells

HSP27 and HSP70

Fig. 3. Cell confluence induces changes in HSP27 subcellular distribution. Confluent (CC) and nonconfluent (NC) HT-29 cells, 0.2 × 10^6 cells and 1 × 10^6 cells, respectively, were hypotonically lysed in the absence (A) or presence (B) of 0.1% Triton X-100 and fractionated as described in "Materials and Methods." The distribution of HSP27 in the different fractions (P2, 2,000 × g pellet; P20 and S, 20,000 × g pellet and supernatant, respectively) was analyzed in immunoblots probed with HSP27 antibody. The accumulation of HSP27 in the different fractions was measured by densitometry. The same experiment was performed to study HSP70 distribution. In this case, 1 × 10^6 cells were lysed in the absence (C) or presence (D) of Triton X-100 and probed with HSP70 antibody. Autoradiographs of ECL-revealed immunoblots are presented. One representative experiment, of the three performed, is shown.

Fig. 2. Quantitative analysis of HSP27 phosphoisoforms in nonconfluent and confluent cells. Two-dimensional immunoblots probed with anti-H5P27 serum of the total proteins of 5 × 10^6 nonconfluent (NC) and 1 × 10^6 confluent (CC) Caco2 cells. Autoradiographs of ECL-revealed immunoblots are presented. The acidic end is to the left. Arrowheads a, b, and c, the three major HSP27 isoforms. The a isoform represents the unphosphorylated form of the protein. Two-dimensional immunoblots were repeated twice. One representative experiment is shown.

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suggests that increased HSP27 content, either by transfection or by cell density, is related to the increased cell survival in the drugs. Supporting the notion that the increase in HSP27 content participates in the resistance phenotype of confluent cells, a similar resistance to the drugs is found between confluent and nonconfluent HSP27-transfected cells in which HSP27 is expressed at the same level (Fig. 5).

We have recently proposed HSP27 as a novel regulator of apoptosis, because its expression in murine L929 cells blocked apoptosis induced by several stimuli (31). Interested in knowing more about the protective effect of HSP27 against chemotherapeutic drugs, we have studied whether HSP27 also inhibits apoptosis induced by CDDP. HSP27- and mock-transfected cells were treated with CDDP (5 μg/ml for 4 h), and 96 h after drug removal, cells were fixed and stained with Hoechst 33242 for chromatin labeling. Overexpression of HSP27 caused a 24 ± 2.3% decrease in the number of apoptotic cells compared to that of control transfectants (percentages were determined from 300 cells counted in triplicate). This result was confirmed by cell staining with deoxynucleotidyl transferase (Apoptag) to detect DNA fragmentation (32). Hence, HSP27 overexpression blocks CDDP-induced apoptotic cell death.

**HSP27 Accumulation Induces Changes in Intracellular ROS Levels.** We next tried to determine the molecular mechanisms that allow HSP27 to decrease the cytotoxic effect of these drugs. Because HSP27 is known to interact with the cytoskeleton (33), it could influence drug uptake and therefore explain the different sensitivity of the cells. However, we found that similar amounts of the drug were taken up by the HSP27-transfected and the control-transfected cells (19.35 ± 2.4 ng CDDP/mg protein after a 4-h treatment with 5 μg/ml CDDP, n = 4). ROS are known mediators of anticancer drug-induced cell death (34). In previous studies, we demonstrated in L929 murine cells that stable expression of human HSP27 correlates with decreased cellular content of ROS (12). This capacity of HSP27 to alter basal ROS levels was associated with its protective activity against agents

had a similar cell cycle distribution with around 80% of the cells in the G₀-G₁ phase. Nevertheless, as shown in Fig. 4A, serum starvation did not provoke an increase in HSP27 content. Similarly, no accumulation of HSP27 in the P₂ fraction was detected in the serum-starved cells (Fig. 4, B and C).

**Overexpression of HSP27 Increases the Resistance of Cells to DXR and CDDP.** We have recently shown that HSP27 participates in the resistance of colorectal cancer cell line HT-29 to DXR (4). Using the stable HT-29 cell clones expressing human HSP27 and the mock-transfected clones as a control described in previous work, we show here that HSP27 expression also protects against CDDP treatment. The immunoblot presented in Fig. 5 shows the steady-state level of HSP27 from nonconfluent and confluent cells from two representative clones of HSP27-transfected and mock-transfected HT-29 cells. Exponentially growing HT-29 cells transfected with human HSP27 are more resistant to CDDP or DXR than are proliferating mock-transfected cells (Fig. 6). This resistance phenotype cannot be explained by differences in the cell cycle distribution. The percentages of HSP27-transfected cells in G₀-G₁ are 48% in the nonconfluent cells and 70.5% in the confluent cells, respectively. The percentages of HSP-27-transfected cells in G₀-G₁ phase are 50.5% in the nonconfluent and 71% in the confluent cells, respectively. Interestingly, confluent mock-transfected cells and exponentially growing HSP27-transfected cells, which contain a similar HSP27 content, are equally resistant to CDDP- or DXR-induced cell death. This result strongly
such as TNF-α. To study whether HSP27 also has this capacity in human colorectal cancer cells, we have compared human HSP27-expressing and mock-transfected HT-29 cell clones. As shown in Fig. 7 there was a 1.6-fold decrease in ROS levels in HSP27-transfected cells compared to that in mock-transfected cells. Remarkably, a decrease in ROS levels was also obtained when there was HSP27 accumulation due to growing the cells at high density. Confluent HT-29 cells had a 1.5-fold decrease in ROS content compared to that of exponentially growing cells (Fig. 7, left panel). Hence, in the same cell line, a similar HSP27 level observed in two different contexts (i.e., proliferative or confluent populations) was associated with a similar intracellular ROS level.

**DISCUSSION**

In this paper, we show that cell culture density modulates HSP27 expression and provokes posttranslational modifications that may affect its activity. HSP27 accumulates as cells reach confluence. Notably, when cells are growth-arrested in G_0-G_1 phase by serum starvation rather than by growing at high density, HSP27 levels do not increase. This suggests that HSP27 accumulation by cell confluence is not cell cycle-dependent. The effect of cell confluence seems to be specific for HSP27, because we found no changes in the intracellular content of the other major HSPs (HSP60, HSP70, and HSP89). Therefore, cell culture density does not induce a general stress response. Interestingly, it is HSP27 that has been specifically implicated in the resistance to anticancer drugs (3–5, 35) and therefore could participate in the higher resistance of colorectal cancer cells when grown at high density.

We also show that high-density cultures are associated with increased content of HSP27 phosphoisoforms and with dramatic changes in the intracellular localization of this protein. In exponentially growing human colorectal cancer cells, HSP27 is mainly dispersed in the soluble phase of the cytoplasm. As cultures reach confluence, HSP27 accumulates around the nucleus. This phenomenon was abolished when lysis was performed in the presence of the nonionic detergent Triton X-100. It can be concluded that in high-density stationary phase cells, an important fraction of HSP27 is probably interacting with the nuclear membrane. This particular behavior of HSP27 is not due to the fact that around 80% of cells in the confluent population are in the G_0-G_1 phase of the cell cycle, because serum starvation provokes a similar recruitment of G_0-G_1-phase cells and does not alter HSP27 localization in these cells. Because HSP27 is known to interact with the cytoskeleton (10, 33), one hypothesis could be that the particular distribution of HSP27 is a consequence of cytomembrane rearrangements that take place in high-density cultures.

Recent studies indicate that phosphorylation plays a key role in the regulation of HSP27 function and its contribution to survival after stress (10, 33). It has been proposed that phosphorylation induces conformational changes in HSP27, resulting in a lower binding efficiency of HSP27 for the barbed end of microfilaments and consequent stabilization of the cytoskeleton. However, other results point out the relative importance of HSP27 phosphorylation in the modulation of its protective function(s) (20). Therefore, although we show here that cell confluence modifies HSP27 phosphorylation and its intracellular localization, the biological significance of these changes in HSP27 function(s) remains to be determined.

To understand the role of HSP27 accumulation during the de-
opment of the resistance of confluent cells against drug-induced cell death, we have used HSP27-transfected HT-29 cells. Overexpression of HSP27 inhibits human colorectal cancer cell death induced by DXR (4) and CDDP, as we have shown. This effect was not due to differences in the intracellular accumulation of CDDP, because, as also found in human testis tumor cells (5), similar amounts of the drug were taken up by HSP27-transfected and control-transfected cells. The increase in the resistance to CDDP and DXR of proliferative HSP27-overexpressing cells is similar to that observed when control cells are grown at a confluent state. In other words, we can mimic the level of resistance of confluent cells just by increasing the HSP27 level in proliferative cells. HSP27-overexpressing cells do not further increase HSP27 content when reaching confluence. This result is not surprising, because in many different HSP27-overexpressing cells, no increased accumulation of HSP27 is detected even after heat shock treatment.4 Remarkably, the absence of induction of HSP27 accumulation when these cells reach confluence is accompanied by the lack of an enhanced cell resistance. Together these data suggest that the protection conferred by HSP27 is strongly involved in the resistance phenotype of confluent colon carcinoma cells.

We have previously shown that expression of HSP27 blocked the apoptotic process generated by several stimuli in murine L929 fibroblasts (31). We show here that HSP27 accumulation also reduces the apoptotic process induced by CDDP in human colorectal cancer cells. Hence, HSP27 may represent a pleiotropic inhibitor of apoptosis in different cell lines.

Concerning the protective activity of HSP27, we have demonstrated in murine L929 cells that HSP27 decreased the cellular content of ROS (12). The intensity of the phenomenon depended on the concentration of the expressed human HSP27. This is probably a conserved property of HSP27, because we show here that HSP27 also decreases ROS levels in a very different cellular model, human colorectal cancer cells. The ability of HSP27 to modulate intracellular redox can lead to protective mechanisms that may not be restricted to oxidative stress resistance and may explain a more general protective role of this protein. Elevated levels of HSP27, by buffering cellular ROS, may protect cellular structures that are damaged by stress and particularly actin microfilaments (36, 37). HSP27 expression, by inducing a proreduced state, may also modify the metabolism and induce changes in cellular structures. Because there is a decrease in ROS levels as cells reach confluence and because we have been able to mimic this ROS decrease in proliferative cells by overexpressing HSP27, we suggest that HSP27 could be a modulator of ROS levels in the development of the resistance of confluent cells. Hence, it is tempting to speculate that in the course of cell confluence, HSP27 accumulation may represent a major event establishing a proreduced state that, in turn, may at least partially explain the confluence-induced resistance against CDDP and DXR, two well-known ROS producers (34).

Confluent cultures are better models than actively growing cells for growth conditions in a solid tumor. Our data support the participation of HSP27 in the inherent resistance of confluent cells to chemotherapeutically active drugs. It would now be interesting to test whether drugs that modulate HSP27 expression and/or posttranslational modifications could also be used to reduce the resistance of confluent cells to anticancer drugs.

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4 P. Mehlken and A-P. Arrigo, unpublished observations.

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