Expression of the c-Ha-ras Oncogene in Mouse NIH 3T3 Cells Induces Resistance to Cisplatin


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ABSTRACT

The effect of expression of the c-Ha-ras oncogene on cisplatin (DDP) sensitivity was examined in murine NIH 3T3 cells transfected with the dexamethasone (DEX)-inducible mouse mammary tumor virus promoter linked to an activated c-Ha-ras gene (LTR H-ras(A) cells). Treatment of these cells with 5 μM DEX for 24 h induced c-Ha-ras expression and produced an 8.2 ± 1.3-fold (SD) increase in DDP resistance as quantitated by clonogenic assay. Induction of the c-Ha-ras oncogene reduced DDP accumulation by 40% and intrastrand adduct formation by 17%. In nontransfected wild-type NIH 3T3 cells, DEX did not induce DDP resistance nor did it decrease DDP accumulation. Induction of c-Ha-ras expression did not alter cellular glutathione content or the activity of glutathione-S-transferase in the LTR H-ras(A) cells. DEX increased cellular metallothionein content by 1.6-fold in NIH 3T3 cells and 3.3-fold in LTR H-ras(A) cells. We conclude that DEX-induced overexpression of a mutant c-Ha-ras gene confers DDP resistance and that this resistance is associated with an impairment of cellular drug accumulation and an increase in metallothionein content.

INTRODUCTION

DDP is the most effective antineoplastic drug for the treatment of ovarian carcinoma (1); however, resistance develops commonly during treatment. Like the situation for multiple drug resistance, the development of the DDP-resistant phenotype is thought to be due to the selection for and overgrowth of cells that arise through spontaneous somatic mutation (2, 3). However, unlike cells exhibiting the multiple drug resistance phenotype (4), DDP-resistant cells do not have any of the cytogenetic hallmarks of gene amplification. Four major mechanisms appear to contribute to the DDP-resistant phenotype in a variety of human and murine cell lines (5–8). A decrease in DDP accumulation has been found in a broad range of cell types (6, 7, 9–11). Elevated levels of GSH in some DDP-resistant cells and sensitization of these cells by GSH depletion suggest that this thiol may play a role either directly or indirectly in mediating resistance (5, 6, 12, 13). Elevation of cellular MTs can result in DDP resistance (14), presumably by direct binding of the drug (15), but not all DDP-resistant cells have elevated MTs. Finally, enhanced repair of DDP-DNA adducts has been noted in resistant murine leukemia cells (16–18), and enhanced DDP-induced unscheduled DNA synthesis has been noted in resistant ovarian carcinoma cells (19). We have recently presented evidence that DDP sensitivity can be modulated by signal transduction pathways. Activation of protein kinase C increases the sensitivity of the 2008 human ovarian carcinoma cell line by 2.5-fold (20). The c-Ha-ras protein is a component of another signal transduction pathway that is incompletely understood. Nevertheless, Sklar (21) reported that NIH 3T3 cells transformed by either the normal or mutant c-Ha-ras oncogene were significantly more resistant to DDP than parental cells. We have used a NIH 3T3 cell line containing a hormone-inducible form of the c-Ha-ras oncogene (22) to study the mechanism by which the c-Ha-ras signal transduction pathway causes DDP resistance. These cells were prepared by transfection of NIH 3T3 cells with a construct containing the c-Ha-ras oncogene coupled to the DEX-inducible MMTV LTR (23). The results indicate that transcriptional activation of this mutant c-Ha-ras oncogene produces a substantial degree of DDP resistance associated with a decrease in DDP accumulation and an increase in MT.

MATERIALS AND METHODS

Materials. DDP was obtained from the Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. DEX was purchased from Sigma Chemical Co., St. Louis, MO. [153mPt]DDP was obtained from Oak Ridge National Laboratories, Oak Ridge, TN. mCB was purchased from Molecular Probes, Inc., Eugene, OR. A stock solution of MCB was prepared in ethanol (20 mm) and kept at 0–5°C. protected from light. [35S]Methionine (specific activity, 1000 Ci/mmol) was obtained from ICN Biomedicals, Costa Mesa, CA. Rat monoclonal anti-v-Ha-ras antibody (Y13-259) was obtained from Oncogene Science, Manhasset, NY. [-H]DEP (specific activity, 16.4 Ci/mmol), an analogue of DDP which produces adducts at identical sites in DNA, was synthesized as previously reported (24).

Cell Lines. The LTR H-ras(A) cell line (22), consisting of a clone of NIH 3T3 cells transfected with plasmid pLTR H-ras(A) containing a codon 12-activated form of the c-Ha-ras under the control of the mouse mammary tumor virus long terminal repeat, was obtained from Dr. Rolf Jaggi. In this line, expression of the c-Ha-ras oncogene was highly inducible by exposure to DEX (22). Another NIH 3T3-derived cell line, 76-21-3, transfected with the plasmid p76/21 containing the c-fos gene (25) under the control of the cadmium-inducible human MT promoter, was kindly provided by Dr. John Atwater (Salk Institute, San Diego, CA). Cell lines were grown on tissue culture dishes in a humidified incubator at 37°C and 5% CO2 atmosphere in medium consisting of DME supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 100 units/ml of penicillin, and 100 μg/ml of streptomycin (Irvin Scientific, Santa Ana, CA). Both LTR H-ras(A) and 76-21-3 cells had been cotransfected with pSV2neo (22) and were grown in the presence of 0.5 mg/ml of G418.

Measurement of DDP Sensitivity. Both colony-forming and [3H]dThd incorporation assays were used to measure DDP sensitivity. Cells were cultured in complete DME in the presence or absence of 0.5 μm DEX.

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2 To whom requests for reprints should be addressed, at the Department of Medicine 0812, University of California, San Diego, La Jolla, CA 92039.

3 The abbreviations used are: DDP, cis-diaminedichloroplatinum(II); DEX, dexamethasone; DME, Dulbecco's modified Eagle's medium; ELISA, enzyme-linked immunosorbent assay; GSH, glutathione; GST, glutathione-S-transferase; [3H]DEP, cis-dichloro[H]ethylenediamineplatinum(II); IC50, 50% inhibitory concentration; mCB, monochlorobimaine; MMTV, mouse mammary tumor virus; LTR, long terminal repeat; MT, metallothionein; PBS, phosphate-buffered saline; dThd, thymidine.

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for 24 h, harvested by trypsinization, exposed to DDP for 1 h, and washed free of all drugs. In the case of the NIH 3T3 cells, they were plated at 2500 cells per 100-mm dish in the absence of drugs and grown for 7 days. The plates were fixed with methanol and stained with Giemsa, and colonies of >60 cells were counted macroscopically (26). Cloning efficiency was 11 ± 1% (SD) and 12 ± 2% (SD) for NIH 3T3 cells with and without DEX, respectively. In the case of the LTR H-ras(A) cells, clonogenic cell survival was assessed in a two-layer soft agarose system (27). Low-level constitutive expression of the c-Ha-ras oncogene from the MMTV promoter permitted colony formation in soft agar even in the absence of DEX induction. After drug exposure, the cells were plated in 0.75% agarose, and colonies of >30 cells were counted after 10 days. Cloning efficiency of the LTR H-ras(A) cells was 22 ± 3% (SD) in the absence of DEX and 18 ± 6% (SD) after treatment with DEX.

DDP sensitivity was also determined using a $[^3H]_{\text{H}}$Tdh incorporation assay. A total of 100,000 cells were seeded into 60-mm culture dishes in 5 ml of medium and allowed to grow for 19 h with or without 0.5 μM DEX. DDP was added to produce various concentrations, and the incubation continued for 1 h at 37°C. The DDP was then removed, and the cells were washed 3 times in drug-free medium and allowed to grow an additional 19 h. $[^3H]_{\text{H}}$Tdh (5 μCi/ml, 28 Ci/mmol; Amersham-Searle, Arlington Heights, IL) was added, and 4 h later the cells were again washed 4 times with PBS containing 5% fetal bovine serum. The dishes were treated with 2 ml of 1 N NaOH for 12 h and neutralized with 2 ml of 1 N HCl, and the $[^3H]_{\text{H}}$Tdh in 0.5 ml was determined by liquid scintillation counting.

Cadmium Treatment and Colony Assays. Subconfluent monolayers of 76-21-3 cells were cultured with complete DME in the presence or absence of 5 μM cadmium chloride. After a 5-h incubation, cells were treated with appropriate concentrations of DDP for 1 h, and then clonogenic cell survival was assessed in plastic dishes in the same manner as for NIH 3T3 wild-type cells.

DDP Accumulation. NIH 3T3 and LTR H-ras(A) cells were harvested from monolayers after a 24-h exposure to DEX or medium alone. Cells were washed and resuspended with DME complete medium in microcentrifuge tubes at a density of approximately 2 × 10^7 cells/ml. The cell suspensions were incubated with DME medium (37°C) containing 10 μM $[^{195}P_{\text{t}}]$DDP for 1 h. The medium was aspirated, and the cells were washed rapidly with 4°C PBS 4 times. One ml of 1 N NaOH was added, and the cells were allowed to digest overnight. A 40-μl aliquot was used for determination of protein content by the method of Bradford (28), and an 0.8-ml aliquot was counted in glass tubes on a Trak 1191 gamma counter (Tracers Analytic, Elk Grove Village, IL).

Glutathione Measurement. Glutathione content was measured by adjusting cells to 10^6/ml and staining them with 25 μM MCB in complete medium at room temperature for the indicated time; relative cellular fluorescence was then immediately measured on a Model 2151 Cytofluorograf II (Orthodiagnostic System, Inc.) flow cytometer with excitation and emission settings of 385 and 480 nm, respectively (29, 30). Values were converted from log fluorescence to linear fluorescence intensity by application of the equation $x = 10^{([y - 20]/60)}$, where $x$ is the relative linear fluorescence intensity and $y$ is the mean log channel number. Cells that were nonviable on the basis of forward and right angle light scatter were excluded from analysis. The forward rate constant for the conjugation of MCB by GST is given by the equation $K = \text{initial rate}[\text{MCB}][\text{GSH}]$. Since the GSH content in the uninduced and induced states turned out to be the same, and since the MCB concentration was identical, the effect of c-Ha-ras induction on $K$ could be estimated from its effect on the initial slope of the conjugation curve.

Determination of MT Content. MT content was assayed by determining the ability of an unknown sample to block the binding of an antibody to purified MT as previously described (14). Microtiter plates were coated with rabbit MT (50 ng/ml) overnight at 4°C. Unbound sites were blocked with PBS containing 1% bovine serum albumin. Cytosolic extracts from DEX-treated or untreated cells, or known amounts of competitor MT used as a standard, were incubated with the anti-MT antibody (31, 32) and then applied to the wells. After several hours at 37°C and washing with PBS-Tween 20 (0.05%), a goat antibody to rabbit immunoglobulin conjugated with horseradish peroxidase was added at 1:3000. After incubation at 23°C for 1 h and three washings, o-phenylenediamine (0.4 mg/ml) containing 0.1% urea peroxide in 0.1 M citrate buffer was added as substrate. Color development was measured by an ELISA autoreader at 492 nm. MT content was determined by comparison to standard curves. The protein content of cytosolic extracts was determined by the method of Bradford (28).

DNA Adduct Formation. Confluent monolayers were cultured with complete DME in 75-cm² culture flasks (Corning Glass Works, Corning, NY) with or without 0.5 μM DEX for 24 h. The medium was changed, and the cells were incubated at 37°C for 6 h with 10 μCi/ml of $[^{3}H]$DDP. Cells were harvested by trypsinization, washed twice with ice-cold PBS, then resuspended in 0.8 ml of 100 mM NaCl/10 mM Tris-Ci/25 mM EDTA/0.5% sodium dodecyl sulfate/0.1 mg/ml of proteinase K, and digested in 50°C for 18 h with shaking. This was followed by extraction with phenol/chloroform and by dissolution of the DNA in 10 mM Tris-Ci/1 mM EDTA containing 0.1 mg/ml of RNase A and digested at 37°C for 3 h. After another extraction with phenol/chloroform, DNA was precipitated with 70% ethanol and stored at −70°C. The platinated DNA was digested to deoxyribonucleosides, and specific DNA-bound adducts were separated by high-performance liquid chromatography (33). The peak height of deoxyctosine measured at 254 nm during elution was converted to nmol by comparison with a standard curve. The level of DNA platination was then expressed as dpm associated with the guanine-guanine intrastrand adduct per nmol of deoxyctosine.

Immunoprecipitation Analysis. LTR H-ras(A) cells were cultured in growth medium with or without 0.5 μM DEX for 24 h, incubated in methionine-free medium for 20 min, and then treated with 250 μCi/ml of $[^{35}S]$methionine for 3 h. Cells were lysed, and nuclear extracts were immunoprecipitated with anti-fos M2 antibody (34). For both types of cells, approximately equal amounts of acid-precipitable radioactive proteins were loaded on a gel, and the products were analyzed by electrophoresis on a sodium dodecyl sulfate-polyacrylamide gel, followed by autoradiography.

RESULTS

Effect of c-Ha-ras Induction on DDP Sensitivity. Fig. 1 shows that in the absence of DEX induction, only the endogenous p21^{cHa-ra} was observed. When LTR H-ras(A) cells were treated for 24 h with 0.5 μM DEX, there was a greater than 10-fold increase in the amount of the mutant c-Ha-ras protein which migrates as a doublet just above the endogenous p21^{cHa-ra} immunoprecipitable from $[^{35}S]$methionine-labeled cells.

The DDP dose-response curves for wild-type NIH 3T3 cells and 3T3 cells containing the inducible c-Ha-ras oncogene were determined with and without DEX treatment. Fig. 2, left, shows that, when the c-Ha-ras-transfected NIH 3T3 cells were treated with 0.5 μM DEX for 24 h, there was a marked decrease in sensitivity to a 1-h exposure to DDP. The slope of the linear portion of this curve in the absence of DEX was −0.304 ± 0.169 (SD; n = 3), whereas it was −0.037 ± 0.025 (SD; n = 3) following DEX treatment. Thus, induction of the c-Ha-ras oncogene caused the cells to become 8.2 ± 1.3 (SD)-fold more sensitive to a 1-h exposure to DDP.
c-Ha-ras ONCOGENE-INDUCED CISPLATIN RESISTANCE

Fig. 1. Accumulation of p21c-Ha-ras in LTR H-ras(A) cells after DEX administration. Crude membrane extracts were prepared from the cells cultured in the growth medium containing (left) or lacking (right) DEX for 24 h prior to 3-h treatment with [35S]methionine.

Fig. 2. Sensitivity of LTR H-ras(A) cells (left) and wild-type NIH 3T3 cells (right) to DDP in the presence (•) or absence (O) of 5 μM DEX. Cells were treated with a 24-h preexposure to DEX, followed by 1-h concurrent exposure to DEX and DDP. Points, mean of 3 experiments performed with triplicate plates; bars, SD.

soft agar; LTR H-ras(A) cells form discrete colonies only in soft agar).

Table 1 shows the effect of DEX treatment on the sensitivity of NIH 3T3 and LTR H-ras(A) cells when DDP sensitivity was measured using [3H]dThd incorporation assay which permitted comparison of the two cell types under exactly the same conditions. With the LTR H-ras(A) cells, DEX reduced the slope of the DDP dose-response curve (P < 0.05) and increased the IC50 (P = 0.06), but had no significant effect on the DDP sensitivity of NIH 3T3 cells. The magnitude of the effect measured with the [3H]dThd incorporation assay was smaller than that observed with the colony-forming assay, a characteristic also observed when comparing cell variants selected for DDP resistance (data not shown).

Effect of c-Ha-ras Induction on Cellular GSH Content and the Activity of GST. MCB reads quantitatively with GSH via GST to form a fluorescent product readily quantitated by flow cytometry (29, 30). Both LTR H-ras(A) and wild-type NIH 3T3 cells were stained with 25 μM MCB for various periods of time, and relative fluorescence was determined immediately by flow cytometry. Fig. 4 shows a representative experiment indicating that maximum staining was obtained by 30 min in both cell lines, and this staining time was used for all subsequent experiments. The GSH content was compared in induced and uninduced wild-type and LTR H-ras(A) cells. In the case of wild-type cells, the GSH content of DEX-treated cells was 102 ±

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* Mean ± SD.  
† P < 0.05.  
‡ P = 0.06.

Effect of c-Ha-ras Induction on Cellular Accumulation of DDP. Both wild-type and LTR H-ras(A) cells were treated concurrently with [195mPt]DDP and DEX for 1 h following a 24-h exposure to DEX. The [195mPt]DDP accumulation was linear over more than 6 h of incubation (data not shown). Fig. 3, left, shows that induction of the c-Ha-ras oncogene produced a significant decrease in the cellular accumulation of [195mPt]DDP in LTR H-ras(A) cells. In the absence of c-Ha-ras oncogene induction, the cells contained 139 ± 19 (SD; n = 3) pmol/mg of protein, whereas those treated with DEX contained just 83 ± 5.7 (SD; n = 3) pmol/mg of protein (P < 0.05). In contrast, similar experiments conducted with wild-type NIH 3T3 cells showed no effect of DEX on DDP accumulation (Fig. 3, right). Thus, induction of c-Ha-ras expression was associated with a 40% reduction in cellular accumulation of DDP; of note is the fact that 10-fold resistant human ovarian carcinoma 2008 cells have a 50% decrease in cellular [195mPt]DDP accumulation (10).

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This technique specifically permits quantitation of the most abundant adduct, the guanine-guanine intrastrand cross-link. DEP-DNA intrastrand adduct formation in DEX-induced LTR H-ras(A) cells was 82.9 ± 9.6% (SD) of that in the absence of DEX (P < 0.05); DEX had no effect on intrastrand adduct formation in the parental NIH 3T3 cells. Thus, c-Ha-ras oncogene expression induced by DEX not only decreased DDP accumulation but also, to a somewhat lesser degree, intrastrand adduct formation.

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Effect of Induction of c-fos Expression on DDP Sensitivity. 76-21-3 cells contain the mouse c-fos coding sequence linked to the human MT promoter inducible by cadmium exposure. Fig. 5 shows that, when 76-21-3 cells were treated for 5 h with 5 μM cadmium chloride, there was a 5-fold increase in the amount of c-fos protein immunoprecipitable from [35S]methionine-labeled cells. However, as shown in Fig. 6, cadmium induction of the c-fos gene failed to change the DDP sensitivity of the cells. Cadmium chloride also caused no significant change in the sensitivity of wild-type NIH 3T3 cells. These results indicate that isolated overexpression of the c-fos gene does not alter DDP sensitivity.

Effect of c-Ha-ras Induction on Cellular MT Level. The MT content of DEX-treated and untreated wild-type and LTR H-ras(A) cells was determined by ELISA with rabbit antiserum to MT (29, 30). As reported for many cell types, DEX treatment of the wild-type 3T3 cells induced MT levels by 1.62 ± 0.18 (SD)-fold. However, in the LTR H-ras(A) cells, DEX treatment induced MT content by 3.32 ± 0.76 (SD)-fold (Fig. 7). Thus, concurrent activation of both the glucocorticoid-responsive element in the endogenous MT promoter and c-Ha-ras expression...
resulted in a 2-fold greater level of MT protein, but it is noteworthy that the basal level of MT expression was lower in the c-Ha-ras oncogene-containing cells.

**DISCUSSION**

*In vitro* selection of mammalian cells readily produces cells with resistance to DDP, but it is difficult to obtain cells with greater than 5- to 10-fold resistance without the use of very long periods of selection with unphysiological concentrations of drug. Most patients with ovarian carcinoma who fail i.v. DDP therapy probably have levels of acquired DDP resistance of no greater than 2- to 3-fold (35). The results reported here indicate that DEX-induced overexpression of the mutant c-Ha-ras oncogene produces a large and highly significant degree of DDP resistance when sensitivity was assayed by clonogenic assay and a smaller but still statistically significant effect when assayed by inhibition of DNA synthesis. One of the problems in studying DDP resistance is that there is a high degree of clone-to-clone variance in DDP sensitivity relative to the level of resistance. Individual 3T3 clones containing a constitutively expressed c-Ha-ras oncogene vary more than 12-fold in sensitivity to DDP. The design of these experiments, wherein it was possible to show the change in DDP sensitivity within a single clone of transfected cells in the uninduced and induced state, avoided this criticism of the initial observation by Sklar (21) that cells transfected with either a normal or activated form of the c-Ha-ras gene driven by strong viral promoter demonstrated DDP resistance.

While there are some advantages to comparing the effect of gene induction within a single clone, this strategy also imposed some limitations on the conclusions that can be drawn. For example, rather than being a direct result of the mutant p21<sup>c-Ha-ras</sup> alone, it is possible that the DDP resistance resulted from a permissive effect of p21<sup>c-Ha-ras</sup> that allowed DEX to induce another unidentified resistance gene. Expression of the c-Ha-ras oncogene clearly permitted DEX to induce MT to higher levels than observed in response to DEX in the untransfected 3T3 cells. The selection of an appropriate control for the LTR H-ras(A) cells is not straightforward. The use of a clone of 3T3 cells transfected with a nonmutated form of the c-Ha-ras gene does not avoid the pitfall of having to compare results between clones. In the studies reported here we have shown that DEX treatment of a clone in which the c-Ha-ras oncogene is induced resistance, whereas treatment of the parental cells fails to produce this effect. Although this comparison required the use of slightly different colony-forming assays for the parental and LTR H-ras(A) cells, each was internally controlled.

We have recently reported that activation of the protein kinase C-mediated signal transduction pathway with 12-O-tetradecanoylphorbol-13-acetate significantly enhanced the sensitivity of human ovarian carcinoma 2008 cells to DDP (20). Although understanding of the function of p21<sup>c-Ha-ras</sup> in cellular homeostasis is incomplete, there are strong indications that it participates in a signal transduction pathway (36). Thus, the ability of the c-Ha-ras oncogene product to modulate sensitivity to DDP in the presence of DEX confirms the concept that sensitivity to DDP may be regulated by a variety of different signal transduction pathways that function normally in cellular homeostasis, some of which are clearly abnormal in the malignant state. This has two implications. (a) It suggests that DDP sensitivity may be modulated by growth factors and hormones for which the cell has receptors, and (b) it identifies elements of these pathways as appropriate targets for strategies to prevent or reverse DDP resistance.

The demonstration that expression of the c-Ha-ras oncogene controls DDP sensitivity has some important implications for the clinical use of this drug. One might expect that those categories of tumor that commonly harbor activated forms of ras would be generally more resistant to DDP. In this regard, it is interesting that both tumors for which DDP is particularly effective, testicular and ovarian carcinoma, have been reported to have a low frequency of mutated ras alleles (37), whereas DDP-resistant tumors such as colon and renal cancer have a higher frequency of mutant ras alleles. Since mutant c-Ha-ras expression produces such a large effect on DDP sensitivity, one might expect DDP treatment to select for the survival and growth of those cells in a tumor expressing a mutant allele as a result of somatic mutation. Since DDP is a weak mutagen in mammalian cells (38), DDP treatment may both generate H-ras mutations and promote their outgrowth. In this regard, it is of interest that H-ras codon 61, one of the sites commonly mutated in activated c-Ha-ras, contains one of the sequences at which DDP characteristically produces DNA cross-links (2).

The finding that DDP sensitivity can be regulated by elements of pathways that normally serve to transduce signals from the cell surface to the nucleus suggests that it may be possible to influence DDP sensitivity with growth factors and hormones. This approach is made attractive by the fact that only transient and possibly relatively small changes in the level of DDP resistance may be needed to effect an improved clinical response rate in cases of acquired DDP resistance. Impairment of DDP accumulation (6, 7, 9-11), elevated levels of MTs (14, 15) or GSH (5, 6, 12, 13, 19), and enhanced DNA repair (39) have been most commonly identified as contributing to DDP resistance. Human ovarian carcinoma 2008 cells selected for 10- to 20-fold DDP resistance in vitro have a 50% decrease in DDP accumulation (10, 11); induction of mutant c-Ha-ras expression in NIH 3T3 cells resulted in a 40% decrease in accumulation. We have shown a similar 40% decrease in accumulation in another clone of 3T3 cells (HSV3A) constitutively expressing the v-Ha-ras oncogene (data not shown) (21). In neither case is it likely that this degree of accumulation impairment by itself accounts for all of the resistance. However, both observations suggest that DDP accumulation can be reduced, although whether this regulation occurs at the level of the plasma membrane or at the level of events that serve to trap DDP within the cell is unknown. Induction of c-Ha-ras oncogene-containing cells produced a 3.32 ± 0.76 (SD)-fold increase in MT levels, while NIH 3T3 wild-type cells had only a 1.64 ± 0.18 (SD)-fold increase. However, it should be noted that MT levels were reproducibly lower in LTR H-ras(A) cells compared with NIH 3T3 wild-type cells, and despite the fact that c-Ha-ras transfection led to a greater inducibility by DEX, the biological significance of the difference in the degree of induction is unknown. Although expression of the ras gene has been reported to alter GST activity in other cell types (40), it did not appear to do so in the LTR H-ras(A) cells under conditions producing a large change in DDP sensitivity. Although a variety of mammalian cells can be sensitized to DDP by severely depleting cellular GSH (26), it is not clear that this is due to failure of a normal pathway of DDP detoxification by conjugation with GSH.

Both impairment of DDP uptake and elevation of MTs would

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* S. B. Howell, unpublished observation.
be expected to limit the amount of reactive DDP reaching the nucleus and, thus, decrease the extent of DDP intranuclear cross-link formation. In fact, induction of the c-Ha-ras oncogene expression did decrease the ability of [3H]DEP to form intranuclear cross-links. Although [3H]DEP may not be metabolized exactly the same way as DDP, we have confirmed that c-Ha-ras induction produces the same degree of impairment in the uptake of both agents (data not shown). Our findings are similar to those reported for DDP-resistant murine leukemia L1210 cells which have an increased MT level (14) and a 40% (39) or more than 60% (9) reduction in drug accumulation. There is currently some evidence suggesting that DDP-resistant cells can remove DDP adducts more rapidly (39), but there are no data linking the c-Ha-ras pathway with the process of excision repair which has recently been shown to be capable of removing DDP intranuclear cross-links in HeLa cells (41).

The fos protein is a nuclear transcriptional activator that presumably lies distal to p21-c-Ha-ras in the signal transduction pathway. Sassone-Corsi et al. (42) and Shönthal et al. (43) presented evidence that activation of c-Ha-ras can activate fos. In this study we found that induction of fos expression to levels capable of causing cell transformation did not alter DDP sensitivity at all. These data indicate that, although the c-Ha-ras pathway may include p55/onc, either the DDP resistance results from other elements of the pathway altogether, or it requires other elements in addition to p55/onc.

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