Increased Sensitivity to Cisplatin by nm23-transfected Tumor Cell Lines

Amy W. Ferguson, Ursula Flatow, Nicholas J. MacDonald, Florence Larminat, Villhelm A. Bohr, and Patricia S. Steeg

Women's Cancers Section, Laboratory of Pathology, National Cancer Institute, Bethesda, Maryland 20892 [A. W. F., U. F., N. J. M., P. S. S.], and Laboratory of Molecular Genetics, National Institutes on Aging, Baltimore, Maryland 21224 [F. L. V. A. B.]

Abstract

We report a functional link between expression of the metastasis suppressor gene nm23 and cancer cell sensitivity to the alkylating agent cisplatin. Cisplatin was 2-15-fold more inhibitory to the growth in vitro of nm23 transfectants of the K-1735 TK murine melanoma, MDA-MB-435 human breast carcinoma, and OVCAR-3 human ovarian carcinoma cell lines as compared to matched control transfectants. Administration of a single dose of cisplatin i.v. after injection of control- or nm23-/-transfected K-1735 TK melanoma cells resulted in a more pronounced inhibition of pulmonary metastatic colonization by the nm23-/- transfectants. The mechanism of nm23-dependent sensitivity to cisplatin is unknown, but was correlated with increased formation of interstrand DNA cross-links in nm23-H1 transfected breast carcinoma cells. These data suggest that elevation of tumor cell nm23 expression may be considered as a potential therapeutic strategy in combination with cisplatin treatment.

Introduction

The nm23 gene was discovered on the basis of its reduced expression in highly metastatic murine K-1735 melanoma cell lines (1). Reduced Nm23 expression has subsequently been correlated with poor patient survival or histopathological evidence of high metastatic potential in several human breast carcinoma cohorts (2-8). Similar correlations were observed in cohorts of several other tumor cell types, including human hepatocellular, ovarian, cervical, and gastric carcinomas and melanoma (reviewed in Ref. 9). A causal relationship between increased nm23 expression and reduced tumor metastatic potential was reported in five transfection studies using either breast carcinoma or melanoma cell lines (10-14), establishing that nm23 can have metastasis suppressive capacity.

We have used Nm23 expression levels as a marker of aggressiveness to examine the in vitro sensitivity of human breast carcinoma and melanoma cell lines to chemotherapeutic compounds. Using the COMPARE algorithm, the relative Nm23 expression of a panel of human breast carcinoma and melanoma cell lines in the National Cancer Institute Developmental Therapeutics Program anticancer drug screening program was correlated with their in vitro sensitivity to 171 agents in either clinical practice or development (standard agents). Data were expressed as Pearson correlation coefficients for each standard agent, which could vary from +1.0 (indicating preferential growth inhibition of only high Nm23-expressing cell lines) to 0 (no association with Nm23 expression) to -1.0 (indicating preferential growth inhibition of only low Nm23-expressing cell lines). Pearson correlation coefficients for the 171 standard agents, as compared to Nm23 expression levels, ranged from +0.845 to -0.631. Surprisingly, of the 30 compounds with the highest positive correlation coefficients, 21 of 30 were alkylating agents.

In the present study, we have asked whether high Nm23 expression is correlated with, or alternatively is causally linked to increased tumor cell sensitivity to cisplatin, an alkylating agent with a Pearson correlation coefficient of +0.512 in the Nm23 COMPARE analysis. We report in vitro and in vivo evidence that nm23-transfected cell lines are more sensitive to cisplatin. We also present data suggesting that cisplatin induces greater numbers of DNA ICLs in nm23-transfected cells.

Materials and Methods

Agents. Cisplatin was kindly provided by Dr. V. Narayanan (Chief, Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, National Cancer Institute). For most experiments, cisplatin was prepared fresh and diluted in the dark. Doxorubicin was purchased from Sigma.

Cell Lines. The murine nm23-/- and control-transfected murine K-1735 TK melanoma cell lines and human nm23-H1- and control-transfected human MDA-MB-435 breast carcinoma cell lines have been described (10, 11, 15, 16). The OVCAR-3 human ovarian carcinoma cell line (gift from Dr. E. Kohn) was transfected with pCMVBamneo expression vector, or the same vector containing the nm23-H1 cDNA (11) using the CaPO4 method, and clones exhibiting resistance to G418 were harvested (Life Technologies, Inc., Gaithersburg, MD). Nm23 expression was determined by the presence of an exogenous transcript on Northern blots and increased Nm23 protein levels on Western blots. Two clones which overexpressed Nm23-H1 in both assays were compared to two randomly selected control transfectants. The control- and nm23-H1-transfected clones exhibited similar growth properties upon s.c. or i.p. injection, but failed to metastasize to the liver, lungs, or other organs, precluding testing of the metastatic potential.

In Vitro Proliferation Assays. Cells were trypsinized from subconfluent flasks, washed in complete medium (DMEM containing 10% FCS, 300 mg/liter glutamine, 100 µg/ml streptomycin, 100 units/ml penicillin) (BioWhittaker), and 1-3 x 10^5 cells in 0.5 ml complete medium was plated per well in TC24 tissue culture dishes. Cells were incubated for 3 to 4 h at 37°C in a humidified atmosphere of 5% CO2, 95% air to permit adherence, and cisplatin was added in 0.5 ml medium, in reduced lighting. Cultures were incubated for the indicated time periods. At harvest the medium was gently removed by aspiration, 0.25 ml 0.05% trypsin (Life Technologies, Inc.) was added, and 1-3 x 10^5 cells in 0.5 ml complete medium was plated per well in TC24 tissue culture dishes. Cells were incubated for 5 min or until the cells were visibly detached by examination under an inverted microscope. An equal volume of complete medium was added to stop the trypsinization, and 10 µl trypsin blue were added to discriminate between viable and nonviable cells. The contents of each well were run up and down a pipette to mix the cells and counted in a hemocytometer. At least two grids of the hemocytometer were counted for each well. Three wells were examined per drug concentration unless otherwise noted. Data were calculated as the percentage of control cultures, which were not exposed to cisplatin.

The abbreviations used are: ICL, interstrand cross-link; dhfr, dihydrofolate reductase gene; ds, double stranded; ss, single stranded.

Received 4/9/96; accepted 5/7/96.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Present address: Department of Pathology, University of Michigan, Ann Arbor, MI.
2 Present address: Developmental Genetics Section, Laboratory of Molecular Biology, National Cancer Institute, Bethesda, MD 20892.
3 To whom requests for reprints should be addressed, at Women's Cancers Section, Laboratory of Pathology, National Cancer Institute, Building 10, Room 2A33, Bethesda, MD 20892.
drug. At least two time points were examined for each drug on each cell line. All experiments were repeated three times with essentially equivalent results.

In Vivo Metastasis Assays. Two sets of clonal control and experimental transfectants of the murine K-1735 TK melanoma cell line were utilized as described previously (10). Subclonant cultures were trypsinized and resuspended in PBS. NIH-nu/nu mice were given i.v. injections of 5 x 10^6 or 7.5 x 10^6 cells in 100 μl in the lateral tail vein, depending on the experiment. At 3 days postinjection, mice which received either the control- or nm23-1-transfected cells were randomly assigned to drug groups, typically using 6-10 animals/dose. Mice were given i.v. injections of 100 μl PBS as a control or 50-100 ng cisplatin in PBS in the lateral tail vein. Mice were sacrificed at 3 weeks postinjection, the appearance of gross metastases was noted at autopsy, and the lungs were inflated and preserved in Bouin’s solution. Gross pulmonary metastases were counted, blind to the treatment regimen. H&E-stained sections of the lungs were examined microscopically to confirm that the lesions were of tumor origin. Data are representative of two replicate experiments. All animal experiments were conducted within the guidelines of an approved NIH Animal Use Proposal.

Formation and Repair of DNA ICLs. Quantitation of gene-specific formation and repair of ICLs was performed essentially as described (17). Cells were grown for 3 to 4 days in complete medium supplemented with 0.3 μCi/ml [3H]thymidine (New England Nuclear) and 10 μM cold thymidine. At 70-80% confluence, they were subcultured in label-free media for 24 h. Cells were treated with DMEM containing 0.2% FCS (v/v) FCS and 300 mg/ml l-glutamine, with or without cisplatin, for 5 h. The culture medium was then aspirated, the cells were washed with two changes of PBS, and either lysed immediately or permitted to repair before lysis. Repair medium contained DMEM supplemented with 10 μM bromodeoxyuridine and 1 μM fluorodeoxyuridine to density label DNA replicated during the repair period. All sections of the lungs were examined microscopically to confirm that the lesions were of tumor origin. Data are representative of two replicate experiments. All animal experiments were conducted within the guidelines of an approved NIH Animal Use Proposal.

Results

In Vitro Antiproliferative Effect of Cisplatin on Control- and nm23-transfected Cell Lines. In the experiments summarized in Table 1, three independent sets of control- and nm23-transfected clonal cell lines were incubated in the presence or absence of semilog concentrations of cisplatin, ranging from 1 ng to 10 μg/ml. Cell concentration and viability were determined at 2–11 days of culture. Data were calculated as a percentage of control cultures, which lacked cisplatin, and are listed as the IDso.

For the MDA-MB-435 breast carcinoma cell line, control transfectants exhibited dose-dependent inhibition by cisplatin with an ID50 of 4200–8200 ng/ml. Four-fold overexpression of nm23-H1 by the H1-170 cell line resulted in a 2-fold increased sensitivity to cisplatin, whereas a 9-fold nm23-H1 overexpression resulted in a 6-fold increase in sensitivity (Table 1). The increased sensitivity of the nm23-H1 transfectants to cisplatin was observed over a wide range of active doses (Fig. 1) and over culture periods as long as 11 days (data not shown).

Similar findings were observed with two independent sets of control- and nm23-1-expressing murine K-1735 TK melanoma cell lines. The nm23-1-expressing lines exhibited a 2.5–15-fold increase in sensitivity to cisplatin (Table 1), which was observable through 5 days of culture (data not shown).

Two nm23-H1 transfectants of the OVCAR3 cell line were identified which overexpressed nm23 RNA on Northern blots by 2.4–6.7-fold and Nm23-H1 protein on Western blots by 3.8–5.3-fold as compared to side-by-side randomly selected control transfectants. The nm23-H1 transfectants exhibited no significant differences in growth in vivo, but the lack of metastatic behavior by control transfectants or the parental cell line in vivo precluded metastasis testing (data not shown). Both nm23-H1 transfectants exhibited a 2-fold reduction in IDso when compared to the most sensitive control transfectant (Table 1).

Other Agents. The in vitro inhibitory effect of several other chemotherapeutic agents was determined. Adriamycin, an agent with microtubule active agent, exerted comparable inhibitory effects on all cell lines. The IDso for Adriamycin varied from 25 to 48 μg/ml in the control transfectants, was stable at 25 μg/ml in the H1-170 cell line, but was reduced 4-fold to 0.6 μg/ml in the highest expressing H1-177 cell line. For the OVCAR3 ovarian carcinoma cell line, both the H1-209 and H1-223 nm23-H1 transfectants exhibited some increased sensitivity to Adriamycin, with IDso values ranging from 7.5 to 8.5 μg/ml as compared to control transfectants at 12–15 μg/ml. No difference in Adriamycin sensitivity was observed among control and nm23 transfecants of the K-1735 TK melanoma cell line. Taxol, a microtubule active agent, exerted comparable inhibitory effects on control- and nm23-1 expressing K-1735 TK melanoma cell lines (data not shown).
Cisplatin Increases DNA ICLs Formation in nm23-H1-Transfected Breast Carcinoma Cells. Studies of cisplatin inhibitory effects and the development of tumor cell resistance have identified multiple parameters which mediate or affect its action, including cellular accumulation, DNA damage and repair, cell cycle arrest, induction of cellular apoptosis, cytoskeletal changes, intracellular glutathione, metallothioneins, mdr and other efflux mechanisms, protein kinases, and other enzyme levels. Cisplatin has been reported to induce at least two different DNA adducts. Intrastress adducts constitute the overwhelming majority of DNA adducts formed by cisplatin, which develop between adjacent purines and are repaired by the excision repair process. ICLs form between DNA strands. Although relatively rare (approximately 1-5% of total DNA adducts formed by cisplatin are ICLs), these lesions require a complex repair process involving a homologous recombination (reviewed in Ref. 20). Given the potential for significant DNA damage and breaks by incomplete repair of ICLs, the complexity of its repair, and its reported role in the development of resistance to cisplatin (21), we investigated the formation and repair of ICLs in control and nm23-H1 transfectants. Also, since DNA repair has been shown to vary in rates between transcribed and nontranscribed DNA (17, 20, 21), we determined gene-specific adduct formation and repair rates.

Two MDA-MB-435 cell lines, a control transfectant C-100, and the highest nm23-H1-expressing transfectant H1-177 were evaluated for formation and repair of ICLs in the nm23-H1 and dhfr gene sequences in response to 100–200 μM cisplatin (Table 3). Briefly, cells were treated with 200 μM cisplatin and permitted to repair for 0 to 24 h in the presence of bromodeoxyuridine. Total DNA was then purified, restricted with EcoRI, and the newly repaired, less dense DNA was isolated from bromodeoxyuridine incorporated replicated DNA on cesium chloride gradients. A modified Southern blot was performed to assess gene-specific ICL formation and repair. DNA was denatured in NaOH, which will only occur if ICLs are not present, electrophoresed in a neutral gel, and processed as a Southern blot using the nm23-H1 cDNA probe. Isolated DNA is then subjected to alkaline treatment, which will only occur if ICLs are not present, electrophoresed on a neutral gel, and processed as a Southern blot using the nm23-H1 cDNA probe. The abundance of ds (cross-linked) and ss (native) DNA fragments was determined with densitometry. Percentage of ICLs was calculated as: % ICL = dsDNA/ddDNA + ssDNA.

In Vivo Effect of Cisplatin. We have asked whether the increased sensitivity of nm23 transfectants to cisplatin in vitro was relevant to metastatic progression in vivo. The K-1735 TK transfectants were selected for in vivo experimentation due to the availability of a rapid, quantitative experimental metastasis assay. The remaining cell lines under investigation herein require spontaneous metastasis assays in vivo, which would need large numbers of animals, surgery to remove the primary tumors, long times postinjection, and would ultimately give "yes-no" answers that are more difficult to quantitate. In the experiments summarized in Table 2, the effect of cisplatin was determined on the outgrowth of pulmonary metastases of two sets of control-transfected and murine nm23-I-transfected K-1735 TK cells. To more closely approximate the course of human disease, tumor cells were injected into the lateral tail vein and permitted to lodge and extravasate in the lungs for several days prior to cisplatin treatment. A single injection of cisplatin or vehicle alone was then delivered i.v. Gross pulmonary metastases were quantitated at autopsy 3 weeks later.

In both sets of control and nm23-I transfectants, the nm23-I transfectants produced quantitatively fewer gross pulmonary metastases, confirming previously published data (10) for the tissue culture passages used herein. Cisplatin treatment exerted a variable effect on the controls, ranging from no inhibition to 26% reductions in gross pulmonary metastases. In both sets, however, the metastatic outgrowth of the nm23-I transfectants was inhibited to a greater extent by administration of cisplatin, confirming the in vitro data for this series of cell lines.

Table 2 Effect of cisplatin in vivo on experimental metastatic potential of control- and nm23-I-transfected K-1735 TK murine melanoma transfectants

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Construct</th>
<th>Cisplatin (ng)</th>
<th>Mean (SEM) pulmonary metastases/mouse</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>2-4</td>
<td>Control</td>
<td>0.09 (5.2)</td>
<td>35.7 (19.0)</td>
</tr>
<tr>
<td></td>
<td>2-4</td>
<td>nm23-1</td>
<td>0.50 (5.3)</td>
<td>18.2 (9.1)</td>
</tr>
<tr>
<td>Experiment 1</td>
<td>2-4</td>
<td>Control</td>
<td>0.09 (5.2)</td>
<td>35.7 (19.0)</td>
</tr>
<tr>
<td></td>
<td>2-4</td>
<td>nm23-1</td>
<td>0.50 (5.3)</td>
<td>18.2 (9.1)</td>
</tr>
</tbody>
</table>

*Seventy-five thousand viable cells were injected i.v. into the lateral tail vein of mice. On day 3 postinjection, mice received a single i.v. injection of cisplatin in PBS or PBS alone. Gross pulmonary metastases were counted at autopsy, 3 weeks postinjection.

*Comparison of control and 100-ng treatments, P = 0.08, t test.

*Comparison of control and 100-ng treatments, P = 0.013, t test.

2933

**In Vivo Effect of Cisplatin**. We have asked whether the increased sensitivity of nm23 transfectants to cisplatin in vitro was relevant to metastatic progression in vivo. The K-1735 TK transfectants were selected for in vivo experimentation due to the availability of a rapid, quantitative experimental metastasis assay. The remaining cell lines under investigation herein require spontaneous metastasis assays in vivo, which would need large numbers of animals, surgery to remove the primary tumors, long times postinjection, and would ultimately give "yes-no" answers that are more difficult to quantitate. In the experiments summarized in Table 2, the effect of cisplatin was determined on the outgrowth of pulmonary metastases of two sets of control-transfected and murine nm23-I-transfected K-1735 TK cells. To more closely approximate the course of human disease, tumor cells were injected into the lateral tail vein and permitted to lodge and extravasate in the lungs for several days prior to cisplatin treatment. A single injection of cisplatin or vehicle alone was then delivered i.v. Gross pulmonary metastases were quantitated at autopsy 3 weeks later.

In both sets of control and nm23-I transfectants, the nm23-I transfectants produced quantitatively fewer gross pulmonary metastases, confirming previously published data (10) for the tissue culture passages used herein. Cisplatin treatment exerted a variable effect on the controls, ranging from no inhibition to 26% reductions in gross pulmonary metastases. In both sets, however, the metastatic outgrowth of the nm23-I transfectants was inhibited to a greater extent by administration of cisplatin, confirming the in vitro data for this series of cell lines.

### Table 3 Formation and repair of gene-specific ICLs in control- and nm23-H1 transfected MDA-MB-435 breast carcinoma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cisplatin concentration (μM)</th>
<th>Repair time (h)</th>
<th>% ICLs</th>
<th>0</th>
<th>4</th>
<th>8</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td>C-100</td>
<td>200</td>
<td>39.4</td>
<td>25.4</td>
<td>24.7</td>
<td>10.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H1-177</td>
<td>200</td>
<td>74.3</td>
<td>46.3</td>
<td>42.3</td>
<td>23.5</td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td>C-100</td>
<td>200</td>
<td>34.2</td>
<td>1.5</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>H1-177</td>
<td>100</td>
<td>32.7</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

*Cells were prelabeled with [3H]thymidine, treated with cisplatin as indicated, and permitted to repair in the presence of bromodeoxyuridine. Total DNA was then purified, restricted with EcoRI, and the newly repaired, less dense DNA was isolated from bromodeoxyuridine incorporated replicated DNA on cesium chloride gradients. A modified Southern blot was performed to assess gene-specific ICL formation and repair. DNA was denatured in NaOH, which will only occur if ICLs are not present, electrophoresed in a neutral gel, and processed as a Southern blot using the nm23-H1 cDNA probe.
(Table 3) and dhfr (data not shown) genes as probes. Formation of ICLs is evident as a dsDNA fragment resistant to NaOH denaturation. With repair, loss of the ds fragment is observed, typically with a concomitant gain of hybridization intensity at the position of the ss native fragment.

At 200 μM cisplatin, formation of ICLs in the nm23-H1 transfected cell line (Table 3) was approximately double that of the control C-100 line, when tested for either nm23-H1 (0-h repair, Table 3) and dhfr (data not shown) gene sequences. Approximately three quarters of the ds fragment was lost (repaired) over 24 h of culture in the C-100 line, and two thirds was lost in the H1-177 line under the same conditions. The data suggest a preferential induction of DNA ICLs in the H1-177 line but no significant difference in repair rates.

Since DNA repair rates can be influenced by the amount of damage sustained, repair rates were also quantified under conditions of equivalent ICL induction (Table 3, experiment 2; data not shown). Culture of C-100 cells in twice the amount of cisplatin as H1-177 cells afforded ICL induction (Table 3, experiment 2: data not shown). Culture of C-100 cells in twice the amount of cisplatin as H1-177 cells induced comparable amounts of ICLs, confirming the increased sensitivity of the H1-177 cell line. Repair rates under these conditions were essentially the same.

Discussion

Using three sets of control- and nm23-transfected cell lines, we report that nm23 overexpression results in increased sensitivity to cisplatin inhibition of cell growth in vitro. Dose-response analyses, shown for the MDA-MB-435 breast carcinoma cell lines but extending to other sets as well, indicate that the increased sensitivity of nm23 transfectants to cisplatin extended over a wide range of active doses. The observation that the cisplatin concentration, at which control transfectants become resistant (i.e., the cells grow in cisplatin as well as without it) is 1–2 logs higher than that of the nm23-H1 transfectants (Fig. 1), may better indicate the potential significance of these data to clinical situations than ID_{50} data. These data were partially confirmed in vivo, where the pulmonary metastatic capacity of nm23-1 transfectants of the K-1735 TK melanoma cell line was more inhibited by a single injection of cisplatin than were control transfectants. Additional in vivo testing of control and nm23 transfectants will be needed to determine the generality of these findings. The long time postinjection and poor quantitation of spontaneous metastasis assays currently used for many human tumor cell lines makes these experiments extremely difficult.

The data presented herein are supported by two independent lines of investigation. First, our previous correlative studies compared the Nm23 expression of a panel of human breast carcinoma and melanoma cell lines to their ability to be growth-inhibited in vitro by 171 standard agents using Pearson correlation coefficients for quantitation. Of the 30 agents with the highest Pearson correlation coefficients, i.e., the greatest preferential inhibitory activity against high Nm23-expressing cell lines, 21 were alkylating agents. In the present report, cisplatin was found to be more preferentially inhibitory in vitro to nm23 transfectants than another agent with alkylating activity, Adriamycin. These data stand in agreement with their Pearson correlation coefficients in the COMPARE analysis.

Using three sets of control- and nm23-transfected cell lines, we report that nm23 overexpression results in increased sensitivity to cisplatin inhibition of cell growth in vitro. Dose-response analyses, shown for the MDA-MB-435 breast carcinoma cell lines but extending to other sets as well, indicate that the increased sensitivity of nm23 transfectants to cisplatin extended over a wide range of active doses. The observation that the cisplatin concentration, at which control transfectants become resistant (i.e., the cells grow in cisplatin as well as without it) is 1–2 logs higher than that of the nm23-H1 transfectants (Fig. 1), may better indicate the potential significance of these data to clinical situations than ID_{50} data. These data were partially confirmed in vivo, where the pulmonary metastatic capacity of nm23-1 transfectants of the K-1735 TK melanoma cell line was more inhibited by a single injection of cisplatin than were control transfectants. Additional in vivo testing of control and nm23 transfectants will be needed to determine the generality of these findings. The long time postinjection and poor quantitation of spontaneous metastasis assays currently used for many human tumor cell lines makes these experiments extremely difficult.

The data presented herein are supported by two independent lines of investigation. First, our previous correlative studies compared the Nm23 expression of a panel of human breast carcinoma and melanoma cell lines to their ability to be growth-inhibited in vitro by 171 standard agents using Pearson correlation coefficients for quantitation. Of the 30 agents with the highest Pearson correlation coefficients, i.e., the greatest preferential inhibitory activity against high Nm23-expressing cell lines, 21 were alkylating agents. In the present report, cisplatin was found to be more preferentially inhibitory in vitro to nm23 transfectants than another agent with alkylating activity, Adriamycin. These data stand in agreement with their Pearson correlation coefficients in the COMPARE analysis.

Second, Bookman and Ozols (22) Scambia et al. (23) recently reported an immunohistochemical and RNA expression analysis of an ovarian carcinoma cohort (n = 106) and correlated the expression data to histopathological and patient clinical course survival. High Nm23 expression was correlated to longer progression-free survival (P = 0.0056). Additionally, among 56 patients with stage II-IV disease, a significantly higher percentage of complete or partial responses to platinum-based therapy was observed in patients with high Nm23-expressing tumors (69%) as compared to those with low Nm23-expressing tumors (44%; P = 0.03). These data suggest that additional cohort studies may be warranted to define further the correlation of Nm23 expression and responsiveness to cisplatin.

Although not used widely in the clinic, cisplatin has received renewed interest with respect to breast and other cancers. Expression of adenovirus E1a (24), the epidermal growth factor receptor (25, 26), Her-2/neu (27, 28), and p53 (29) have been reported to influence cellular sensitivity to cisplatin, some of which may have application in cancer therapy. The mechanism(s) of altered breast cancer cell sensitivity to cisplatin induced by gene overexpression or underexpression is unknown, but has been associated with alterations in DNA repair rates, cell cycle checkpoint control, or apoptosis. Our data suggest that higher levels of interstrand DNA cross-links are formed after exposure to cisplatin in nm23-transfected breast carcinoma cells, but that repair rates remain constant. Further definition of these effects with respect to other cisplatin-induced DNA lesions and other sets of control- and nm23-transfected cell lines are warranted. We observed that apoptosis, induced by DNA laddering on agarose gels, was highest in the highest nm23-H1-expressing H1-177 MDA-MB-435 breast carcinoma cell line (data not shown). However, the nm23-H1-transfected H1-170 cell line exhibited DNA laddering levels equivalent to those of the control transfectants. Multiple and complex mechanisms may therefore explain these data.

Based on transfection and cohort studies, we have postulated that elevation of tumor cell Nm23 expression may be of clinical benefit to limit metastatic spread and colonization of breast carcinoma and melanoma cells and possibly other cancer cell types as well. Current research is attempting to define those elements of the nm23-H1 promoter responsible for its higher level of expression in nonmetastatic breast carcinoma cell lines. Screening experiments are planned to identify compounds which can enter a cell and activate this promoter. If found, the current data suggest that they may be of use in combination with cisplatin or other agents identified with high Pearson correlation coefficients in the COMPARE analysis.

References


2934

Downloaded from cancerres.aacjournals.org on July 20, 2017. © 1996 American Association for Cancer Research.
nm23 AND CISPLATIN SENSITIVITY


Increased Sensitivity to Cisplatin by \textit{nm23}-transfected Tumor Cell Lines

Amy W. Ferguson, Ursula Flatow, Nicholas J. MacDonald, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/56/13/2931

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.