Aberrant p53 Expression Predicts Clinical Resistance to Cisplatin-based Chemotherapy in Locally Advanced Non-Small Cell Lung Cancer

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ABSTRACT

The development of cisplatin-based induction chemotherapy followed by surgical resection or radiation has improved the poor prognosis of stage III non-small cell lung cancer (NSCLC). In vitro studies indicate that p53 alterations lead to cisplatin resistance in NSCLC cells, but the molecular genetic features determining response or resistance to cisplatin in vivo must be defined. For this reason, tumor specimens from 52 patients with stage III NSCLC entered in a prospective clinical trial of cisplatin-based induction chemotherapy followed by surgical resection were examined for p53 expression by immunohistochemical staining before and after induction chemotherapy. p53 expression was correlated with clinical and pathological response using Fisher's exact test. No correlation was established between p53 expression and clinical response because 47 of the 52 patients studied had a major response. However, a significant association was observed between aberrant p53 expression and resistance to chemotherapy as assessed by pathological response. Only 3 of the 20 patients whose tumors exhibited a high level (++) of p53 staining experienced a major (+ + + to + + + +) pathological response to chemotherapy. Only 7 of 52 cases examined before and after chemotherapy treatment exhibited a change in the level of p53 expression after cisplatin-based chemotherapy. These results indicate that cisplatin alters p53 expression infrequently and suggest a direct link between aberrant p53 expression and resistance to cisplatin-based chemotherapy in NSCLC.

INTRODUCTION

Lung cancer is a formidable problem. There are more than 170,000 cases of lung cancer each year in the United States, and it is the most common cause of cancer-related deaths in both men and women. Each year, approximately 40,000 patients with NSCLC are present with locally advanced tumors (stage IIIA or IIIB). Traditional treatment of stage III tumors with surgical resection or radiation alone yielded 5-year survival rates of only 5–10% (1). Recently, multimodality treatment using induction chemotherapy or chemoradiotherapy followed by surgical resection or chemotherapy has improved the poor prognosis of stage III NSCLC. The details of this trial, performed from 1984 to 1991, were described previously (7). In brief, the patients enrolled in this trial had stage IIIA NSCLC that was confirmed pathologically and untreated at clinical presentation and response to cisplatin chemotherapy. The study was conducted primarily in fibroblast cell lines, showing that p53-dependent apoptosis mechanisms appear to be involved in the cytotoxicity induced by ionizing radiation and by several anticancer agents including 5-fluorouracil, etoposide, and doxorubicin (10–12). In addition, adenovirus-mediated transfer of the wild-type p53 gene into monolayer or multicellular spheroid tumor cultures of a human NSCLC cell line with a homozygous deletion of p53 markedly increased the sensitivity of the cells to cisplatin (13). This suggests a direct link between wild-type p53 expression and cisplatin-mediated cytotoxicity in lung cancer. Additional clinical evidence for a relationship between wild-type p53 and resistance to chemotherapy is found in another solid tumor, germ cell cancer. Germ cell tumors rarely exhibit p53 mutations and are quite sensitive to cisplatin-based chemotherapy (14).

Taken together, these findings indicate that p53 might regulate cisplatin response in NSCLC. Although this hypothesis is consistent with the available in vitro data, our knowledge of the cisplatin response has not as yet been tested in the in vivo setting. A large clinical trial at Memorial Sloan-Kettering Cancer Center (New York, NY) evaluating the use of induction cisplatin-based chemotherapy in stage IIIA NSCLC permitted us to explore the relationship between p53 expression and response to cisplatin chemotherapy. The patients who participated in this single-institution trial were carefully staged and were a clinically homogeneous group. Almost all patients had an initial mediastinoscopy, which made tissue available for immunohistochemical staining before chemotherapy treatment. The induction regimen consisted of high-dose cisplatin-based chemotherapy without concurrent radiation therapy. The surgical management of patients who responded to chemotherapy was uniform and included thorough intrathoracic staging. The clinical and histopathological evaluations of response and the long-term outcome of patients enrolled in the study have been analyzed and reported previously (7). This study reports findings indicating that aberrant p53 expression is linked to clinical resistance to cisplatin-based chemotherapy in these NSCLC patients.

MATERIALS AND METHODS

Clinicopathological Features. The tumors analyzed in this study were obtained from patients enrolled in a prospective clinical trial of neoadjuvant therapy for stage IIIA NSCLC. The details of this trial, performed from 1984 to 1991, were described previously (7). In brief, the patients enrolled in this trial had stage IIIA NSCLC that was confirmed pathologically and untreated previously. These patients received two or three cycles of an induction chemotherapy regimen containing cisplatin (120 mg/m²) and mitomycin (8 mg/m²) on days 1, 29, and 71 and vinblastine (4–4.5 mg/m²) or vindesine (3 mg/m²) on days 1, 8, 15, 22, and 29 and subsequently every 2 weeks. Patients experiencing a partial or complete radiographic response after induction therapy underwent thoracotomy to resect residual tumor 4–6 weeks after the final dose of cisplatin. Of 136 patients entered in this study, 105 (77%) experienced a major radiographic response, 98 (72%) underwent thoracotomy, and 82 (60%) had a complete resection. With a minimum follow up of 12 months, the...
were baked at 60°C for 2 h immediately before staining. The sections were
expression.

Cases were selected for this study only when tumor specimens were available
both before and after chemotherapy. This criterion permitted both corre-
lution of the pathological response with p53 expression before chemotherapy
and a determination of whether induction chemotherapy altered p53
expression.

Histopathological Methods. Five-μm sections were cut from archival
paraffin blocks and placed on superfrost/Plus microscope slides. The slides
were baked at 60°C for 2 h immediately before staining. The sections were
deparaffinized in xylene and rehydrated through graded alcohol steps
to distilled water. The sections were placed in a citrate buffer solution (2.1 g
of citric acid in 1 liter of distilled water, buffered to pH 6.0 with NaOH)
in a microwaveable container. The slides were microwaved in a citrate
buffer at 560 W for two 5-min cycles, and distilled water was added if
needed to correct for evaporation. The sections were cooled in the solution
for 20 min at room temperature, rinsed in distilled water, and transferred to
a PBS bath. The sections were then placed in a 0.05% BSA/PBS bath for
1 min, and normal horse suppressor serum diluted 1:20 in 2% BSA/PBS
was added for 10 min. The normal horse suppressor serum was removed,
and the sections were incubated overnight with the primary antibody
diluted in 2% BSA/PBS at 4°C. The following day the primary antibody
was removed from the slides by rinsing in three changes of PBS. The
secondary antibody (biotinylated horse anti-mouse IgG diluted 1:500 in 1%
BSA/PBS) was applied for 60 min. The slides were rinsed in three changes
of PBS, and peroxidase-conjugated streptavidin was applied for 60 min
diluted 1:500 in 1% BSA/PBS). Diaminobenzidine was used as the chro-
mogen. The slides were counterstained with hematoxylin, dehydrated, and
coverslipped. The mAb used in this study was pAb1801 (Oncogene, Union-
dale, NY) at a dilution of 1:500.

A negative control for the antibody was performed using nonimmune
instead of the primary antibody. The positive control consisted of a pulmonary
squamous cell carcinoma known to exhibit nuclear p53 expression using the
p53 mAb.

Assessment of Histopathological Response. Pathological changes in the
resected posttherapy tumors varied from case to case and between different
regions of individual tumors. Changes regarded as reflecting a response to
therapy included evidence of necrosis and fibrosis. Foci of necrotic tumor were
noted less frequently and were occasionally difficult to distinguish from tumor
necrosis, which is often encountered in untreated high-grade NSCLC. The
areas of fibrotic tumor were distinct. Paucicellular regions of hyalinized
massive with abundant small vessels and sparse inflammatory cells were found (Fig.

Fig. 1. a, b, and c. histological appearance of
NSCLC after postoperative chemotherapy. a. tu-
mors showing near-complete response consist
largely of paucicellular fibrous tissue with numer-
ous small vessels and scattered lymphocytes. A
focus of residual viable adenocarcinoma is present
(arrows). b, in tumors with minimal treatment ef-
flect, there is focal inflammation and fibrosis only:
the tumor cells show cytomegaly and nuclear en-
largenent (arrows), which is better seen at higher
magnification (c; X 40).

RESULTS

Adequate pathological material for this study was available from
52 of the 82 patients who underwent resection of their tumor after
induction chemotherapy. The 30 cases viewed as unsuitable for
this study either had inadequate archival material for analysis or
had outside slides of mediastinal nodal biopsies that were unavail-

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Table 1  Correlation between partial or complete clinical response and p53 immunohistochemical staining before chemotherapy

<table>
<thead>
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<th>Immunohistochemical staining before chemotherapy</th>
<th>Complete response</th>
<th>Partial response</th>
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<td>1+</td>
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<td>7</td>
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</tr>
<tr>
<td>Total</td>
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</tr>
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</table>

Table 2  Correlation between pathological response and p53 immunohistochemical staining

<table>
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<th>Pathological Responsea</th>
</tr>
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<td>0-1 2-4</td>
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<tr>
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<td>2+</td>
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<tr>
<td>3+</td>
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<tr>
<td>4+</td>
<td>0 3 1 0 0</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>16 12 7 10 7</td>
<td></td>
</tr>
</tbody>
</table>

a When tumor specimens are grouped together into high and low categories only 3 of the 17 patients with a +++ or ++++ pathological response had a high level (++ to ++++) of p53 staining before chemotherapy.

Table 3  Comparison between level of p53 immunohistochemical staining before and after chemotherapy

<table>
<thead>
<tr>
<th>Before chemotherapy</th>
<th>After chemotherapy</th>
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</thead>
<tbody>
<tr>
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<td>1+ 2+ 3+ 4+</td>
</tr>
<tr>
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<td>18 1 0 1 0</td>
</tr>
<tr>
<td>1+</td>
<td>3 3 0 0 0</td>
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<tr>
<td>2+</td>
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<tr>
<td>3+</td>
<td>1 2 2 1 0</td>
</tr>
<tr>
<td>4+</td>
<td>0 1 0 1 2</td>
</tr>
</tbody>
</table>

a Cases in which there were complete pathological responses after induction chemotherapy could not be assessed because there was no residual viable tumor on which to perform immunohistochemical staining.

The comparison between pre-p53 expression and clinical response is shown in Table 1. A total of 47 patients had a major objective response, but the response was partial in 42 of these patients. Of the 47 patients, absent p53 staining was found in 22 specimens and positive staining was observed in 8 specimens before chemotherapy. Seventeen specimens revealed ++ to ++++ staining.

The correlation between pre-p53 expression and pathological response is shown in Table 2. A total of 36 patients had a pathological response. This was scored as + to ++ in 19 patients and as +++ to ++++ in 17 patients. Because of the small numbers of patients, the levels were collapsed before a Fisher's exact test was used to test for association. The 20% cutpoint in the percentage of cells staining was chosen by the pathologist involved in this study (D. K.) as the level at which staining could be consistently defined as positive across all samples examined. No association between p53 expression and clinical response was found because of the large proportion of clinical responses which were partial. A statistically significant association was found when p53 expression and pathological response were grouped into the low and high categories. Only 3 of the 20 patients who had tumors exhibiting a high level (+++ to ++++) of p53 tumor staining before chemotherapy had a ++++ to ++++ pathological response (P = 0.04).

The comparison between p53 expression before and after chemotherapy is shown in Table 3. This comparison could not be made in cases in which a major pathological response to chemotherapy offered little or no viable tumor on which to perform staining for p53. In 27 of the 44 evaluable specimens, there was no change in the level of p53 staining (Fig. 2). In 14 cases, the level of p53 staining decreased, and in 3 cases it increased. However, in only 7 cases did the level of p53 positivity change by more than one level from the prechemotherapy to the postchemotherapy specimen. Therefore, most of the changes observed were small enough to be caused by technical factors, such as variations in fixation or variable sampling in tumors with heterogeneous p53 expression.

![Fig. 2. a. and b, immunohistochemical staining for p53. Both pretreatment tumor from mediastinal lymph nodes (a) and posttreatment tumor (b) show intense nuclear positivity. The surrounding lymphocytes and stromal cells are negative.](image-url)
DISCUSSION

Patients with stage III NSCLC constitute a large and important segment of the lung cancer population, representing approximately 40,000 cases annually in the United States. Previously, treatment with radiation therapy or surgical resection alone offered most patients with stage III disease only a 10% or lower chance of survival at 5 years. These poor survival rates are related to the locally advanced nature of the primary tumor and the presence of micrometastatic disease. Recently, combined modality regimens using chemotherapy as the induction therapy have significantly improved the prognosis of stage III NSCLC patients (2-7). The chemotherapy drug common to all of these regimens is high-dose cisplatin. A complete pathological response to induction chemotherapy identifies those patients with the most favorable long-term survival (7). However, only 20% or fewer of patients develop a complete pathological response, and 20–40% of patients do not respond to induction chemotherapy. Understanding the molecular genetic features that determine response or resistance to cisplatin-based chemotherapy should permit selection of the most suitable patients for neoadjuvant therapy. This would avoid exposing some patients to ineffective treatment and could tailor chemotherapy treatment to those stage III NSCLC patients most likely to respond. This could enhance development of innovative treatment for patients most likely to be refractory to cisplatin-based chemotherapy.

The precise mechanisms responsible for cisplatin-mediated toxicity are not fully understood. In vitro studies indicate cisplatin inhibits DNA synthesis by causing double-strand breaks (15), leading to apoptosis at the G2-M transition (16). In vitro studies also indicate a link between wild-type p53 and chemotherapy-induced apoptosis. A similar relationship is reported for cisplatin in NSCLC cell lines (13), esophageal adenocarcinoma, and gastric carcinoma cell lines (17). Related in vitro studies suggest that wild-type p53 is involved in growth suppression of malignant cell lines induced by radiation and several chemotherapeutic agents thought to function through apoptosis (11, 12, 18–21). The hypothesis that p53 is an important regulator of the clinical response to cisplatin is supported by the observation that germ cell cancer, a solid tumor that is highly sensitive to cisplatin, almost never exhibits p53 mutations (14). This report extends previous in vitro work by exploring the relationship between p53 expression in tumors and clinical treatment response to cisplatin-based chemotherapy in stage III NSCLC.

The results of this study reveal that the absence of p53 immunostaining before chemotherapy does not correlate statistically with pathological response to induction with cisplatin-based chemotherapy in this clinical setting. However, aberrant p53 expression was significantly associated with a lack of pathological response to cisplatin-based chemotherapy. It is recognized that there often is a discrepancy among clinical, radiographic, and pathological responses after induction therapy of NSCLC. Pathological response provides the most precise measure of the clinical effects of induction treatment (1, 5, 8). Although cisplatin may act through p53-independent pathways (22–25), aberrant p53 expression predicts resistance to cisplatin treatment of the lung cancer patients examined in this study. The number of cases available for analysis in this study is too small to allow statistically valid correlations between the immunohistochemical results and overall survival. However, the previous analysis of the clinical trial from which these samples were drawn showed a significant correlation between complete pathological response and overall survival (7). Therefore, our results have important clinical implications.

Some factors could complicate the interpretation of the findings of this study. Most of the prechemotherapy specimens available for analysis were mediastinal nodal metastases. It is conceivable that p53 expression in nodal metastases does not fully reflect the pattern of p53 expression found in the primary tumor. Arguing against this possibility are previous reports indicating that p53 mutations found in a primary lung cancer are conserved in the metastases from that tumor (26). Therefore, the specimens used in this study should be useful for analysis.

A larger prospective study is needed to confirm the findings of this report. It is notable that the induction regimen in this trial included a Vinca alkaloid and mitomycin. How this combination regimen modulates the interactions between cisplatin and p53 remains to be determined. However, the finding of a correlation between p53 expression and a response to cisplatin chemotherapy reported in this study is more likely to be accurate than an analysis performed in other neoadjuvant trials using concurrent cisplatin and radiation for induction therapy. A chemotherapy-only induction regimen prevents the potentially complicating effect of radiation, which also is thought to inhibit tumor cell growth via p53-dependent apoptosis mechanisms (12).

Finally, immunohistochemical staining does not distinguish between p53 expression that is abnormal because of p53 mutations and expression that is abnormal because of deregulated expression of a structurally normal p53 protein (27). Several mechanisms can lead to p53 protein overexpression in primary NSCLC, and the concordance rate between mutations and overexpression as assessed by immunohistochemical staining is reported to be only 67% (28). It is viewed that aberrant p53 protein expression as assessed by immunohistochemical techniques provides a clinically useful measure of deregulated p53 function in NSCLC.

In summary, the results reported here show a statistically significant link between aberrant p53 expression and cisplatin-based chemotherapy resistance. This link is not yet strong enough to dictate clinical treatment decisions in stage III NSCLC. These findings also indicate that this cisplatin-based treatment infrequently alters p53 expression, and that the absence of p53 staining does not predict increased clinical sensitivity to this combination chemotherapy regimen. Which other cell cycle regulators act in concert with or independent of p53 to elicit these beneficial clinical responses remains to be determined. The role of Rb, cyclin-dependent kinases, or inhibitors in mediating these clinical responses warrants study. Additional work is needed to identify those molecular pathways that mediate major clinical chemotherapy responses in NSCLC, particularly in the setting of wild-type p53.

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