Accumulation of p53 Protein Correlates with a Poor Prognosis in Human Lung Cancer

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Abstract

Mutations in the gene coding for the p53 tumor suppressor protein are common in a variety of human cancers. To assess the role of a putative mutated p53 protein in human lung cancer, a monoclonal antibody recognizing it was used in an immunoperoxidase detection system. A total of 114 cases of Stage I and II adenocarcinomas and squamous cell carcinomas were studied. The staining pattern was always intranuclear and heterogeneous. When the median or mean survival time was compared between cases, p53 accumulation had a statistically significant negative prognostic value. This was supported by a Kaplan-Meier survival plot of p53 producers and nonproducers. In 7 of 24 Stage II cases that were negative for p53 in the primary tumor, metastatic regional lymph nodes were p53-positive. These latter cases had greatly reduced survival times. Thus, p53 accumulation in primary tumors (and regional lymph nodes) may identify a subgroup of lung cancer patients with a prognosis of more aggressive disease.

Introduction

The p53 gene was originally identified as an oncogene (1) but is now recognized as an antiproliferation gene. The wild-type p53 tumor suppressor gene codes for a protein which exhibits growth and division suppressive activity against a variety of transformed cell types, including a human glioblastoma cell line (2) and a human colorectal adenocarcinoma cell line (3). Mutated forms of the p53 gene are found in numerous human neoplasms (4, 5). For example, in the inherited Li-Fraumeni syndrome (6), affected offspring have a mutated p53 allele in every somatic and germline cell. These individuals have a 50% probability of developing one of at least 5 tumors by the age of 30 years: cancers of the breast, bone, and brain as well as leukemia and several soft tissue sarcomas. Some of the p53 mutations are dominant-negative. That is, a mutation in one allele also results in a loss of function for any p53 coded for by the remaining wild-type allele. One model to explain this (7) postulates that normal p53 functions as a homodimer or homologomer. It is known that this protein is a nuclear transcription regulator (8), displaying DNA base sequence-specific binding (9). As such, it may regulate the expression of other social control genes. Evidence has been presented that cotranslation of mutant and wild-type p53 allele mRNAs can result in a dimer or oligomer with an altered conformation, such that its normal function is abolished. Alternatively, p53 mutations may result in a gain-of-function whereby the protein actually stimulates growth (10). Numerous studies have shown an association between human NSCLC and p53 gene mutations (11-16). p53-specific monoclonal antibodies (potentially reactive with both wild-type putative mutated forms of p53) have been used immunocytochemically to detect the putative mutated protein in human lung cancer tissue (15-17). No unequivocal evidence exists to show whether the accumulation of p53 protein has prognostic value in lung cancer. The data presented here represent a retrospective study in which 114 cases of NSCLC were evaluated in terms of p53 accumulation. Statistical analysis demonstrates a strong correlation between p53 deposition in lung cancer cell nuclei and subsequent early death. Therefore, we believe p53 detection can be a useful prognostic tool in human NSCLC.

Materials and Methods

Source of Tissue. Paraffin blocks of primary tumors and regional lymph nodes of cases of NSCLC were used. Of a total of 114 cases, 48 were adenocarcinoma and 66 were squamous cell carcinoma. All cases, obtained from the West Virginia Department of Pathology, were Stage I (n = 72) or Stage II (n = 42) and had been operated on by the West Virginia University Health Sciences Center, Department of Surgery, from 1974 to 1988. Patient age ranged from 37 to 79 years (mean, 61 years). Data on tumor histology, tumor size, and lymph node status were obtained from the pathology reports. Survival information was obtained from hospital charts and the West Virginia University Health Sciences Center Tumor Registry. Survival was expressed as number of months from the date of primary surgery until the date of death from recurrent disease.

Immunohistochemistry. Five-μm sections were cut from paraffin blocks and allowed to air dry. A modification of the method of Hsu et al. (18) was used for the immunostaining. The slides were deparaffinized in xylene (3 times for 5 min each) and absolute alcohol (2 times for 2 min each) and treated with 0.3% hydrogen peroxide-methanol (10 min) to remove endogenous peroxidase activity. Following a H2O rinse, the slides were pretreated with a 1 mg/ml solution of trypsin (Difco) for 5 min. Then slides were treated for 30 min with PBS (Gibco) containing 0.15% bovine serum albumin (Sigma A-9647). To reduce background staining, normal horse serum (5% in PBS; Gibco) was placed on the slides for 30 min. The normal horse serum was allowed to run off, and the slides were incubated overnight with the primary antibody at 4°C in a moist chamber. The primary anti-p53 monoclonal antibody was PAb-1801 (Ab-2; Oncogene Science). This antibody recognizes both wild-type and mutant p53 forms. A dilution of 1:200 (0.5 μg/ml) was used for this antibody as well as the negative control antibody (MOPC-21; BTI). After washing with PBS for 30 min at room temperature, the slides were incubated with the secondary antibody (biotinylated anti-mouse IgG; Vector Laboratories). A dilution of 1:250 (0.6 μg/ml) was used. Following a PBS wash, the slides were then incubated with the avidin-biotin-peroxidase reagent (Vector Elite) for 30 min. After a PBS wash the antigen-antibody complex was visualized using a 0.05% solution of diaminobenzidine tetrahydrochloride in PBS. The slides were then counterstained with Mayer’s hematoxylin, blued with NH4H2O2, dehydrated, and coverslipped with Permount.

Analysis. Nuclear p53 staining of primary tumors and metastatic lymph nodes was assessed prior to obtaining survival information. All p53-reactive tumors were rated as positive, including those with only a
few positive cells. Patient survival data were then used to determine the possible correlation between p53 accumulation and early death. Survival curves were constructed using Kaplan-Meier analysis. Statistical significance of these data was measured by analysis of variance and $\chi^2$, using a SAS software package.

Results

The anti-p53 monoclonal antibody (PAb 1801) used in these studies recognizes both wild-type and mutant forms of p53. However, no reactivity was observed in any normal lung epithelial tissue. As presented in Fig. 1, both NSCLC adenocarcinomas and squamous cell carcinomas are reactive. For both of these primary tumor types, approximately 40% were positive for p53 production (Table 1). p53 localization was exclusively limited to the nucleus. For almost all reactive cancers, staining was heterogeneous but nonrandom. That is, p53 was generally concentrated in those cancer cells bordering uninvolved tissue. Again, the latter was completely negative for p53 production as detectable by this methodology.

As indicated in “Materials and Methods,” a particular NSCLC case was coded as positive even if only a few cells were reactive or p53 producers. Once all 114 Stage I and Stage II cases were designated “positive” or “negative,” survival data were obtained. As seen in Table 1, p53 production is correlated, in a statistically significant manner, with short-term survival. This applies to all cases, as well as Stage I and Stage II, when analyzed separately. To further support the validity of these data, Kaplan-Meier survival plots were generated for both p53 producers and nonproducers. Fig. 2 exhibits those results. Clearly, p53 accumulation is a predictor of a poor prognosis. The 50% survival time for p53 producers was 16 months; for nonproducers it was 38 months. The Kaplan-Meier plots were analyzed statistically by $\chi^2$. That number was 11.26, generating a $P$ value of $<0.001$.

Of the 24 Stage II NSCLC cases which were p53-negative (Table 1), 7 of those cases had regional lymph node metastatic sites that were p53-positive. This is presented in Fig. 3. Multiple tissue blocks and multiple sections were evaluated to ensure that the primary tumors were indeed p53 nonproducers. Interestingly, this subset of Stage II cases was composed of short-term survivors. Their mean survival time was 11 months, compared to a mean survival time of 34 months for those cases p53-negative in both primary tumor and metastatic regional lymph nodes (analysis of variance $P$ value $< 0.009$).

Discussion

These results demonstrate, for the first time, we believe, a strong correlation between p53 accumulation and poor survival for NSCLC cases. This applies to both Stage I and II primary adenocarcinomas and squamous cell carcinomas. Of particular interest is the finding that patients with p53-negative primary tumors but p53-positive metastatic lymph nodes also have a poor prognosis. Evidence that this p53 protein is the product of a mutated gene is indirect, since the PAb 1801 monoclonal antibody recognizes both mutant and wild-type forms of p53.
A study to assess p53 accumulation as a prognostic indicator in these cancers found no significant correlation (23). Patients with p53-negative and -positive tumors had mean survival times of 49.2 and 48.1 months, respectively.

Alterations in the p53 gene in human lung cancer involve a variety of abnormalities (11, 24, 25), including allele deletions, point mutations, and other small mutations. While the commonness of p53 gene changes in human lung cancer is well documented (11–16), no clear evidence has yet been reported that this could have prognosis value. In this report we present evidence that the production and intranuclear accumulation of p53 in both adenocarcinomas and squamous cell carcinomas is a strong indicator of short-term survival. High levels of p53 could be due to increased expression or extended half-life. The latter could be due to a reduced sensitivity to a general or p53-specific protease. It is possible that a putative mutated form of p53, by way of forming a functionally inactive homologimer, may eliminate the tumor suppressor effects even for p53 coded by the nonmutated allele. Of particular interest were those p53-negative Stage II cancers which converted to a p53-positive state at lymph node metastatic sites. For those individuals, the mean survival time was only one-third that of Stage II patients who remained p53-negative at nodal metastatic sites. Thus, p53 accumulation may be playing a role in the early steps of tumor progression. Perhaps of more importance is the possibility that p53 accumulation, as a prognostic factor, may eventually have clinical value. While aggressive therapeutic intervention has been used in lung cancer, 90% of patients with this disease die from it. If p53 accumulation is an indicator of poor prognosis, then a better understanding of the mechanism(s) of this accumulation, as well as elucidating in some detail the biological implications of these defects, may lead to better treatment modalities. In addition, studies are needed to determine whether hypothesized p53 accumulation in premalignant lung disease also has diagnostic and prognostic potential.

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p53 ACCUMULATION IN HUMAN LUNG CANCER


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