

p53 EXPRESSION IN NON-SMALL CELL AND SMALL CELL LUNG CARCINOMAS: RELATIONSHIP WITH PROLIFERATING CELL NUCLEAR ANTIGEN AND CIGARETTE SMOKING

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The p53 gene is a tumor suppressor gene which is located on band 13p of chromosome 17. It encodes a 53-kd nuclear phosphoprotein, and has been found in all mammalian cells. It is thought to play a role in the control of the cell cycle. The wild type p53 protein inhibits cell proliferation by arresting cells in the G1 phase of the cell cycle, and loss of this activity can lead to neoplastic transformation.¹⁻³ Mutations in p53 gene are the most common genetic alterations in several human malignancies, including lung, breast, colorectal, ovarian and cutaneous neoplasms, although the prognostic value of altered p53 expression is still debated.⁴⁻⁶

Under physiological conditions, the wild type protein has a short half-life to allow immunohistochemical detection.⁷ However, the mutant type is more stable than the wild type, and is detectable in cell nuclei by standard immunohistochemical staining procedures.^{1,7} To investigate the role of p53 in cell proliferation, we focused on markers of the cell cycle whose expression could be correlated with p53 immunohistochemical detection. One such marker is the proliferating cell nuclear antigen (PCNA). PCNA is a 36-kd nuclear polypeptide that is related to cell proliferation. PCNA, an auxiliary protein for DNA polymerase, is identical to cyclin, synthesized during the late G1 to S phase. The synthesis of PCNA is reported to be directly correlated with DNA replication and cell proliferation.^{8,9}

Our hypothesis was that p53 overexpression may be associated with increased cell proliferation, which might affect the clinical outcome of non-small cell lung carcinomas (NSCLC) and small cell lung carcinomas (SCLC). We examined the expression of p53 and PCNA immunohistochemically. The aim of this study was to evaluate the relationship of p53 and age, sex, nodal involvement, tumor stage, tumor size and proliferative activity in NSCLC and SCLC.

Materials and Methods

This study was based on 60 cases of primary lung carcinoma taken from the files of the Pathology Department, School of Medicine, Akdeniz University. Tumor tissue was obtained by biopsy (n=22, SCLC) or surgical resection (n=30, NSCLC and n=8, SCLC). The pathologic features of the surgical specimens were classified and staged according to the World Health Organization criteria and TNM staging system. Cases of NSCLS consisting of 24 squamous, 1 adenosquamous and 5 adenocarcinomas were examined. In the resection specimens, the size of the tumor and the tumor stage, vessel and nerve invasion, lymph node and pleura involvement were evaluated.

All specimens were fixed in 10% formalin and routinely processed for paraffin wax embedding. Sections were cut into 5 μ pieces, mounted on glass and dried overnight at 37°C. All sections were then deparaffinized in xylene, rehydrated through alcohol and washed in phosphate-buffered saline. This buffer was used for all subsequent washes and for dilution of the antibodies. Sections for p53 detection were heated in a microwave oven twice for 5 minutes at 700 W in citrate buffer (pH: 6). Monoclonal mouse antihuman p53 protein antibody DQ-7 (dilution: 1:50) was used. PCNA expression was investigated by PC 10 monoclonal mouse antibody (dilution: 1:50). Incubation time was 60 minutes at room temperature. The conventional avidin-biotin peroxidase method was performed with both antibodies. 3-amino-9-ethyl-carbozole was used as a chromogen. Negative controls were obtained by leaving out primary antibodies.

In each case, nuclei from about 200 tumor cells were counted, and labelling index was calculated as the percentage of positive neoplastic nuclei for PCNA.⁹ In the histological assesment of nuclear staining of tumor cells, the intensity of nuclear staining was assessed on a semiquantitative 4-point scale as follows: negative: no staining; +: weak; ++: moderate; +++: intense staining. The results were scored for the percentage of positive nuclei: score 0, no positive staining; score 1, from 1% to 30% positive cells; score 2 from 31% to 60% positive cells; score 3 more than 61% of positive cells.

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Accepted for publication 2 January 2001. Received 1 July 2000.

FIGURE 1A. Positive nuclear staining for p53, NO7, squamous cell carcinoma (200x).

FIGURE 2A. Positive nuclear staining for PCNA; PC 10, small cell carcinoma (200x).

Results

Tumor samples from 30 patients with NSCLC and 30 patients with SCLC were studied. In the NSCLC cases, 26 patients (86.6%) were male and four (13.4%) were female. Their ages ranged from 37 to 78 years (mean, 60.9). A review of the medical records showed that 18 patients (60%) were smokers and 12 (40%) were nonsmokers. Thirteen patients (43.3%) were at stage II, 16 (53.3%) were at stage III, and one was at stage IV of the disease. In the SCLC cases, 21 patients (70%) were male and nine (30%) were female. Their ages ranged from 27 to 85 years (mean, 55.5). Twenty-four (80%) were regular daily smokers, and six (20%) were nonsmokers.

Positive nuclear staining was found in 13 patients (43.3%) of the NSCLCs and in 6 (20%) of the SCLCs, with variable intensity of the p53 staining and variable distribution of the immunoreactivity (Figures 1A and 1B). PCNA nuclear immunoreactivity was shown in all 30 (100%) of NSCLC cases and in 22 of 30 SCLC cases (73.3%) (Figures 2A and 2B). The percentage of positive

FIGURE 1B. Positive nuclear staining for p53, NO7, small cell carcinoma (400x).

FIGURE 2B. Positive nuclear staining for PCNA; PC 10, adenocarcinoma (200x).

nuclei varied in different specimens. No staining was detected in normal tissue. We also found a significant positive correlation between p53 and PCNA expression in NSCLCs ($P=0.047$). But in the group of SCLCs, correlation was not found between p53 and PCNA expression ($P>0.05$). There was no significant correlation between the level of p53 expression and sex, age, histopathological findings, tumor stage, tumor size in the groups of NSCLC and SCLC. Statistically significant correlation was not found between the percentage of PCNA and sex, age, histopathological findings, tumor size, and tumor stage in the groups of NSCLC and SCLC.

There was a highly significant correlation between PCNA immunoreactivity and p53 expression in the group of NSCLC ($P=0.047$), whereas this correlation was not detected in the group of the SCLC ($P>0.05$).

A relation was found to exist between p53 immunohistochemical detection and a smoking history in the group of the NSCLC. None of the 12 nonsmoking patients showed p53 nuclear positive staining, whereas positive p53 staining was detected in 13 of 18 smoking patients ($P=0.005$). A relation was not detected between the percentage of PCNA and a smoking history in either group.

Discussion

The p53 gene is thought to be the most frequently mutated gene in human tumors. On examination by

immunohistochemical techniques, mutated p53 protein is generally detectable, whereas wild-type p53 protein is undetectable because of the unstable nature of this protein.¹⁻³ Mutations of p53 are not restricted to a single site, therefore, immunohistochemistry can be considered a more straightforward method for identifying p53 mutations than the tedious nucleic acid-based method.

In the study, nuclear staining was found in 43.3% of NSCLCs and in 20% of SCLCs, with variable intensity and distribution of the staining. Significant statistical difference was found between the expression of p53 in NSCLCs and SCLCs. The percentages were compared favorably with the results from another study on lung tumors. Perhaps these different findings might be related to the use of different antibodies.^{5,10-14}

It is not clear whether there is some correlation between p53 overexpression and clinicopathological parameters in lung tumors. Some authors have not found any significant association between p53 overexpression and cancer progression, while others have reported that tumors with a high percentage of p53-positive cells were more likely to be associated with negative prognostic factors.¹⁵⁻¹⁸ In lung cancers, the nodal involvement and the stage of disease relate to survival. In our series of cases, no significant association could be found between p53 expression and age, sex, histopathological findings, tumor stage, tumor size and nodal status. Passlick et al. detected p53 nuclear staining in 45.2% of 73 NSCLC patients, and could not find a relationship between p53 expression and histopathological findings and tumor stage.¹⁹ Fontanini et al. investigated p53 nuclear staining in 101 lung carcinomas and observed more p53 overexpression in tumors with metastatic nodal involvement than in tumors with nonmetastatic nodal involvement, although no correlation was found between p53 overexpression and overall survival.²⁰ Irie et al. detected p53 nuclear staining in 51.6% of 211 lung carcinomas, and found that there was no significant relationship between p53 overexpression and sex, clinicopathological stage and size of the tumor.²¹ Coppola et al. found p53 nuclear staining in 21% of 14 typical carcinoid tumors, in 64% of 11 atypical carcinoid tumors, and in 88% of 8 small cell carcinomas. They reported that in the spectrum of neuroendocrine tumors of the lung, p53 overexpression correlates with more aggressive histologic cell types.²²

Casey et al. determined that 88 of 154 non-small cell lung cancers (57%) contained DNA sequence mutations, whereas positive immunohistochemical staining was detected in 63 of 137 lung cancers (46%). They observed a high concordance between the presence of p53 missense mutations and positive immunohistochemical staining.²³ Wiethge et al. analyzed p53 accumulation and the expression of the PCNA by standard immunohistochemistry. Among 46 SCLCs, 35% were positive for p53 and 51% were positive for PCNA. Of 279 NSCLCs, 43% showed positive p53 immunoreaction and 72% showed a positive PCNA. They concluded from their results that it seemed appropriate to assess the p53 status exclusively in

the specimens positive for PCNA.²⁴ Lee et al. found p53 nuclear staining in 66% of 103 NSCLCs. They reported that high expression of the p53 oncoprotein is a favorable prognostic factor in a subset of patients with NSCLC.²⁵

Proliferative activity of normal and neoplastic tissues may be assessed by monoclonal antibodies or by flow cytometric analysis. PCNA is now considered to be a negative prognostic indicator in many different neoplasms.^{10,11} In this study, PCNA nuclear staining was detected in all 30 NSCLCs (100%), and in 22 SCLCs (73.3%). Statistically, no significant correlation was found between the percentage of PCNA and sex, age, histopathological findings, tumor stage, and tumor size in either of the groups ($P>0.05$).

It has been reported that wild-type p53 can downregulate PCNA mRNA and protein expression selectively. It has also been shown that the mutant p53 may activate the PCNA promoter directly.⁴ In our study, we observed that the expression of p53 in NSCLC cases appears to be related to the proliferating activity of the tumors ($P=0.047$), whereas the expression of p53 in SCLCs does not appear to be related to the proliferating activity of the tumors ($P>0.05$). We can hypothesize that in some cases, the absence of correlation with proliferating activity may be dependent upon a remaining amount of normal p53 gene levels.

Kawai et al. studied the proliferative activity by using PCNA in 165 lung carcinomas.²⁶ They investigated a relationship between PCNA and sex, age, tumor stage, survival, histologic type, degree of cell differentiation and cellularity. They found that there was concordance between PCNA and clinical stage, cellularity and DNA content. Esposito et al. found p53 nuclear staining in 36% of 61 NSCLCs. They did not find p53 nuclear staining in nonsmoking patients, but they detected altered p53 expression in 40.7% of smoking patients.⁴ Dosaka-Akita et al. observed that p53 nuclear staining in the group of smoking patients was more than in the group of nonsmoking patients.²⁷

In this study, a relationship was found to exist between p53 immunohistochemical detection and smoking history in the group of NSCLCs. None of the 12 nonsmoking patients showed p53 nuclear positive staining, whereas positive nuclear staining was detected in 13 of 18 smoking patients. We believe that the target gene of tobacco-associated lung carcinogenesis may be the p53 gene which upregulates the PCNA in NSCLC cases.

References

- Hollestein M, Sidronsky D, Volgelstein B. P53 mutations in human cancers. *Science* 1991;253:49-53.
- Caamano J, Ruggeri B, Momiki S, et al. Detection of p53 in primary lung tumors and nonsmall cell lung carcinoma cell lines. *Am J Pathol* 1991;139:839-45.
- Lane P. P53 guardian of the genome. *Nature* 1992;358:15-6.
- Esposito V, Alfonso B, Luca A, et al. Prognostic value of p53 in nonsmall cell lung cancer: relationship with proliferating cell nuclear antigen and cigarette smoking. *Hum Pathol* 1997;28:233-7.
- Jiarig Y, He AG, Dai XC. The immunohistochemical study of p53 protein in primary lung cancer. *Chung-Hua-Chieh-Ho-Ho-Hsi-Tsa-Chih* 1994;17:45-6.
- Memis L. Lung carcinomas. *Patoloji Bülteni* 1996;13:5-7.
- Fontanini G, Bigini D, Vignoti S, Macchiarini R. P53 expression in nonsmall cell lung cancer. Clinical and biological correlation. *Anticancer Res* 1993;13:737-42.
- Hall PA, Levison DA, Woods AL. Proliferating cell nuclear antigen (PCNA) immunolocalisation with evidence of deregulated expression in some neoplasms. *J Pathol* 1990;162:285-94.
- Linden MD, Torres FX, Kubus J, Zaibo RJ. Clinical application of morphologic and immunocytochemical assesment of cell proliferation. *Am J Clin Pathol* 1990;162:285-94.
- Carbone DP, Mitsomi T, Chiba I, et al. P53 immunostaining positivity is associated with reduced survival and is imperfectly correlated with gene mutations in resected nonsmall cell lung cancers. *Chest* 1994;106:3775-815.
- Melhem MF, Law JC, El-Ashmawy L, et al. Assessment of sensitivity and specificity of immunohistochemical staining of p53 in lung and head and neck cancers. *Am J Pathol* 1995;146:1170-7.
- Guinne DG, Travis WD, Trivers GE, et al. Gender comparison in human lung cancer analysis of p53 mutations, anti-p53 serum antibodies and c-erb B-2 expression. *Carcinogenesis* 1995;16:993-1002.
- Mitsudomi T, Oyama T, Nishida K, et al. P53 nuclear immunostaining and gene mutations in nonsmall cell lung cancer and their effects in patient survival. *Ann Oncol* 1995;6(Suppl 3):9-13.
- Gorgoulis VG, Zoumpoulis V, Rassidakis GC. A molecule and immunohistochemical study of the MDM2 protein isoforms and p53 gene product in bronchogenic carcinoma. *J Pathol* 1996;180:129-37.
- Chiba I, Takahashi T, Nau MM, et al. Mutations in the p53 gene are frequent in primary resected non-small cell lung cancer. *Lung Cancer Study Group. Oncogene* 1990;5:1603-10.
- Korkolopoulou P, Oates J, Crocker J. P53 expression in oat and nonoat small cell lung carcinomas: correlations with proliferating cell nuclear antigen. *J Clin Pathol* 1993;46:1093-6.
- Nuorva K, Soini Y, Kamel D, et al. Concurrent p53 expression in bronchial dysplasia and squamous cell carcinomas. *Am J Pathol* 1993;142:725-32.
- Ebina M, Steinberg SM, Mushine JL, et al. Relation of p53 overexpression and up regulation of proliferating cell nuclear antigen with the clinical course of nonsmall cell lung cancer. *Cancer Res* 1994;54:2496-503.
- Passlick B, Izbicki JR, Haussinger K, et al. Immunohistochemical detection of p53 protein is not associated with poor prognosis in nonsmall cell lung cancer. *J Thorac Cardiovasc Surg* 1995;109:1205-11.
- Fontanini G, Vignodi S, Bigini D, et al. Bcl-2 protein: a prognostic factor inversely correlated to p53 in nonsmall cell lung cancer. *Br J Cancer* 1995;1:1003-7.
- Irie K, Ishida H, Furukawa T, et al. Clinicopathological study expression of p53 suppressor gene and bcl-2 oncogene in relation to prognosis. *Rinso-Byori* 1996;44:32-41.
- Coppola D, Clarie M, Landreneau R, et al. Bcl-2, p53, CD44, CD44v6 isoform expression in neuroendocrine tumors of the lung. *Mod Pathol* 1996;9:484-90.
- Casey G, Lopez ME, Ramos JC, et al. DNA sequence analysis of exons 2 through 11 and immunohistochemical staining are required to detect all known p53 alterations in human malignancies. *Oncogene* 1996;13:1971-81.
- Wiethege T, Voss B, Müller KM. P53 accumulation and proliferating cell nuclear antigen expression in human lung cancer. *J Cancer Res Clin Oncol* 1995;121:371-7.
- Lee JS, Yoon A, Kalapurakal SK, et al. Expression of p53 oncoprotein in nonsmall cell lung cancer: a favorable prognostic factor. *J Clin Oncol* 1995;13:1893-903.
- Kawai T, Suzuki M, Kono S, et al. Proliferating cell nuclear antigen and Ki-67 in lung carcinoma. *Cancer* 1994;74:2468-75.
- Dosaka-Akita H, Shindoh M, Fujino M, et al. Abnormal p53 expression in human lung cancer is associated with histologic subtypes and patients' smoking history. *Am J Clin Pathol* 1994;102:660-4.