P53 Expression in Stage I Squamous Cell Lung Cancer*

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P53 expression was studied using immunohistochemistry in patients (n=94) with pathologic stage I squamous cell lung cancer treated surgically between 1991-1992. The overall p53 positivity ratio was 48/94. 83 of the cases proved to be suitable for follow-up analysis carried out in November, 1995. 46/83 were p53 positive, and 25/46 patients were alive at the time of analysis. The patients who died (21/46) had a mean survival time of 17.5 months. In p53 negative cases (37/83), however, 29/37 patients were still alive at the time of follow-up, and 8/37 had died with a mean survival time of 23.1 months. A significant correlation could be found between p53 immunopositivity and reduced survival time (p=0.0125). Interestingly, out of 83 cases analyzed histologic evidence of tuberculous sear tissue was present in 9 tumors with a p53 positivity ratio of only 1/9. When flow cytometry was used to examine tumor samples from all subgroups mentioned above (n=32), no correlation was found between the p53 immunopositivity or the prognosis and the DNA content of tumor tissues. Our results suggest that in the early stage of squamous cell lung cancer the p53 positivity may be an indicator of a more aggressive tumor behavior and a shortened survival time. (Pathology Oncology Research Vol 4, No 1, 8–13, 1998)

Key words: p53, immunohistochemistry, lung cancer, flow cytometry

Introduction

Lung cancer is the most common cause of cancer death worldwide, and it is showing a steadily increasing incidence. Despite the rapid development of imaging techniques and preoperative biopsy methods as well as the use of combined cytostatic and irradiation treatments, there has been no improvement in the overall 5-year-survival, which is still under 15%.1 Many studies using molecular biological methods are ongoing to better understand the cellular mechanisms of lung carcinogenesis, and to find tumor markers that may have diagnostic, prognostic and therapeutic implications.

In the present study we have investigated the p53 protein in lung tumors, which is the product of p53 tumor suppressor gene very commonly altered in human malignancies. The p53 gene, located on chromosome 17 (17p13.1), encodes a 53 kDa nuclear phosphoprotein consisting of 393 amino acids that plays a central role in the regulation of normal cell cycle and in carcinogenesis. Cell damaging effects increase the activity of wild-type p53 protein, which arrests the cell in the G1-S phase, and either enables the repair mechanisms to act, or may trigger apoptosis.2,3 Physiologically, the p53 protein has a short half-life, therefore it can not be detected by immunohistochemical methods. In case of protein dysfunction caused by genetic alteration (missense mutation, allelic loss) or in case of intracellular protein accumulation by inhibited excretion (e.g. in viral infection), the half-life of p53 is prolonged and the protein becomes immunohistochemically detectable. The role of p53 in carcinogenesis is still under investigation. P53 overexpression detected by

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Abbreviations: NSCLC: non-small cell lung cancer; SCLC: small cell lung cancer; SCC: squamous cell carcinoma
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immunohistochemistry has been observed in almost all types of malignancies and also in many premalignant lesions.4

According to the molecular analyses of the p53 gene, immunopositivity does not necessarily mean simultaneous genetic alteration(s) and inversely, not all mutations in the genom result in p53 overexpression. However, marked positive correlation has been observed between tumors with missense mutations in p53 gene and p53 immunostaining, and positively staining tumors not carrying missense mutations were found only occasionally, pointing to other p53 stabilizing mechanisms. In cases of other types of mutations (nonsense/chain-terminating, splicing abnormalities or deletions) the resultant p53 protein is usually absent, truncated or unstable, therefore it is undetectable immunohistochemically.5

In some cancers (esophageal, gastric, colon and bronchial carcinoma) the p53 alteration occurs at an early stage of tumorigenesis, and its frequency is found to increase during the malignant transformation.6 In other tumor types (prostatic cancer and melanoma) the abnormal p53 expression is a late event in the multistep carcinogenesis.7,10,11 A correlation between p53 overexpression and smoking habits has been observed in patients with head and neck or lung cancer, suggesting a tobacco induced p53 alteration. The most common type of association in human lung cancer is G:C to T:A, which can also be induced in the p53 gene in vitro by benzo(a)pyrene, one of the major carcinogens in tobacco.12-15

Positive correlation between immunodetectable p53 and increased aggressivity in tumor behavior has been found in many carcinomas (breast, stomach, ovary, endometrium and prostate), but in primary bronchial cancer the prognostic relevance of p53 overexpression is still debated.13-16 In human lung cancer the p53 immunostaining appears at an early stage of carcinogenesis and correlates with the severity of bronchial dysplasias. It is maintained in the process of metastasis and is associated with the histologic subtypes.20-22 P53 expression has also been investigated in pulmonary neuroendocrine tumors ranging from typical carcinoids of relative better prognosis – through atypical carcinoids – to highly malignant small cell lung cancers (SCLC). Many authors have reported abnormal p53 expression in the majority of SCLCs and in some cases of atypical carcinoids. However, none of the typical carcinoids examined were found to show positive reaction, although negative immunostaining for p53 proved not to be an indicator for the absence of p53 missense mutation. These observations might be of clinical importance in the differential diagnosis of pulmonary neuroendocrine neoplasms.23-26

It is well known that the presence of DNA aneuploidy in a tumor can be of clinical relevance. In bladder and prostate cancer there is a positive correlation between DNA aneuploidy and tumor grade and stage, while in colorectal cancer there is no clear association between these parameters. Moreover, in certain malignancies (e.g. neuroblastoma, rhabdomyosarcoma, and childhood acute lymphoblastic leukemia) DNA hyperploidy is found to be related to a better prognosis.27

Many investigators have observed a correlation between p53 immunopositivity and a shortened survival in non-small cell lung cancer (NSCLC), but contrarily recent publications have shown that high expression of p53 is a favourable prognostic factor in this subtype of bronchial carcinoma.28,33

In the present study we have investigated the p53 expression with immunohistochemistry in primary bronchial cancer focusing on the surgically resectable squamous cell carcinomas (SCC) of early stage (n=94), and have also examined the possible association between p53 expression and survival. DNA content in selected tumor tissues was also analyzed.

Materials and Methods

Patients and tumor characteristics

94 consecutive patients were examined (74 men, 20 women, mean age: 58.3 years, 33–71 years) with pathologic stage I squamous cell lung cancer. They had all undergone surgical resection between January, 1991 and December, 1992. Apart from three cases (two wedge resections and one pneumonectomy), all tumors were removed with lobectomy. 83 patients participated in the follow-up analysis in November, 1995 (66 men, 17 women, mean age: 57.9 years, 33–70 years). 11 patients were excluded for the following reasons: 3 patients had other malignant diseases simultaneously (breast cancer [2], prostatic cancer [1]), in one patient duplex bronchial carcinoma was found, there were 4 deaths not related to lung cancer (brain stroke [1], bilateral pneumonia [1], ischemic heart disease [1], and sepsis [1]), and in 3 cases postoperative documentation was not available.

Immunohistochemistry

4-5 μm-thick sections from formaline fixed, paraffin embedded tumor tissues were immunostained with monoclonal mouse anti p53 IgG (DO-1, Oncogene Science, Ab-6), which detects both the wild-type and the mutant variants of the p53 protein. Immunoreactions were performed by using standard methods as well as Antigen Retrieval Solution (BioGenex) and microwave oven heating for antigen demasking. Sections were incubated with primary antibody (1:100 in phosphate-buffered saline solution) overnight at 4°C. Subsequently, avidine-biotin complex reagent kit (Vectastain Elite ABC, Vector Laboratories)
was used, then diaminobenzidine as the chromogen. After hematoxylin counterstaining the slides were dehydrated and coverslipped.

**Flow cytometry**

Hematoxylin-eosin stained sections taken from the paraffin embedded blocks were screened to select the well-preserved and representative samples suitable for flow cytometry, excluding necrotic or haemorrhagic tissues. Using 50 μm-thick sections, paraffin was removed with repeated xylene and descending concentrations of ethanol, then sections were washed in distilled water and cut into small pieces. Tissue pieces were digested with 0.25% trypsin in 0.01 M TRIS-HCl, pH 7.5, containing 0.025% ribonuclease, 0.03% EDTA, 0.1% Triton X-100 and 0.05% sodium azide, at room temperature, overnight. A suspension of $10^3$–$10^6$ nuclei/ml was stained with propidium iodide (50 μg/ml) for 20 min at room temperature and filtered through a silk gauze prior to flow cytometry. The analysis was performed on a FACStar flow cytomter (Becton-Dickinson) in conjunction with an HP-200 computer and Consort 30 data acquisition package.

**Statistical analysis**

Survival curves were estimated with the use of Kaplan-Meier method, and differences in survival were evaluated by the generalized Wilcoxon test.

**Results**

The p53 expression was examined in 94 consecutive patients with surgically resected stage I squamous cell lung carcinoma. Overall, about half of the tumors (48/94) showed positive nuclear immunostaining, which was never observed in the adjacent normal lung tissue. In all p53 positive cases at least 20% of tumor cells were stained, and usually very intensively (Figure 1). Moreover, in about one third of positive cases the proportion of immunopositive tumor cells exceeded 50%. 83 patients took part in the survival analysis, which showed a significant difference between p53 positive (n=46) and negative (n=37) cases (p = 0.0125) (Figure 2). According to the Kaplan-Meier survival analysis, in our study the p53 immunopositivity referred to a poor prognosis and a shortened postoperative survival in stage I squamous cell lung cancer.

When tissue samples (n=32) were examined from all follow-up categories with flow cytometry, no significant differences were found in DNA content between the p53 positive and negative subgroups. Nevertheless, there was a slightly higher proportion of aneuploid tumors in p53 positive cases with poorer prognosis, and a higher number of diploid tumors was detectable in p53 negative cases with better prognosis (Table 1). It is of interest to mention that in contrast to the usual p53 positivity ratio in squamous cell lung carcinomas, only 1 positive tumor was found out of 9 cases with tuberculous scar tissue (Table 1).

**Discussion**

The role of oncogenes and tumor suppressor genes in malignant transformation, carcinogenesis and tumor progression is still under intensive investigation. The alteration of the p53 gene and p53 protein is one of the most
Table 1. Follow-up data of patients with surgically resected, pathologic stage I squamous cell carcinoma (n=83)

<table>
<thead>
<tr>
<th></th>
<th>p53 positive</th>
<th></th>
<th>p53 negative</th>
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<tbody>
<tr>
<td></td>
<td>A/D*</td>
<td></td>
<td>A/D</td>
<td></td>
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<tr>
<td>Patients alive</td>
<td>25** (1)</td>
<td>5/4</td>
<td>29 (7)</td>
<td>3/6</td>
</tr>
<tr>
<td>Patients died</td>
<td>21 (0)</td>
<td>5/2</td>
<td>8 (1)</td>
<td>4/3</td>
</tr>
</tbody>
</table>

* Flow cytometry was performed in selected tumor tissue samples from all follow-up categories (n=32, A: aneuploidy, D: diploidy)
** Two of them subsequently developed metastasis (1 brain, 1 lymph node) and one had tumor recurrence
( ) Indicates the number of tumor samples with histology referring to presence of tuberculous scar tissue (n=9)

common lesions in human malignancies including primary bronchial cancer.\(^{34-36}\) Positive correlation between p53 immunopositivity and increased clinical aggressiveness has been observed in many solid tumors, even in less frequent types such as parotid gland carcinoma and male breast cancer.\(^{37,38}\)

Previous studies in NSCLC have also shown an association between p53 immunopositivity and either a more aggressive tumor behavior (increased metastatic hilar and mediastinal lymph node involvement) or a shortened survival.\(^{3,16,39}\) On the contrary, in NSCLC improved disease-free survival has been found in p53 positive cases, supposing that the detected protein possesses the wild-type tumor suppressor function.\(^{3,14}\) It has been reported that the concordance between p53 overexpression and p53 gene mutation is not perfect, however, concerning missense mutations in exons 5-8 in NSCLC, close association has been found with immunostaining.\(^{40,41}\)

It is important to mention, that the group „NSCLC“ always consists of various proportions of lung cancers with different histologic subtypes and clinical behaviors, especially in cases of tumors with diverse stages. Nonomura et al. showed in a retrospective study of 993 lung cancers that of peripheral tumors no SCC with a diameter of 15 mm or less had lymph node metastasis, whereas 18.9% of the adenocarcinomas of this size were positive for nodal involvement.\(^{42}\)

We examined the association between p53 expression and survival in a homogenous group of patients with pathologic stage I SCC to eliminate the survival influences of diversity of tumor histologies and stages. Our results are in accordance with the recently published data in which p53 overexpression was found to be an independent and better prognostic factor but only in node-negative NSCLC.\(^{43}\) In our present retrospective study, immunopositivity can only be an approximate indicator of the tumors with altered p53 protein, since selective antibody to detect mutant p53 gene product – although commercially available – can only be used in fresh frozen tissue.

Changes in the normal cell cycle as well as alterations in cell cycle regulation are far more complex processes than to be characterized by a single factor or event. Many studies have been reported in which p53 overexpression was investigated in lung cancer together with other markers, such as proliferating cell nuclear antigen expression or DNA content to find more accurate tumor characteristics of predictive clinical value.\(^{44,46}\)

In the present study, no significant correlation was found between either p53 status or postoperative survival and the DNA content of tumor samples. On the contrary, p53 immunopositivity was found to be a suitable marker to identify – even preoperatively, by using bronchoscopic specimens – lung SCC patients with more aggressive tumor behavior. It is interesting to mention that all but one SCC sample with tuberculous scar were p53 negative. Further investigations with more tissue samples and analyses of collagen types of scars are necessary to determine the importance of this observation.\(^{47}\) Concerning the future perspectives of p53 status analysis, it may become an important indicator of lung cancer of early stage using sputum cytology specimens in screening programs, at least among high risk individuals.\(^{38}\)

As until now radical surgical treatment at a very early stage of lung cancer remains the only chance of complete recovery; p53 immunopositivity of preoperative biopsy specimens might influence the extension of lung resection or may necessitate close follow-up in order to introduce adjuvant chemo- or radiotherapy, in time.

It has been found that serum antibody against mutant p53 protein could be detected in a certain percent of patients with different malignancies including lung cancer.\(^{49}\) Further studies are still required to evaluate the applicability of this method in the detection of lung cancer and in follow-up analyses. It has been shown that p53 influences the expression of the mdr1 gene, responsible for drug resistance.\(^{49}\) In case of the functional alteration of the p53 gene, overexpression of the mdr1 gene occurs resulting in failure of chemotherapy as well as radiotherapy. The restoration of the p53 function, therefore, might be a possible way to eliminate drug resistance and enable the induction of apoptosis in altered cells, thus the p53 gene can become a tumor suppressor gene again.\(^{51}\)

References


