Effect of Cytokeratin Typing on Response to Chemotherapy and Survival Time in Non-Small Cell Lung Cancer

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Abstract

In view of the fact that certain objective determinants are needed to make a more reliable classification of nonsmall cell lung carcinomas (NSCLC) and aiming to assess the relationship between immunohistochemical typing and histopathological typing, immunohistochemical typing was done in patients with stage IV NSCLC using monoclonal anti-cytokeratin 18 (CK18) antibody. Also, to examine the role of immunohistochemical typing in predicting the prognosis of NSCLC, the relationships between the immunohistological type and response to chemotherapy, as well as length of survival, were evaluated.

Tissue samples of 27 patients with stage IV NSCLC were examined immunohistochemically with anti-cytokeratin 18 (CK18) monoclonal antibody using "biotin-streptavidin" method.

There was no relationship between immunohistochemical typing and histopathological typing, but the relationship between CK18 positivity and adenocarcinoma was statistically significant. The relationship between immunohistochemical typing and survival time, and that of histopathological typing and response to chemotherapy were not statistically significant, while a statistically significant relationship was found between immunohistochemical typing and response to chemotherapy. Response to chemotherapy was better in patients with CK18 (-) than in those with CK18 (+).

We conclude that determination of CK18 pattern can be a useful criterion in deciding on the initiation of chemotherapy.

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Introduction

Non-small cell lung carcinomas (NSCLCs) comprise 70-80 percent of all lung carcinomas. At the time of diagnosis, distant metastases are reported to have already occurred in 40 percent of the patients (1). Cytokeratins are the most important class of intermediate filament proteins (IFPs), a type of protein present in the cellular skeleton. The cellular cytokeratin patterns of different tissues can be determined immunohistochemically. Generally, a cellular cytokeratin pattern is an indicator of the cellular immunohistochemical composition (2,3,4).

The subjective opinion of the pathologist plays the most important role in the histopathological classification of NSCLCs. It has been reported that in the histopathological examination of NSCLCs interobserver consensus is 56% in adenocarcinomas, 48% in squamous cell carcinomas, and 4.8% in large cell carcinomas (5). In another study, the histopathologists were very successful in distinguishing between small cell and non-small cell lung carcinoma, but they were not equally good at subclassifying the non-small cell lung carcinoma group (6). These observations indicate that objective determinants are required to obtain a

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Table 1. Histopathological and immunohistochemical typing in 27 patients						
	Histopathological typing					
Immunohistochemical typing	Squamous cell carcinoma		Adenocarcinoma		TOTAL	
	Number%		Number %		Number %	
CK18 (+)	**12**	**63.2**	**8**	**100.0**	**20**	**74.0**
CK18 (-)	7	36.8	0	0	7	26.0
TOTAL	19	100.0	8	100.0	27	100.0
Fisher exact test P=0.134.						

more reliable classification. The results of some immunohistochemical studies suggest that cytokeratin typing can be used as a complementary method to routine histopathological typing (7,8).

It is well known that the stage of the disease is the most important prognostic factor in NSCLC. However, differences in survival among patients who are at the same stage indicate that there must be several other factors affecting prognosis (10). A relationship between length of survival and presence of some cytokeratin fragments in tissue and in serum have been reported in NSCLC patients (9,10,11).

In view of the fact that certain objective determinants are needed to make a more reliable classification of NSCLC, we performed immunohistochemical typing in patients with stage IV NSCLC using monoclonal anti-cytokeratin 18 (CK18) antibody. Thus we aimed to assess the relationship between immunohistochemical typing and histopathological typing, Also, to examine the role of immunohistochemical typing in predicting the prognosis of NSCLC, we attempted to evaluate the relationship between the immunohistological type and length of survival in these patients. It is generally reported that the response to chemotherapy is not satisfactory in NSCLC, but results of studies investigating the relation between histopathological typing and response to chemotherapy have been controversial. (12,13,14). This point was also taken up in our study.

Materials and Methods

Of 156 patients with stage IV non-small cell lung carcinoma (NSCLC) diagnosed at the Department of Pulmonary Medicine, Ondokuz Mayıs University School of Medicine in the period between 1994 and 1998, 27 patients who fulfilled all of the criteria listed below were included in the study:

a) To have received two or more cycles of chemotherapy with a cisplatin-based protocol. The following regimens were used:

MIC- Mitomycin 6 mg/m^2(1st day i.v.) - Ifosfamide 3 g/m^2(1st day i.v.) - Cisplatin 50 mg/m^2 (1st day i.v.)

NC- Navelbine 30 mg/ m^2 (1-8th days i.v.)- Cisplatin 80 mg/m^2 (1st day i.v.)

TC- Taxol 175 mg/ m^2(1st day i.v.) , Carboplatin 30 mg/m^2 (1st day i.v.)

b) To have not been exposed to palliative thoracic radiotherapy.

c) To have a satisfactory tissue sample available for immunohistochemical examination.

Slides of 4-6 μ were prepared from the tissues which had been fixed in 10% phosphate with neutral formalin and these samples were then placed in paraffin.

The immunohistochemical study was performed by "biotin streptavidin" method using anti-cytokeratin 18 (CK18) monoclonal antibodies (monoclonal, mouse, antikeratin 18 clone DC 10, Zymed Laboratories San Francisco) in the tissue samples. In the pathological examination, the presence of intracytoplasmic granular staining was regarded as positive staining. Survival time was accepted as the time interval from the first day of chemotherapy to death. Three patients were not included in the survival analysis because we were not able to contact them. Response to chemotherapy was evaluated according to the recommendations of Miller et al (15). Complete and partial response were both accepted as "response", while stable disease and progression were accepted as "no response".

In the statistical analysis chi-square test, Fisher's exact test and Kaplan-Meier survival analyses were used. "p" values less than 0.05 were considered significant.

Results

Of the 27 NSCLC patients, 21 (78%) were men and 6 (22%) were women . Mean age was 59.4 ± 11.4 (35-82) years. All patients had developed one or more distant metastases,

In the immunohistochemical examination, it was demonstrated that the staining pattern of cytokeratin 18 (CK18) positive cells was intracytoplasmic and near the cytoplasmic membrane. The staining pattern of normal bronchial epithelium was apical rather than intracytoplasmic.

Of the 27 patients with stage IV non-small cell lung carcinoma (NSCLC) 19 (70.4%) were squamous cell carcinoma and 8 (29.6%) were adenocarcinoma. Twelve (63.2%) of patients with squamous cell carcinoma and all of the patients with adenocarcinoma were CK18 (+). All of the 7 patients with CK18 (-) were squamous cell carcinoma. There was no statistically significant difference between patients with squamous cell carcinoma and patients with

Table 2. Immunohistochemical typing and response to chemotherapy						
	Immunohistochemical typing					
Response to chemotherapy	CK18 (+)		CK18 (-)		Total	
	Number%		Number %		Number %	
Response(+)	5	25.0	6	85.0	11	40.7
Response(-)	15	75.0	1	15.0	16	59.3
TOTAL	20	100.0	7	100.0	27	100.0
*Fisher's exact test P=0.009.						

adenocarcinoma regarding immunohistochemical staining features ($p>0.05$). Twenty patients were CK18 (+) and seven patients were CK18 (-) (Table 1).

Response to chemotherapy was demonstrated in 5 (25%) of 20 patients with CK18 (+) and 6 (85%) of 7 patients with CK18 (-). The rates of response to chemotherapy in patients with CK18 (-) were significantly higher than in those with CK18 (+) ($p < 0.01$) (Table 2).

Nine (47%) of 19 patients with squamous cell carcinoma and two (25%) of eight patients with adenocarcinoma responded to chemotherapy. Although the rates of response to chemotherapy were worse in patients with adenocarcinoma than those of patients with squamous cell carcinoma, there was no statistically significant difference ($p > 0.05$) (Table 3).

Mean survival time of patients with CK18 (-) and CK18 (+) patients were 13.2 ± 2.6 months (median 9 months) and 10.1 ± 1.7 months (median 7 months) respectively. Although the mean survival time of patients with CK18 (+) was shorter than the mean survival time of patients with CK18 (-), there was no statistically significant difference ($p > 0.05$).

In our study, there was no difference between patients with CK18 (+) and patients with CK18 (-) with regard to factors that may affect response to chemotherapy, such as the presence or absence of metastases in the liver or in the bones.

Discussion

Non-small cell lung carcinomas (NSCLCs) comprise 70-80% of lung carcinomas(1,16). Histopathological subgroups of NSCLCs are squamous cell carcinoma, adenocarcinoma, large cell carcinoma, adenosquamous carcinoma, carcinoid tumors and bronchial gland carcinoma. In the past 20 years, after the introduction of immunohistochemical studies to tumor diagnosis, immunohistochemical classification of lung carcinomas has been attempted. Cytokeratins which constitute a group of intermediate filament proteins (IFPs), play an extremely important role in this classification. Cytokeratins are located in the perinuclear region of the cell and are among the main elements constituting the cellular skeleton (17). It has been shown that all respiratory tract epithelial cells contain cytokeratins (18). Due to the fact that the cellular cytokeratin pattern is an indicator of cellular differentiation and that this pattern does not change in neoplastic proliferations, histopathological subtypes of lung carcinoma have characteristic cytokeratin patterns (17).

Histopathological classification of NSCLC depends on cellular differentiation. In this classification the subjective opinion of the pathologist plays a major role. Some studies indicate that in the histopathological classification of NSCLC, interobserver consensus is quite low (5,6). These results point to a need for objective determinants for a more accurate classification.

It has been reported that cytokeratin 18 (CK18) positivity is specific for glandular tissues and adenocarcinoma (7,8). In another study, since CK7 was positive in lung adenocarcinoma and negative in lung squamous cell carcinoma, it has been reported that CK7 positivity can be used in the differential diagnosis of small biopsy specimens (19). In our study, 63.2% of patients with squamous cell carcinoma and 100% of patients with adenocarcinoma were CK18 (+). There was no statistically significant relationship between histopathological typing and immunohistopathological typing. Our results are consistent with results of other studies reporting that CK18 positivity is specific for glandular differentiation and adenocarcinoma(7,8).

The most important factor affecting the natural history in NSCLC is the stage of the disease (9). Differences in survival times among patients who are in the same stage indicate that there must be some factors related to prognosis other than stage of the disease. Niklinski and colleagues (9) reported a negative relationship between serum levels of CYFRA 21-1 (a soluble fragment of CK19 in serum) and survival time in NSCLC. Cohen and colleagues (8) investigated the relationship between CK18 positivity in tissue and survival time after surgical therapy in patients with stage I and II NSCLC. They reported that the survival of patients with CK18 (+) were significantly shorter than survival of patients with CK18 (-) regardless of the histologic types. In addition, they also reported that shorter survival times in patients with CK18 (+) was due to a relationship between CK18 positivity and the nature of the tumor. In our study, although survival times in patients with CK18 (+) were shorter than those of CK18 (-), differences in survival times between two groups were not statistically significant. Our results are not entirely consistent with results of Cohen and colleagues. In their

Table 3. Histopathological typing and response to chemotherapy						
	Histopathological typing					
Response to chemotherapy	Squamous cell carcinoma		Adenocarcinoma		TOTAL	
	Number	%	Number	%	Number	%
Response(+)	9	47.0	2	25.0	11	40.7
Response(-)	10	53.0	6	75.0	16	59.3
TOTAL	19	100.0	8	100.0	27	100.0
Fisher's exact test P=0.405.						

study patients were of stage I and II NSCLC and in our study patients were of stage IV NSCLC. In stage IV patients, some factors other than stage of disease such as presence of metastases, site and number of metastases, performance status of patients, and chemotherapy may affect survival time. Accordingly, it can be considered that these factors might have changed the effect of cytokeratin pattern upon survival time in our study.

The relationship between histopathological typing and response to chemotherapy in NSCLC is controversial. "Eastern Cooperative Oncology Group (ECOG)" investigators (12) and Cellerino (11) report that all patients except those with large cell lung carcinoma and all patients except those with squamous cell carcinoma respond to chemotherapy better than other groups. However, the great majority of combination chemotherapy trials reveal that the histology of tumor is not an important factor in predicting response to chemotherapy (13). In our study, 47% of patients with squamous cell carcinoma and 25% of patients with adenocarcinoma responded to chemotherapy. While the response rate appears to be lower in patients with adenocarcinoma, the difference between the two groups was not statistically significant. This result is consistent with results of other studies (13).

To obtain better results from systemic chemotherapy in NSCLC, patients must be selected for chemotherapy. Some factors indicating better response to chemotherapy are well known, such as good performance status, no prior chemotherapy, and female sex (20). In our study, regardless of histopathological typing, the relationship between immunohistochemical features of tumor and response to chemotherapy in 27 patients with stage IV NSCLC was investigated. Twenty-five percent of patients with CK18 (+) and 85 percent of patients with CK18 (-) responded to chemotherapy. The rates of response to chemotherapy in patients with CK18 (-) were significantly higher than those of patients with CK18 (+). Nisman et al observed that levels of cytokeratin reflected the clinical response to chemotherapy and suggested that cytokeratins can be used as tumor markers in non-small cell lung cancer (22). van der Gaast A et al evaluated the use of cytokeratins as tumor markers and found that rising levels of cytokeratin indicate disease progression. They suggested that such knowledge obtained at an early stage may be used in guiding the treatment program (23). Brambilla et al, in their study on chemotherapy in small cell lung cancer, reported that CK (+) patients showed a good response to chemotherapy and that immunohistochemical analysis showed an increase in cytokeratin expression in the treated patients (24). Yasumoto et al found that the presence of CK(+) cells in the bone marrow was related to a poor prognosis for NSCLC patients in stages II to IIIA, but did not predict the prognosis of patients in stage I. For stage I NSCLC patients, the detection of CK(+) cells in the lymph nodes implied a poor prognosis (25).

In our study, patients with CK18 (-) responded to chemotherapy better than patients with CK18 (+). The interpretation of this finding may be linked to the degree of the differentiation of the tumour. In earlier studies, the relationship between differentiation of the tumor and response to chemotherapy has not been explained clearly (26,27). However, in more recent years it has been shown that histologically poorly differentiated lung cancers (like small cell lung cancers) usually have neuroendocrine features (19) and it is also reported that response to chemotherapy is better in patients with NSCLC presenting neuroendocrine differentiation (20). Accordingly, it might be thought that poorly differentiated lung cancers may respond to chemotherapy better than others. Thus we can speculate that because cellular cytokeratin pattern is an indicator of cellular differentiation, sensitivitiy or resistance to cytotoxic drugs may be associated with cellular differentiation.

In stage IV NSCLC, chemotherapy causes a decrease quality of life in some patients. The majority of stage IV patients will also show no response to chemotherapy. Thus, in such patients, it is important to evaluate all factors predicting the response and weigh the advantages accordingly before starting chemotherapy. We believe that, in addition to other factors, determination of the CK18 pattern is useful in deciding on chemotherapy.

We believe this study is the first to show a relationship between cytokeratin typing of tumor and response to chemotherapy in NSCLC patients. Further studies including large groups of patients are needed to reveal this relationship more accurately.

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