The Prognostic Application of Cytokeratin Typing of Nonsmall Cell Lung Carcinoma

A Retrospective Study

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BACKGROUND. In a previous study, the authors used a variety of anticytokeratin monoclonal antibodies to show that a large proportion of lung tumors cytologically diagnosed as squamous cell carcinoma contain cells expressing simple epithelial cytokeratins, suggesting that these tumors have their origin in adenocarcinoma. These findings raised the possibility that cytokeratin (CK) typing might have a diagnostic capacity not attainable by standard histopathology. The aim of the current study was to assess the value of CK typing for this purpose by determining the correlation between the diagnosis of lung tumors based on CK typing and the survival rate of the patients.

METHODS. Paraffin embedded tissue sections of 66 nonsmall cell lung carcinoma (NSCLC) specimens were examined. These included 18 adenocarcinomas, 32 squamous cell carcinomas, and 16 undifferentiated carcinomas, all diagnosed surgically and histopathologically, and further classified as either Stage I or II. CK typing was performed using the streptavidin-biotin-peroxidase method, employing the following anti-CK monoclonal antibodies: Ks.B.17 (which reacts with CK 18), A3-3 (which reacts with CK 13), and E5-9 (which reacts with CK 10).

RESULTS. Comparison between the 5-year survival rates (5 ysr) of patients with different NSCLC indicated that all types of Stage II tumors had a much poorer prognosis than Stage I tumors. Differences found in the 5 ysr among patients with different types of Stage I tumors were not statistically significant (adenocarcinomas, 33% 5 ysr; squamous cell carcinomas, 59% 5 ysr; undifferentiated carcinomas, 36% 5 ysr; all diagnosed by conventional histopathology). Similarly, no significant differences were noted in 5 ysr between patients with tumors stained positively or negatively with monoclonal antibodies A3-3 or E5-9 (anti-CK 13 and anti-CK 10, respectively). In contrast, highly significant differences ($P = 0.002$) were found in the 5 ysr between patients with Stage I tumors positively or negatively stained with monoclonal antibody Ks.B.17 (23% vs. 75% 5 ysr, respectively) regardless of the histologic types of tumors. Especially informative was a combination of immunohistochemical and histologic diagnoses, with best survival rates (87% 5 ysr) in Ks.B.17 negative tumors histologically diagnosed as Stage I squamous cell carcinomas and worst survival rates (14% 5 ysr) in Ks.B.17 positive tumors diagnosed as adenocarcinomas.

CONCLUSIONS. The current study showed that CK 18 typing of lung tumors can provide a more accurate diagnosis and therefore facilitate the planning of more suitable therapeutic approaches. ***Cancer* 1997; 79:468–73.** *© 1997 American Cancer Society.*

KEYWORDS: cytokeratin typing, nonsmall cell lung carcinomas, survival rate, prognostic markers.

Lung carcinoma is the most common cause of cancer death in the adult population of Western countries.[3] Lung carcinomas are

classified into several groups, including small cell and nonsmall cell lung carcinomas (NSCLC). The latter group, which accounts for approximately 75% of all lung carcinomas, includes squamous cell carcinomas, adenocarcinomas, and undifferentiated large cell carcinomas. The most successful treatment for these entities remains surgical removal of the tumor.[4–7] This treatment is especially applicable to patients with Stage I tumors, in whom the average 5-year survival rate (5 ysr) is approximately 50–60%.

Most patients with lung carcinoma (>60%) present to their physician in the late phases of the disease when the average 5 ysr after resection declines sharply to approximately 30% and 20% for Stage II and IIIA disease, respectively.[6–8] The average 5 ysr of inoperable clinical Stage IIIA, IIIB, and IV patients assigned to receive radiotherapy, chemotherapy, or a combination is <15%.[6]

Survival curves and clinical observations enable physicians to select treatment, estimate prognosis, and compare the results of different therapies. However, the marked heterogeneity of the expected progression of the disease in different individuals, even after successful surgical removal of Stage I tumors, poses a major difficulty in providing a reliable prognosis and selecting optimal therapy for these patients.[8,9] Thus it was desirable to obtain additional information on the nature of Stage I tumors, beyond the histologic typing, to distinguish the more aggressive primary tumors from the less aggressive ones.

Previous studies,[10–15] including studies from this institution,[1,16] have established the usefulness of cytokeratin (CK) typing for cancer immunodiagnosis in a large variety of tumors, including tumors of the respiratory tract. It has been shown that the pattern and combination of CK polypeptides expressed in either normal or cancerous tissues are indicative of the origin and state of differentiation of the cells examined.[12,14,15]

As previously reported, CK typing was also shown to be potentially useful in lung carcinoma diagnosis.[1,15,17] The major types of lung carcinomas show the differentiation specific CK expression patterns. Squamous cell carcinomas may express moderate levels of CKs 4, 10, 11, 13, and 16 and high levels of CKs 14, 15, and 17. The expression of stratification-related CKs 4, 10, 11, 13, and 16 diminishes with increasing histologic grade of the malignant tumor, which does not influence the expression of the basal cell CKs 14, 15, and 17.

Conversely, adenocarcinoma and undifferentiated large cell carcinoma are characterized by the simple CKs 7, 8, 18, and 19. Unexpected expression of specific CKs in the different histologic types may be due to progression of simple epithelial tumors into squamous differentiation, which often occurs in lung carcinomas.[15]

In a previous study,[1] the authors used anti-CK antibodies for the immunocytochemical characterization of squamous cell carcinomas and adenocarcinomas in fine-needle aspiration biopsies. This study revealed a remarkable discrepancy between the cytologic diagnosis and the diagnosis based on the CK profile. Specifically, nearly 60% of the tumors, cytologically diagnosed as squamous cell carcinomas, appeared to contain cells expressing simple epithelial CK 18 and were devoid of keratins specific for keratinizing squamous epithelium. This study suggested that CK typing may indeed provide significant prognostic information.

The objective of the current study was to directly test the prognostic significance of this finding and to examine retrospectively the correlation between the expression of specific CKs (mainly CK 18) by different types of lung carcinomas and patient survival. This study will show that the expression of CK 18 is an excellent prognostic indicator in patients with Stage I NSCLC.

MATERIALS AND METHODS

Tissue Samples

Immunoperoxidase labeling was performed on formaldehyde fixed paraffin embedded tumors, prepared and originally examined between 1979–1988. A total of 66 NSCLC samples were examined in this study, both macroscopically and histopathologically. Tumor staging was based on surgical, histopathologic, and radiologic data, according to the staging system of the American Joint Committee on Cancer.[7] In all cases studied the tumors were surgically removed and the patients were not subjected to radiotherapy. Patients with metastases or involved mediastinal lymph nodes were not included in this study. They were staged and diagnosed at the Sapir and Sheba Medical Centers in Israel. Of the 66 lung carcinoma patients, 48 were male and 18 were female with an average age of 63 ± 9 years.

Immunohistochemical Procedures

CK typing was performed by the streptavidin-biotin-peroxidase method[2] using the following anti-CK mouse monoclonal antibodies: 1) antibody Ks.B.17, which reacts with human CK polypeptide 18 and stains tumors expressing this polypeptide; 2) antibody A3-3, which reacts with CK 13 and stains tumors from stratified non-keratinizing epithelia; and 3) antibody E5-9, which reacts with CK polypeptide 10 and stains keratinizing epithelia.

Immunoperoxidase labeling was performed as previously described.[2] Labeled slides were examined

TABLE 1
Five-Year Survival (%) and Median Survival (Years) Values of Patients with Nonsmall Cell Lung Carcinoma According to Tumor Stage and Type

Stage/type	SQ	AD	UND	Total
I	59%[a]	33%[a]	36%[a]	50%[b]
	(27)	(12)	(11)	(50)
				5.5 yrs
II	0%	17%	20%	12%[b]
	(5)	(6)	(5)	(16)
				2.5 yrs
Total	50%	28%	31%	45%
	(32)	(18)	(16)	(66)
	5.0 yrs	3.5 Yrs	3.0 yrs	

SQ: squamous cell carcinoma; AD: adenocarcinoma; UND: undifferentiated carcinoma; yrs: years.
[a] $P = 0.26$.
[b] $P = 0.024$.
Number in parentheses indicates number of patients.

and evaluated by two independent observers. In rare cases of observer disagreement, additional sections were prepared and stained.

Statistical Analysis

Survival data of the patients were obtained from the computerized records of the Ministry of Health reporting overall survival, death due to all causes, and noncancer specific survival. The survival period was defined as the time from the date of surgery to the date of death. The Mantel-Haenszel test was used to define specific survival parameters for subgroups of patients according to histologic type, stage, and CK profile. The chi-square test was used to compare the subgroups with respect to 5 ysr.

RESULTS

A total of 66 independent tissue samples of lung carcinoma were staged and typed by regular histopathology. Comparison of the various cases (Table 1) indicates that the 5 ysr of patients with Stage II tumors was significantly lower than that of patients with Stage I tumors. However, differences between tumors of different types within each stage were insignificant. Similarly, the median survival was significantly lower (2.5 years) in Stage II tumors compared with Stage I tumors (5.5 years). The level of differentiation of the squamous cell carcinomas, as determined by histopathology, did not significantly affect the 5 ysr. The median survival of patients with squamous cell carcinomas (all stages, combined) was somewhat higher (5 years) than that of patients with adenocarcinomas (3.5 years) or undifferentiated carcinomas (3 years). It is noteworthy that the majority of patients included in this study had Stage I tumors (Table 1).

The 5 ysr and median survival, according to CK typing alone, regardless of the histologic typing and staging, is summarized in Table 2. As shown, staining with antibodies A3-3 and E5-9 did not provide any additional information regarding prognostic value because both positive and negative samples had similar 5 ysr and median survival values. Conversely, a marked difference was found between the 5 ysr of patients with Ks.B.17 positive and negative lung tumors (21% and 68%, respectively), as well as their median survival (13.5 years vs. 3.5 years), regardless of the stage and histologic type of the tumor.

The authors further examined the added prognostic value of CK typing (with Ks.B.17 antibody), combined with clinical staging and histopathologic typing (Table 3) (Fig. 1). As shown, the best survival rates (87% 5 ysr) were found in Ks.B.17 negative, Stage I squamous cell carcinoma. In the Stage I adenocarcinoma group, which is known to have a poor prognosis (33% 5 ysr), patients with tumors lacking expression of CK 18 had significantly higher survival rates (60% 5 ysr) compared with those with positively labeled tumors (14% 5 ysr).

In all histologic groups, patients with Ks.B.17 negative tumors also showed a much broader scatter and higher mean survival values (Fig. 2) than patients with positively labeled tumors. The number of patients that were still alive after >5 years of follow-up was higher in the Ks.B.17 negative tumor group (13 patients) compared with the Ks.B.17 positive tumor group (6 patients).

DISCUSSION

The primary objective of this study was to explore novel approaches for the reliable prognostic characterization of NSCLC. The need for an improved diagnostic procedure is evident from the limited prognostic information provided by conventional histologic typing of such tumors, especially Stage I tumors. As shown in the current study (Tables 1 and 3), and in other reports,[8,9] the variability in survival of patients with Stage I lung tumors is very high, regardless of the exact histologic definition of the tumor.

This problem is well illustrated in Table 1 and Figure 2, which show that although the mean 5 ysr and median survival values of patients with the 3 main types of tumors (squamous cell carcinoma, adenocarcinoma, and undifferentiated carcinoma) were similar, the range of survival values was quite broad, ranging from 2 months to >15 years. Clinical staging of these tumors did provide additional information, manifested mainly by the low (12%) 5 ysr of patients with Stage II tumors, yet the variability within each group was still very high.

TABLE 2
Survival of Lung Carcinoma Patients According to Cytokeratin Typing

Monoclonal antibody	Ks.B.17[a]		A3-3[b]		E5-9[c]	
Positive/negative	+	−	+	−	+	−
No. of cases	38	28	14	20	8	26
% 5-year survival rate	21	68	36	35	38	34
Median survival (yrs)	3.5	13.5	3.5	3.5	2.5	2.5
P value	0.004		0.97		0.88	

[a] Ks.B.17 stains tumors from pseudostratified epithelia (cytokeratin 18).
[b] A3-3 stains tumors from stratified nonkeratinizing epithelia (ctyokeratin 13).
[c] E5-9 is a marker for keratinization (cytokeratin 10).

TABLE 3
5-Year Survival (%) of Patients with Nonsmall Cell Lung Carcinoma According to a Combination of Histology, Staging, and Cytokeratin Typing

Histology/CK typing	SQII	SQI	ADII	ADI	UND II	UND I	Stage I	Stage II	Stage I and II
Ks.B.17(+)	0%	25%[a]	20%	14%[b]	33%	29%[c]	23%[d]	16%	21%[e]
	(4)	(12)	(5)	(7)	(3)	(7)	(26)	(12)	(38)
Ks.B.17(−)	0%	(87%)[a]	0%	60%[b]	0%	50%[c]	75%[d]	0%	68%[e]
	(1)	(15)	(1)	(5)	(2)	(4)	(24)	(4)	(28)
Both									
Ks.B.17(+)	0%	59%	17%	33%	20%	36%	50%	12%	45%
Ks.B.17(−)	(5)	(27)	(6)	(12)	(5)	(11)	(50)	(16)	(66)

CK: cytokeratin; SQII: Stage II squamous cell carcinoma; SQI: Stage I squamous cell carcinoma; ADII: Stage II adenocarcinoma; ADI: Stage I adenocarcinoma; UNDII: Stage II undifferentiated carcinoma; UNDI: Stage I undifferentiated carcinoma.
[a] $P = 0.001$.
[b] $P = 0.09$.
[c] $P = 0.48$.
[d] $P = 0.002$.
[e] $P = 0.004$.
The number in parentheses indicates the number of patients.

The choice of CK typing as a complementary diagnostic tool was based on the vast experience using intermediate filament and especially CK-typing for tumor diagnosis[10–15,17] and on the authors' previous experience with CK typing of cytologic samples of lung tumors.[1,16] As previously shown, each type of epithelial cell (both normal and transformed) expressed a specific and characteristic set of CK polypeptides.[10,15] Identification of the expressed CKs, using either an immunohistologic approach or electrophoretic analysis, may thus provide valuable information regarding the origin and state of differentiation of tumors, potentially reflecting their clinical properties.

The choice of CK antibodies for the current study was based on the rationale that although the primary tissue of origin of all carcinomas of the upper respiratory tract is simple or pseudostratified epithelia, these epithelia may further undergo squamous differentiation that might affect the biologic and pathologic properties of the tumor. The authors' previous study[1] indicated that lung tumors, including tumors cytologically diagnosed as adenocarcinomas, often contain cells with squamous differentiations and that squamous cell carcinomas frequently contain cells expressing CKs of simple or pseudostratified epithelia. This apparent discrepancy between the cytologic or histologic appearance of the tumors, and their cytokeratin profile was very intriguing, emphasizing the fact that the variability in cellular composition of the tumors, as revealed by CK labeling, has prognostic significance and value, as well as the fact that positive labeling for CK 18 is associated with poor prognosis.

In keeping with this rationale, the authors selected for the current study a set of monoclonal antibodies that selectively react with CKs present in keratinizing squamous epithelia (CK 10), nonkeratinizing squamous epithelia (CK 13), and simple epithelia (CK 18).

As shown earlier, immunostaining for CK 13 and

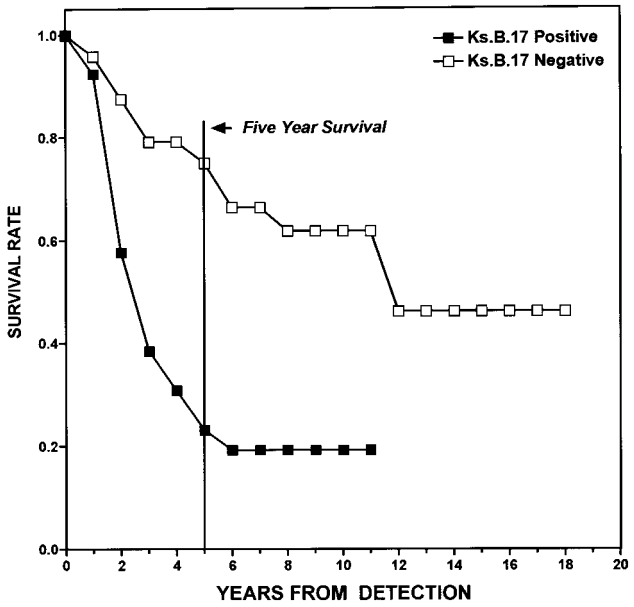

FIGURE 1. Survival of patients with nonsmall cell lung carcinoma (NSCLC) correlated with Ks.B.17 staining. Cumulative survival curves of Stage I NSCLC according to cytokeratin (CK) 18 typing. The average 5-year survival of CK 18 negative patients (monoclonal antibody Ks.B.17 negative) was significantly higher (75%) compared with CK 18 positive patients (23%).

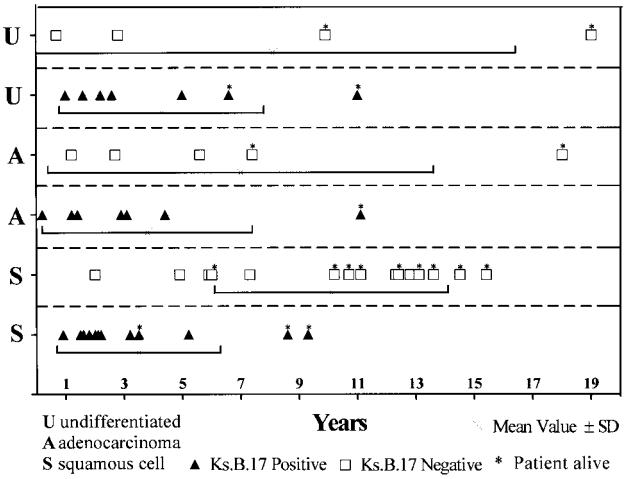

FIGURE 2. Survival of patients with nonsmall cell lung carcinoma (NSCLC) correlated with Ks.B.17 staining and histologic typing. Mean and scatter survival of the Ks.B.17 negative and positive Stage I patients according to the different histologic groups. In all histologic groups, patients with negatively stained tumors showed a much broader scatter and higher mean survival values than patients with positively stained tumors.

CK 10 provided information that was generally in agreement with the histologic typing (namely, it was present in squamous cell carcinoma but not adenocarcinoma), but the prognostic value of the labeling was quite limited. Conversely, the presence of simple epithelial characteristics, namely the continuing expression of CK 18, was a more highly reliable indicator for an aggressive tumor phenotype than the predominant morphology.

The rationale underlying the selection of cytokeratin antibodies for the current retrospective study was that transformed cells in the lung may either retain their simple epithelial characteristics or undergo squamous differentiation, and that this transition may have a strong effect on the malignancy of the tumor. To differentiate between simple and squamous epithelial cells, the monoclonal antibody Ks.B.17.2 was employed, which recognizes CK 18 (which is broadly expressed in simple epithelia and absent from squamous cell epithelia[12,15,18]), as well as antibodies that react with CK 13 and CK 10, which are expressed in nonkeratinizing and keratinizing epithelia, respectively.

The current study results indicate that the presence of cells expressing squamous epithelial keratins is largely in agreement with the histopathologic appearance of the tumors, and thus does not add much new information beyond the conventional histology. Conversely, staining for CK 18 did provide the most valid information because many tumors with a predominant squamous morphology contained Ks.B.17.2 positive cells and some histologically diagnosed squamous cell carcinomas were devoid of labeling. It is interesting to note that the expression of CK 18 has a much clearer prognostic capability than the dominant histology of the tumor. Thus, the best correlation with patient survival was found between CK 18 labeling and histologic tumor typing. Ks.B.17.2 positive adenocarcinomas had, by far, the poorest prognosis whereas Ks B.17.2 negative squamous cell carcinomas were correlated with the longest survival.

In conclusion, CK 18 immunolabeling of Stage I lung carcinomas may provide valuable information regarding the state of differentiation of the tumors and their aggressiveness and may thus be of great prognostic value. Moreover, combined with the histologic data, CK 18 typing allows even better distinction between tumors of more favorable and poorer prognosis.

On the basis of these findings, it is only appropriate to raise the question of whether high risk patients with CK 18 positive Stage I NSCLC should also receive adjuvant chemotherapy shortly after undergoing surgery, even though patients with primary Stage I tumors are considered, at this time, to be at low risk for recurrence and therefore do not warrant adjuvant therapy. Further studies including large patient popu-

lations are needed to answer this provocative, important, and practical question.[9,19]

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