Research Article

Tissue Expression of Low and High Molecular Weight Cytokeratins in Lung Carcinoma Sections: Its Correlation with Some Clinic-Pathological Features

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The tissue expression of low (LMW) and high (HMW) molecular weight cytokeratins and Ber-EP4 antigen in both small (SCLC) and non-small (NSCLC) cell lung carcinomas, as well as its correlation with a variety of clinic-pathological features, was evaluated. In general, 43/52 (82.7%) of NSCLC sections showed the expression of at least one type of cytokeratin while only 7/16 (43.7%) of SCLC were stained with both LMW cytokeratin and pan-cytokeratins antibodies. Remarkably, 18/52 (34.6%) of NSCLC were positive to both types of cytokeratins. However, none of SCLC showed this pattern of expression. In NSCLC patients, the increasing levels of HMW cytokeratins expression, as shown by 34 β E12 antibody, correlated with the occurrence of disease recurrence ($P = 0.0057$; Fisher's exact test). Consequently, the expression of HMW cytokeratins was found to be associated with a poor 4-year overall survival of NSCLC patients ($P = 0.0315$; Log rank test), not taking into account the histopathological classification of tumors. Similar results were obtained when 8-year overall survival was assessed ($P = 0.0103$; Log rank test). Our results could suggest the assessment of HMW cytokeratins in a larger series of NSCLC samples in order to confirm the potential prognostic value of them.

1. Introduction

Lung cancer is the most frequent cancer in the world and usually has a very poor prognosis [1, 2]. There are two main variants of the disease, non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). But, NSCLC constitutes about 80–85% cases of all lung carcinomas [3, 4]. NSCLC describes a histologically heterogeneous group of tumors with variable clinical behavior. Disease stage and performance status at time of diagnosis have been considered the two most noticeable prognostic factors in patients with NSCLC [5]; however, clinical outcomes in individual patients remain imprecise [6].

Consequently, novel biomarkers are currently assessed for early diagnosis and predicting treatment response and

prognosis in NSCLC patients. Between them, markers originating from cytoskeleton, especially the cytokeratins (CK), are of great practical interest to the clinicians and researchers [7]. CKs are the intermediate filament-forming proteins of epithelial cells, although they are expressed in a tissue-specific manner in normal organs and tumors that arise from them. Two types of cytokeratins are distinguished that form heterodimers, namely, acidic type I (cytokeratins 9–19) and basic to neutral type II (cytokeratins 1–8). Cytokeratins may also be classified as low molecular weight (commonly 8, 18, and 19) and high molecular weight (commonly 1, 5, 10, and 14) [8].

Due to the fact that epithelial tumors largely maintain the features of specific keratin expression associated with the respective cell type of origin, keratins have long and

extensively been used as immunohistochemical markers in diagnostic tumor pathology [9]. Since the development of monoclonal antibodies against several of the CK polypeptides, they are used to evaluate the pattern of CK expression in a variety of epithelial tumors [10] permitting the identification of particular types of cell differentiation and becoming useful diagnostic tools [9].

Additionally, these molecules have been considered important protectors of epithelial structural integrity under conditions of stress, regulators of other cellular functions, including motility, signaling, growth, and protein synthesis [8]. Moreover, accumulating evidence points to their importance as prognostic markers and, more interestingly, as active regulators of epithelial tumorigenesis and treatment responsiveness [8]. For that reason, in this study the tissue expression of both low and high molecular weight cytokeratin in lung carcinoma sections, as well as its association with a variety of clinic-pathological features, was evaluated. In addition, the expression of a transmembrane glycoprotein functioning as a cellular adhesion molecule (Ber-EP4) was assessed in the same samples.

2. Materials and Methods

2.1. Monoclonal Antibodies. Monoclonal antibodies against high molecular weight cytokeratins (1, 5, 10, and 14) (clone 34 β E12, Dako) and low molecular weight cytokeratins (cytokeratin 8) (clone 35BH11, Dako) were used. Additionally, the pan-cytokeratins (clone MNF116, Dako) that react with cytokeratins 5, 6, 8, 17, and probably also 19 as well as a monoclonal antibody that recognize a transmembrane glycoprotein functioning as a cellular adhesion molecule, Ber-EP4 (clone Ber-EP4, Dako), were also evaluated. All Mabs were diluted 1:20 using an antibody diluent solution (Dako S0809).

2.2. Tissue Specimens. A number of 52 and 16 routinely processed formalin-fixed and paraffin-embedded archival samples with diagnosis of non-small cell carcinoma and small cell carcinoma, respectively, were taken from the pathology department of both Hermanos Ameijeiras General Hospital and the National Institute of Oncology and Radiobiology, after obtaining approved consent by the institutional ethical committees.

2.3. Pathological Features Evaluation. Some morphological parameters such as histopathological classification, grade of differentiation, degree of cellular pleomorphism, and mitotic and necrosis indexes were evaluated for an expert pathologist (ChER) in each section using hematoxylin and eosin (H/E) staining as described in [11]. Briefly, the degree of cell pleomorphism was evaluated considering the cytomorphologic characteristics of tumor cells and it was grouped as low and high degree cell pleomorphism. Mitotic index was recorded by the evaluation of 10 high-power fields with 400x magnification (10x ocular, 40x objective) and it was calculated by dividing cells out of total cells counted and expressed as previously described for cell pleomorphism. For the

evaluation of the degree of tumor necrosis (necrosis index) on each section a low-power field with 100x magnification (10x ocular, 10x objective) was used. It was scored subjectively as follows: low necrotic index (less than 50% of necrosis areas per field) and high necrotic index (more than 50% of necrosis areas per field).

2.4. Immunohistochemical Staining. Five micrometer serial sections from each paraffin block were obtained in a micrometer (Leitz 1512) and they were mounted on plus slides (Dako S2024). All sections were attached to the slide by heating in a 70°C oven for 1 h. Afterwards the slides were kept at room temperature until they were used.

The slides were dewaxed in xylene and rehydrated in graded ethanol series as usual and endogenous peroxidase activity was blocked with 0.03% hydrogen peroxide in absolute methanol for 30 minutes. All sections were rehydrated in tap water, distilled water, and Tris/saline buffer solution (TBS) for 15, 10, and 5 minutes, respectively. The slides were pretreated with 0.4% pepsin in 0.1N hydrochloric acid solution at 37°C for 30 minutes. After pretreatments, the slides were washed gently in tap water and then with distilled water and TBS, as were described above.

Afterward, the tissues were incubated with primary monoclonal antibodies (see Section 2.1) in a humid chamber for 1 h at room temperature. After two rinses in TBS, the slides were incubated at room temperature with a rabbit anti-mouse biotinylated secondary antibody (Dako E0354) and ABCComplex/HRP (Dako E0355) during 30 minutes both at a dilution 1:100. Between incubations, slides were washed with TBS for 10 minutes. Afterward, enzymatic activity was visualized with DAB substrate chromogenic solution (Dako K3465) and the tissues were counterstained with Mayer's hematoxylin (Dako S2020). The samples were dehydrated and mounted with a synthetic medium. The analysis was performed using an Olympus BX51 bright-field microscope.

For conventional fluorescent and confocal microscopies, after the incubation with the primary antibody, a rabbit anti-mouse fluoresceinated secondary antibody (Dako E0354) (green signal) was used and finally the nuclei were counterstained with propidium iodide (PI) (Sigma P4170) at a final concentration of 4 μ g/mL (red signal). The analysis was performed using a Leica DMRXA fluorescence microscope and a Leica SP2 confocal microscope.

2.5. Immunohistochemical Evaluation. All tissue markers were evaluated for percentage of positive cells (0–100%) and the intensity of reaction (0–3+). The final results were considered according to two observers agreement (ChER and RB). Afterward, the IHC score (H-score) was used as was previously described [12] with minor modifications. The H-score was calculated for each specimen by multiplication of the intensity of reaction and the grade of positive cells, resulting in a score ranging from 0 to 300. Subsequently, these scores were grouped as follows: low expression (scores < 150) and high expression (scores $\geq$ 150).

2.6. Statistical Analysis. GraphPad Prism 5 Software (2007 GraphPad Software Inc., La Jolla, CA, USA) was used for data

analysis. Survival distribution was estimated by the Kaplan-Meier method. Two-sided Log-rank tests were performed to compare overall survival (OS) distribution according to cytokeratins and Ber-EP4 expressions as well as the clinic-pathological features. Chi-square and Fisher's exact tests were used to compare two or three different parameters. The correlation between biomarkers and clinic-pathological features was assessed by Spearman ranks correlation coefficients. For all tests, the criterion for statistical significance was $P < 0.05$.

3. Results

3.1. Patient Description and Pathological Features. Table 1 shows a summary of patient characteristics and some pathological features. The gender ratio was close to 2:1 in favor of males, with a mean age of 55.0 years (range 23–86 years). In general, the 4-year overall rate of NSCLC patients was 75.0% (29/52) while the mean overall survival of this population was 37.6 months (ranged from 3.0 to 54.3). Concerning SCLC, the overall survival was 56.3% (9/16) with a mean of 25.9 months (ranged from 0.9 to 41.6). Statistically significant difference was obtained when the survival of SCLC patients was compared with that from NSCLC ($P = 0.0478$; Log rank test).

When disease recurrence, grade of tumor differentiation, and both necrosis and mitotic index were compared between NSCLC and SCLC statistically significant differences were obtained ($P = 0.0216$, $P = 0.0001$, $P = 0.0009$, and $P = 0.0006$; Fisher's exact test, resp.). Tumor stage as well as the occurrence of both lymph node and disease recurrence was associated with poorer overall survival of NSCLC patients ($P = 0.0372$, $P = 0.0140$, and $P = 0.0024$; Log rank test, resp.). In addition, a tendency of association between the appearance of other site metastasis and overall survival of patients was detected ($P = 0.0522$; Log rank test, resp.).

3.2. Distribution of Cytokeratins in NSCLC and SCLC Samples. Table 2 shows the tissue expression of cytokeratins according to the histopathological classification of tumors. In general, 43/52 (82.7%) of NSCLC sections showed the expression of at least one type of cytokeratin. The expression of these molecules was mainly evidenced in the cytoplasm of malignant epithelial cells, although the cell membrane was also decorated (Figure 1). According to cytokeratin types, 35/52 (67.3%) of NSCLC were positive with pan-cytokeratins antibody, 37/52 (71.1%) expressed low molecular weight cytokeratin, and 22/52 (42.3%) expressed high molecular weight cytokeratin (Figure 2). No expression of high molecular weight cytokeratins was detected in SCLC (0/16) while 7/16 (43.7%) of cases were stained with both low molecular weight cytokeratin and pan-cytokeratins antibodies.

When the reactivity of high molecular weight cytokeratin in NSCLC and SCLC was compared statistically significant differences were evidenced ($P = 0.0015$, Fisher's exact test). No differences were detected when the reactivity of low molecular weight cytokeratin and pan-cytokeratins antibodies was compared in these types of tumors ($P = 0.4001$ and $P = 0.1403$, Fisher's exact test; resp.). No significant

differences were observed when the expression of high molecular weight cytokeratins was compared in epidermoid carcinomas, adenocarcinomas, and large cell carcinomas ($P = 0.1045$, Chi-square test). Similar results were obtained with low molecular weight cytokeratin and pan-cytokeratins Mabs ($P = 0.9505$ and $P = 0.6969$, Chi-square test; resp.).

As expected, the expression of high and low molecular weight cytokeratins correlates with the reactivity of pan-cytokeratins monoclonal antibody ($P = 0.0366$, $r_s = 0.2906$, and $P < 0.0001$, $r_s = 0.7715$; Spearman test). Nevertheless, the expression of high molecular weight cytokeratin tended to correlate with the expression of low molecular weight cytokeratin ($P = 0.0929$, $r_s = 0.2354$; Spearman test).

3.3. Considerable Overlapping of Cytokeratins Was Evidenced in NSCLC but Not in SCLC. Table 3 shows the tissue expression of both high and low molecular weight cytokeratins according to the histopathological classification of tumors. In general, 18/52 (34.6%) of NSCLC were positive to both types of cytokeratins. However, none of SCLC (0/16) showed this pattern of expression. Large cell carcinoma displayed the higher rate of cytokeratins overlapping (5/10) followed by epidermoid carcinomas (7/16) and the other histological type minority represented 2/6. Concerning adenocarcinomas, these tumors showed the lower rate of cytokeratins overlapping (4/20).

3.4. Ber-EP4 Expression Correlates with Low Molecular Weight Cytokeratin in Both NSCLC and SCLC Patients. The tissue expression of Ber-EP4 according to the histopathological type is shown in Table 4. In general, the expression of this antigen was evidenced in 33/52 (63.5%) and 7/16 (43.7%) of NSCLC and SCLC, respectively. No differences were detected when the expression of Ber-EP4 was compared in this group of tumors ($P = 0.5678$; Fisher's exact test). Significant differences were found when the expression of Ber-EP4 in adenocarcinoma was compared with epidermoid carcinomas and large cell carcinomas ($P = 0.0002$ and $P = 0.0004$; Fisher's exact test, resp.). No differences were detected when the expression of this antigen in epidermoid and large cell carcinomas was compared ($P = 0.8876$; Fisher's exact test).

On the other hand, the expression of Ber-EP4 antigen in NSCLC samples correlates with the expression of low molecular weight cytokeratin ($P = 0.0113$, $r_s = 0.3485$; Spearman test). A tendency to correlate with pan-cytokeratins expression ($P = 0.0597$, $r_s = 0.2629$; Spearman test) was also evidenced, while no correlation with the expression of high molecular weight cytokeratin was detected ($P = 0.5631$, $r_s = 0.0820$; Spearman test). In SCLC, a statistically significant correlation was obtained when the expression of Ber-EP4 antigen was compared with the reactivity of low molecular weight and pan-cytokeratins antibodies ($P = 0.0009$, $r_s = 0.7460$; Spearman test, in both cases).

3.5. The Expression of High Molecular Weight Cytokeratins Was Associated with Disease Recurrence in NSCLC Patients. In order to correlate the expression of cytokeratins with the clinic-pathological features, the expression of these molecules

TABLE 1: Clinic-pathological characteristics.

Features	NSCLC (<i>n</i> = 52) Number of cases (%)	SCLC (<i>n</i> = 16) Number of cases (%)	<i>P</i> value
Gender			
Female	15 (28.8)	6 (37.5)	0.5460
Male	37 (71.2)	10 (62.5)	
Age			
<60	37 (71.2)	13 (81.2)	0.5294
≥60	15 (28.8)	3 (18.8)	
Tumor size (cm)			
<3	12 (23.1)	7 (43.7)	0.1227
≥3	40 (76.9)	9 (56.3)	
Tumor stage			
Limited disease	—	13 (81.2)	—
Extended disease	—	3 (18.8)	
I-III A	44 (84.6)	—	
IIIB-IV	8 (15.4)	—	
Recurrence			
Yes	18 (34.6)	11 (68.7)	0.0216
No	34 (65.4)	5 (31.3)	
Lymph node metastasis			
Yes	17 (32.7)	2 (12.5)	0.2012
No	35 (67.3)	14 (87.5)	
Other site metastases			
Yes	5 (9.6)	2 (12.5)	0.6640
No	47 (90.4)	14 (87.5)	
Grade of differentiation			
Well/moderate	32 (61.5)	1 (6.2)	0.0001
Poor/undifferentiated	20 (38.5)	15 (93.8)	
Degree of cell pleomorphism			
Low	25 (48.1)	7 (43.7)	0.7834
High	27 (51.9)	9 (56.3)	
Necrosis index			
<50%	38 (73.1)	4 (25.0)	0.0009
≥50%	14 (26.9)	12 (75.0)	
Mitotic index			
Low	24 (46.2)	0 (0)	0.0006
High	28 (53.8)	16 (100)	

NSCLC, non-small cell lung carcinoma; SCLC, small cell lung carcinoma; %, percentages.

TABLE 2: Cytokeratins profile expression according to histopathological type.

Anti-CKs antibodies	<i>n</i>	34βE12 ^a		35BH11 ^b		MNFI16 ^c	
Low/high expression (%)		—	+	—	+	—	+
Epidermoid carcinoma	16	50.0	50.0	25.0	75.0	25.0	75.0
Adenocarcinoma	20	75.0	25.0	25.0	75.0	25.0	75.0
Large cell carcinoma	10	40.0	60.0	30.0	70.0	40.0	60.0
Others	6	50.0	50.0	50.0	50.0	40.0	60.0
Small cell carcinoma	16	0	0	56.3	43.7	56.3	43.7

^a34βE12 reacts with high molecular weight cytokeratins (1, 5, 10, and 14); ^b35BH11 stains cytokeratin 8 (low molecular weight cytokeratin); ^cMNFI16 reacts with cytokeratins 5, 6, 8, 17, and probably also 19.

FIGURE 1: Confocal microscopy microphotographs of non-small cell lung carcinoma. (a) FITC-labeled cytokeratin (green signal). Observe the intense reactivity of MNF116 antibody (reacts with cytokeratins 5, 6, 8, 17, and probably also 19) mainly located in the cytoplasm of malignant epithelial cells. (b) Nuclei counterstained with propidium iodide (red signal). (c) Overlay of microphotographs (a) and (b). Yellow bars = 100 μm .

FIGURE 2: Microphotographs of squamous carcinoma of the lung. (a) Hematoxylin and eosin coloration. Immunohistochemical localization of cytokeratin was performed by means of MNF116 (reacts with cytokeratins 5, 6, 8, 17, and probably also 19) and 34 β E12 antibodies (reacts with high molecular weight cytokeratins 1, 5, 10, and 14). Observe the intense reactivity of these Mabs located mainly in the cytoplasm of malignant epithelial cells. Conventional immunoperoxidase technique counterstaining with Carrazzi hematoxylin. Black bar = 50 μm .

TABLE 3: Expression of both high and low molecular weight cytokeratins according to histopathological type.

Tissue samples	Number of cases	^a 34 β E12 + ^b 35BH11
Low/high expression (%)		– +
Epidermoid carcinoma	16	56.3 43.7
Adenocarcinoma	20	80.0 20.0
Large cell carcinoma	10	50.0 50.0
Other	6	66.7 33.3
Small cell carcinoma	16	100 0

^a34 β E12 reacts with high molecular weight cytokeratins (1, 5, 10, and 14);
^b35BH11 stains cytokeratin 8 (low molecular weight cytokeratin).

TABLE 4: Expression of Ber-EP4 according to histopathological type.

Tissue samples	Number of cases	Ber-EP4 ^a
Low/high expression (%)		– +
Epidermoid carcinoma	16	52.5 47.5
Adenocarcinoma	20	25.0 75.0
Large cell carcinoma	10	50.0 50.0
Other	6	66.7 33.3
Small cell carcinoma	16	56.3 43.7

^aBer-EP4 antibody recognizes a transmembrane glycoprotein functioning as a cellular adhesion molecule.

was scored (see Section 2.5) according to both intensity of reaction and percentage of positive cells (Figure 3). In the group of NSCLC patients with increased expression of high

molecular weight cytokeratins, the occurrence of disease recurrence (11/18; 61.1%) was significantly more frequent than in patients with low expression of these cytokeratins (7/34; 20.6%) ($P = 0.0057$; Fisher's exact test). There were no

FIGURE 3: Conventional fluorescence microphotographs of non-small cell lung carcinomas. (a) Intense, (b) intense but focal, and (c) moderate and diffuse expression of high molecular weight cytokeratins (CKs 1, 5, 10, and 14) mainly located in the cytoplasm of malignant epithelial cells. FITC-labeled cytokeratin (green signal). Nuclei contrasted with propidium iodide (red signal). Yellow bars = 100 μ m.

TABLE 5: Survival of NSCLC patients according to antigens expression profile.

Antigens expression	34 β E12 ^a		35BH11 ^b		MNF116 ^c		Ber-EP4 ^d	
Low/high reactivity	–	+	–	+	–	+	–	+
Number of cases	41	11	26	26	24	28	29	23
% 4-year survival rate	87.8	27.3	73.1	76.9	66.7	82.1	82.8	65.2
Mean survival (months)	36.0	29.3	35.2	33.9	32.7	37.0	34.3	34.8
P value	0.0315		0.8380		0.1340		0.8268	

^a34 β E12 reacts with high molecular weight cytokeratins (1, 5, 10, and 14); ^b35BH11 stains cytokeratin 8 (low molecular weight cytokeratin); ^cMNF116 reacts with cytokeratins 5, 6, 8, 17, and probably also 19; ^dBer-EP4 antibody recognizes a transmembrane glycoprotein functioning as a cellular adhesion molecule.

differences in the frequencies of low and high expression of high molecular weight cytokeratins when gender, age of patients, pathologic stage, tumor size, occurrence of lymph node and other site metastasis, histopathological type, grade of differentiation, degree of cell pleomorphism, index of tumor necrosis, and mitotic index were considered.

No correlation between low molecular weight cytokeratin and Ber-EP4 expressions and clinic-pathological features was obtained in NSCLC patients. Concerning SCLC, no association between cytokeratin and Ber-EP4 tissue expressions and clinic-pathological characteristics was evidenced.

3.6. Tissue Expression of High Molecular Weight Cytokeratins Was Significantly Predictive of Survival Outcome. Table 5 shows the survival distribution in NSCLC patients according to antigens expression profile. The mortality of NSCLC patients displaying high and low expression of high molecular weight cytokeratins was 19.5% and 45.5%, respectively. Consequently, the expression of high molecular weight cytokeratins was found to be associated with a poor 4-year overall survival of NSCLC patients ($P = 0.0315$; Log rank test)

FIGURE 4: Kaplan-Meier estimate of 4-year overall survival among NSCLC patients showing different levels of high molecular weight cytokeratins expression ($P = 0.0315$; Log rank test).

(Figure 4), not taking into account the histopathological classification of tumors. Similar results were obtained when 8-year overall survival was assessed ($P = 0.0103$; Log rank test) (data not shown).

Differences in the reactivity of low molecular weight cytokeratin (Figure 5), pan-cytokeratins, and Ber-EP4 monoclonal antibodies were not significantly predictive of survival outcome ($P = 0.8380$, $P = 0.1340$, and $P = 0.8268$; Log rank test, resp.).

4. Discussion

Lung cancer is classified into small cell carcinoma (SCLC) and non-small cell carcinoma (NSCLC). The profile of molecular and genetic alterations considerably differs between SCLC and NSCLC, as well as among the subtypes of NSCLC [13].

FIGURE 5: Kaplan-Meier estimate of 4-year overall survival among NSCLC patients showing different levels of low molecular weight cytokeratins expression ($P = 0.8380$; Log rank test).

However, SCLC is the most aggressive lung cancer and almost fatal despite a good initial response to chemoradiation therapy [14]. In spite of recent progresses in the diagnosis and treatment, the prognosis of lung cancer still remains poor [1, 2]. A variety of prognostic factors have been identified in patients bearing lung cancer. Disease stage and performance status at time of diagnosis are considered among the most important prognostic factors in patients with NSCLC [5].

In this study, we showed an increased overall survival of patients at stages I-II as compared with the group of patients at stages III-IV, as well as in patients with absence of metastasis (lymph node and other site) and local recurrence. Concerning SCLC, patients showed increased disease recurrence, grade of tumor differentiation, and both necrosis and mitotic index displaying a poorer overall survival as compared with NSCLC. Nevertheless, the pathology-based TNM stage classification (tumor, node, and metastasis) has been considered to provide imprecise information about the survival rates [6]. Consequently, numerous studies are currently focusing in the selection of newer biological and molecular prognostic factors as a potential complement of TNM staging system [15–17].

In line with this, markers originating from the cytoskeleton, a complex network that influences the dynamic morphology of all eukaryotic cells in their tissue environment, are of great practical interest in lung cancer [18]. Among them, cytokeratins (CK), a group comprising of at least 29 different proteins, are intermediate filament proteins present in epithelial cells [19, 20]. They are expressed in a tissue-specific manner in normal organs and the tumors that arise from them [10]. However, under the influence of intrinsic or extrinsic factors, each cell will express different types of cytokeratins in course of its evolution which is also affected by the growth and differentiation rate [21].

It is known that the immunophenotypes of the main histologic types of lung carcinoma are stable and highly

reproducible. However, in the present work, it was observed as an overlapping of low and high molecular weight cytokeratins in about 30% of NSCLC samples. Between them, squamous and large cell carcinomas displayed the higher rates of cytokeratins overlapping, while the lower rates of overlapping were evidenced in adenocarcinomas. Usually, squamous cell carcinomas, independently of their site of origin, are characterized by the expression of the stratified epithelial keratins CK5, CK14, and CK17 and the hyperproliferative keratinocyte-type keratins CK6 and CK16 [9]. However, in poorly differentiated squamous cell carcinomas, coexpression of the simple epithelial keratins CK8, CK18, and CK19 is often observed [8]. Concerning large cell carcinomas, they have been considered as poorly differentiated non-small cell tumors coexpressing, in some cases, low (CK7) and high (CK5) molecular weight cytokeratins [22]. Adenocarcinomas express the simple epithelial keratins CK8, CK18, and CK19, whereas CK7 and CK20 expression is variable [9]. Because of considerable overlapping, immunophenotyping should not be used alone for histopathologic classification of lung cancer, but only as an adjunct to light microscopy [23].

Additionally, about half of the SCLC samples were stained with both pan-cytokeratins and low molecular weight (CK8) antibodies. Staining for pan-cytokeratin AE1/AE3 and low molecular weight cytokeratins (CK8, CK18, and/or CK19) antibodies has been previously demonstrated in SCLC samples [24, 25]. However, CK7 has been considered not very useful cytokeratin for SCLC diagnosis because only about half of these tumors stain with it [26, 27]. In line with this, in SCLC usually the diagnosis can be established based on the observation of high-quality hematoxylin and eosin stained sections without other immunostains [25]. Nevertheless, we obtained no expression of high molecular weight cytokeratins in SCLC samples. Similar results were previously published by Zhang et al. [28]. Although in the present work not so much cases were evaluated, high molecular weight keratin seems to be highly effective for distinguishing between small cell carcinoma and poorly differentiated squamous cell carcinoma.

Concerning Ber-EP4, this antigen was expressed in about 60% and 40% of NSCLC and SCLC, respectively. Malignant epithelial cells of different subtype of lung carcinoma displayed varying levels of Ber-EP4 antigen expression. But, adenocarcinomas were strongly positive (75% of cases) as compared with other lung carcinomas, including SCLC. Ber-EP4 antigen was reported to stain between 60% and 100% of lung adenocarcinomas [29]. In this study, the tissue expression of Ber-EP4 also correlated with the expression of the low molecular weight cytokeratin (CK8), the most common cytokeratin type found in our series of lung adenocarcinomas. Our results confirmed the increasing levels of this antigen in lung adenocarcinomas as compared with other lung tumors. The strong expression of Ber-EP4 in lung adenocarcinomas has permitted considering it a useful tool in distinguishing between these tumors and malignant mesothelioma [30, 31].

Finally, some authors have reported the prognostic value of cytokeratins in patients with non-small cell lung cancer. Nevertheless, the majority of these work included low

molecular weight cytokeratins such as CK8 [32], CK18 [33], and CK19 [34]. Here, the reactivity of 34 β E12 antibody was grouped into low and high levels of CKs expression, following the score previously described by our group [12]. Using this score, it was found that increased expression of high molecular weight cytokeratins measured by means of 34 β E12 antibody was associated with disease recurrence in NSCLC patients. Consequently, the expression of a group of high molecular weight cytokeratins by means of the clone 34 β E12 that reacts with 1, 5, 10, and 14 was associated with a poorer overall survival of NSCLC, without taking into account the histopathological type.

In previous studies, the increased expression of high molecular weight CK5/6, CK14, and CK17 proteins was associated with lower grading of NSCLC [35]. However, in esophageal squamous carcinoma, the reactivity of 34 β E12 antibody correlated with the occurrence of lymph node metastasis, depth of tumor invasion, and degree of tumor differentiation [36]. The abnormal expression patterns of staining using the 34 β E12 antibody have been also related with disease recurrences and disease-free survival in patients with low-grade papillary bladder neoplasms [37] as well as an independent predictor of disease-free survival in patients with triple-negative tumors of the breast [38]. Our results could suggest the assessment of high molecular weight cytokeratins in a larger series of NSCLC samples in order to confirm the potential prognostic value of them.

5. Conclusions

The use of cytokeratins as diagnostic tool in tumor pathology is by far their most common application in the field of cancer. In this study, the tissue expression of low and high molecular weight cytokeratins and Ber-EP4 antigen, in both SCLC and NSCLC, was reported. Considerable overlapping of cytokeratins was evidenced in NSCLC but not in SCLC. Although in the present work a limited number of SCLC samples was evaluated, tissue expression of high molecular weight cytokeratins seems to continuously be a highly effective tool for distinguishing between SCLC and poorly differentiated squamous cell carcinoma. Additionally, the expression of high molecular weight cytokeratins was associated with disease recurrence in NSCLC patients and was significantly predictive of survival outcome. Our results could suggest the assessment of high molecular weight cytokeratins in a larger series of NSCLC samples in order to confirm the potential prognostic value of them.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Charles E. Rengifo and Rancés Blanco contributed equally to this work.

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