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Research Article

Tumor Expression of the Carcinoembryonic Antigen Correlates with High Mitotic Activity and Cell Pleomorphism Index in Lung Carcinoma

Rancés Blanco,[1] Charles E. Rengifo,[2] Mercedes Cedeño,[2] Milagros Frómeta,[2] Enrique Rengifo,[2] and Mayra Ramos-Suzarte[3]

[1] *Laboratory of Specific Recognition and Biological Activity, Department of Quality Control, Center of Molecular Immunology, 216 Street and 15 Avenue Atabey, Playa, P.O. Box 16040, 11600 Havana, Cuba*
[2] *Department of Pathology, Manuel Fajardo General Hospital, Zapata and D Street Vedado, Plaza de la Revolución, 10400 Havana, Cuba*
[3] *Division of Clinical Trials, Center of Molecular Immunology, 216 Street and 15 Avenue Atabey, Playa, P.O. Box 16040, 11600 Havana, Cuba*

Correspondence should be addressed to Rancés Blanco; rances@cim.sld.cu

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At present, some research efforts are focusing on the evaluation of a variety of tumor associated antigens (TAAs) for a better understanding of tumor biology and genetics of lung tumors. For this reason, we evaluated the tissue expression of carcinoembryonic antigen (CEA) and ior C2 (a cell surface O-linked glycoprotein carbohydrate chain TAA) in lung carcinomas, as well as its correlation with a variety of clinicopathological features. The tissue expression of CEA was evidenced in 22/43 (51.16%) lung carcinomas and it was correlated with mitotic activity, cell pleomorphism indexes, and age of patients. The expression of ior C2 was observed in 15/43 (34.88%) tumors but no correlation with the clinicopathological features mentioned above was obtained. No correlation between both CEA and ior C2 antigens expression and the overall survival (OS) of non-small-cell lung cancer patients was also observed. However, CEA-negative patients displayed higher OS rates as compared with positive ones (69.74 versus 58.26 months). Our results seem to be in agreement with the role of CEA expression in tumor cell proliferation, inhibition of cell polarizations and tissue architecture distortion. The significance of ior C2 antigen in these malignancies and it potential use in diagnosis, prognosis, and/or immunotherapy must be reevaluated.

1. Introduction

Lung tumors are one of the leading causes of cancer-related mortality around the world [1]. There are two main variants of the disease, non-small cell lung cancer (NSCLC) and small-cell lung cancer (SCLC). However, NSCLC represents more than 80% of all lung carcinomas [2]. In patients with NSCLC, some genetic and regulatory abnormalities have been considered responsible for the tumor survival advantage. The alterations in gene expression that occur in cells during the malignant transformation usually conduce to the aberrant expression of antigens whether existing or not in normal cells. In this way, some research efforts are focusing on the evaluation of a variety of tumor associated antigens (TAAs) for a better understanding of tumor biology and genetics of lung tumors [3].

Carcinoembryonic antigen (CEA) is a glycoprotein expressed during embryonic and fetal development. It is frequently expressed on the apical surface of gastrointestinal epithelium, although it can also be found in other human epithelium, including lung tissues [4, 5]. Serum expression of CEA has been considered a sensitive and valuable tumor marker for diagnosis, prognosis, and therapy monitoring in lung cancer [6, 7]. Nevertheless, up to now, the evaluation

of CEA expression has been mainly restricted to the serum analysis [6–9] and the function of tumor CEA expression has remained unelucidated [10].

On the other hand, the ior C2 is an O-linked-tumor-specific glycoprotein with very limited presence in most normal tissues and overexpressed in the surface of some human malignancies. This TAA was demonstrated in these malignancies using the ior C5 Mab, a highly specific IgG1 against ior C2 antigen, by means of immunohistochemical methods [11, 12]. Additionally, immunoscintigraphy with 99mTc-labeled Mab ior C5 has been considered a useful procedure for the diagnosis and followup of the patients with colorectal tumors, its metastasis, and recurrences [13–17].

In the present work, we evaluated the tissue expression of CEA and ior C2 antigens in lung carcinoma, as well as its correlation with a variety of clinicopathological features such as mitotic activity index, cell pleomorphism, and overall survival of patients.

2. Materials and Methods

2.1. Monoclonal Antibodies. We used the ior cea1 [18] and ior C5 [12] Mabs, both obtained and produced by the Center of Molecular Immunology (Havana, Cuba). These Mabs are two murine IgG1 highly specific against the carcinoembryonic and a cell surface O-linked glycoprotein carbohydrate chain (ior C2) antigens, respectively.

2.2. Tissue Specimens and Previous Processing. A number of 43 routinely processed, formalin-fixed, and paraffin-embedded archival samples with diagnosis of lung cancer were obtained from the pathology department of the National Institute of Oncology and Radiobiology. The samples were taken after obtaining the approval consent by the institutional ethical committee. Five micrometer serial sections from each block were obtained in a micrometer (Leitz 1512, Germany) and mounted on plus slides (Dako S2024, Carpinteria, CA, USA). All sections were attached to the slide by heating in a 70°C oven for 1 h. Afterward, the slides were kept at room temperature until they were used.

2.3. Immunohistochemical Staining. The slides were dewaxed in xylene and rehydrated in decreasing ethanol series as usual and endogenous peroxidase activity was blocked with 0.03% hydrogen peroxide in methanol for 30 minutes at room temperature. Afterward all sections were washed in distilled water for 10 minutes, then were rinsed with TBS (Tris/saline buffer solution) for 5 minutes, were placed in a humid chamber, and incubated with the primary mouse ior cea1 and ior C5 Mabs for 1 h at room temperature. Negative controls were performed by substituting primary antibody for TBS and sections of colonic adenocarcinoma of known positivity for these antigens were taken as positive control. After two rinses in TBS, the slides were incubated with a rabbit anti-mouse biotinylated secondary antibody (Dako E0354, Carpinteria, CA, USA) and ABComplex/HRP (Dako E0355, Carpinteria, CA, USA) both for 30 minutes at room temperature 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, Carpinteria, CA, USA) and the tissues were counterstained with Mayer's hematoxylin (Dako S2020, Carpinteria, CA, USA). The samples were dehydrated and mounted with a synthetic medium.

2.4. Immunohistochemical Evaluation. The presence of brown staining (DAB reaction product) on both cell membrane and cytoplasm of malignant cells was considered as positive reactivity for ior cea1 and ior C5 Mabs. Sections only stained with Mayer's hematoxylin (blue color) were taken as negative.

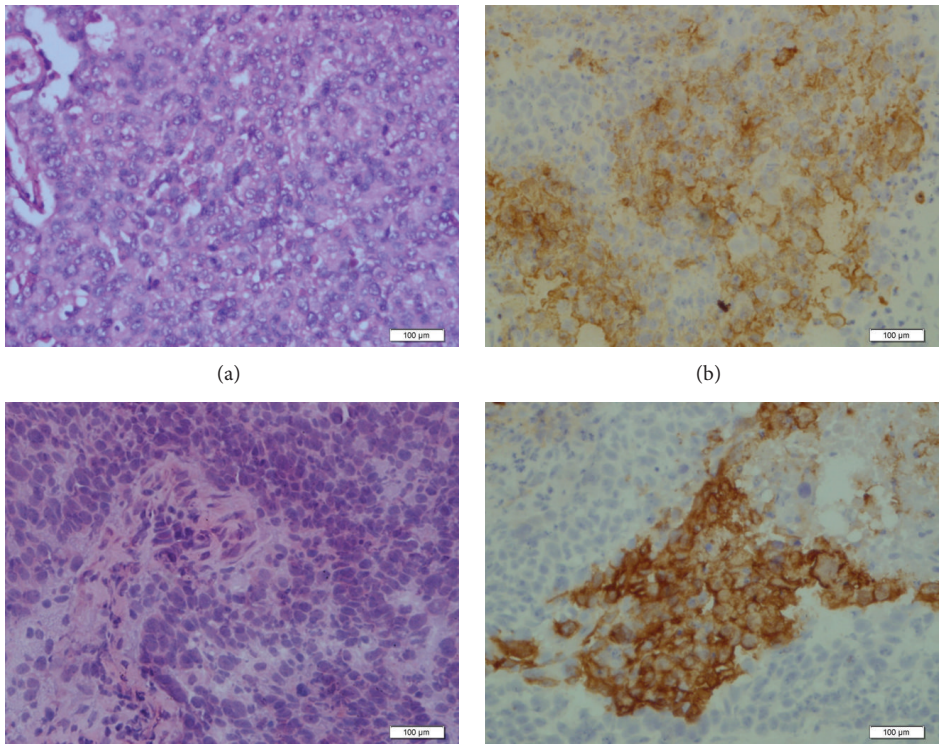
All evaluations were performed using an Olympus BX51 brightfield microscope with magnification of 200x and 400x (10x ocular with 20x and 40x objectives, resp.). The intensity of reaction of each tumor tissues was qualitatively estimated and expressed as follows: negative (−), weak (+), moderate (++), and intense (+++). A combination of these patterns was used to express intermediate levels of immunostaining. The most representative regions of each section were selected and the percentage of tumor cells stained with Mabs in them was estimated using the 10x objective lens (100x magnification). It was classified as 0 (negative to less than 5%), 1 (6%–50%), and 2 (more than 50%). All microscopic analyses were performed by two different observers.

2.5. Pathological Features Evaluation. The evaluation of some pathological features was performed for each tumor tissues using the hematoxylin and eosin (HE) staining. Morphologic parameters such as histopathological classification, grade of differentiation, and degree of cellular pleomorphism as well as mitotic and necrosis indexes were evaluated for an expert pathologist (Charles E. Rengifo) as previously described in [19]. Briefly, the degree of cell pleomorphism was expressed by the evaluation of some cytomorphologic characteristics such as cell and nuclear size, cellular shape, chromatin pattern, nucleoli, and amount of cytoplasm and was scored as follows: 0 (no evident cell pleomorphism) 1 (low), 2 (moderate), and 3 (high) cell pleomorphism.

For the mitotic activity index (MAI), the most cellular area of each tumor section (containing the subjectively highest number of mitoses) was selected. Then, only unequivocal mitotic figures were counted in 10 high-power fields with 400x magnification (10x ocular, 40x objective) of each sample. Finally, the MAI was calculated by dividing mitotic cells out of total cells counted and expressed as previously described for cell pleomorphism.

In the case of the degree of tumor necrosis (necrosis index), a low-power field with 100x magnification (10x ocular, 10x objective) of each section was used. It was scored subjectively as follows: 0 (no necrosis), 1 (less than 50% of necrosis areas per field), and 2 (more than 50% of necrosis areas per field).

2.6. Statistical Analysis. GraphPad Prism 5 software (2007 GraphPad Software Inc., La Jolla, CA, USA) was used for data analysis. The correlation between the reactivity of both ior



(a) (b)

(c) (d)

Figure 1: Microphotographs of lung squamous cell carcinomas sections. (a) and (c) represent hematoxylin and eosin staining. Vote malignant cells exhibiting a moderate mitotic activity index and high degree of cell pleomorphism, respectively. (b) and (d) represent tissue detection of CEA antigen. Note the moderate (b) and intense (d) reactivity of ior cea1 Mab. The reactivity of this Mab was mainly located in the membrane and also in the cytoplasm of malignant cells. White bar = 100 μm.

cea1 and ior C5 Mabs with clinical and pathological features was assessed by Spearman ranks correlation coefficients. Survival distribution was estimated by the Kaplan-Meier method. Survival comparison was performed by two-sided logrank tests. For all tests, $P < 0.05$ was considered statistically significant.

3. Results

3.1. Patient Description and Pathological Features. Tables 1 and 2 showed a summary of patient characteristics and some pathological features. The gender ratio was close to 2 : 1 in favor of males. The median of patient age at presentation was 55.58 years (ranged from 23 to 76 years). Median overall survival of patients was 65.55 months (ranged from 3 to 93.0 months) and 35.02 months for NSCLC and SCLC, respectively.

3.2. High Tumor Expression of CEA Was Evidenced in Lung Tumors. The tissue expression of CEA was evidenced in 22/43 (51.16%) lung carcinomas (Table 3). In 15/22 (68.18%) of tumors, a weak or focal reaction located in both cell clusters and its secretion with ior cea1 Mab was detected, while in 6/22 (27.27%) of samples a moderate-to-intense staining with this Mab was observed. In the latter, the pattern of staining of ior cea1 Mab was finely granular and was highly expressed in both the plasmatic membrane and the cytoplasm of malignant cells (Figure 1).

3.3. CEA Expression Correlates with Age of Patient, High Mitotic Activity Index, and Cell Pleomorphism in Lung Carcinoma. A significant correlation was detected between the tissue expression of CEA and both MAI ($P = 0.0218$, rs = 0.3489; Spearman test) and pleomorphism index ($P = 0.0159$, rs = 0.3655; Spearman test). In addition, the expression of CEA correlates with the age of patients ($P = 0.0497$, rs = −0.3012; Spearman test). No correlation between CEA expression and the rest of the clinicopathological features was observed.

3.4. The Expression of Ior C2 Antigen Was Limited in Lung Tumors. The expression of ior C2 antigen was observed in 15/43 (34.88%) lung tumors and was mainly localized in cell clusters and its secretion (score 1). According to the histopathological classification, 4/14 (28.57%) squamous cell carcinoma, 5/14 (25.71%) adenocarcinoma, and 3/9 (33.33%) large cell carcinoma as well as 2/4 (50.00%) of other minor types represented were recognized by the ior C5 Mab (Figure 2). The reactivity of ior C5 Mab was also evidenced in 2/2 of SCLC.

No correlation between the expression of ior C2 antigen and the clinicopathological features was obtained.

Table 1: Patients characteristics.

Features	Number (%) ($n = 43$)
Gender	
Female	15 (34.88)
Male	28 (65.12)
Age (years)	
<60	31 (72.09)
60–70	9 (20.93)
>70	3 (6.98)
Tumor size (cm)	
<3	7 (16.28)
>3	36 (83.72)
Tumor stage	
LD	1 (2.32)
I	24 (55.81)
II	8 (18.60)
III A	5 (11.63)
III B	2 (4.65)
IV	3 (6.98)
Recurrence	
Yes	36 (83.72)
No	7 (16.28)
Overall survival	
Alive	33 (76.74)
Dead	10 (23.26)
Median (months)	63.07

Legend: no.: number of cases; %: percentages; LD: limited disease.

Table 2: Tumor characteristics.

Features	Number (%) ($n = 43$)
Histopathological type	
Small-cell lung carcinoma	2 (4.65)
Non-small cell lung carcinoma	
Squamous cell carcinoma	14 (32.56)
Adenocarcinoma	14 (32.56)
Large cell carcinoma	9 (20.93)
Other	4 (9.30)
Grade of differentiation	
Well	5 (11.63)
Moderate	18 (41.86)
Poor	15 (34.88)
Undifferentiated	5 (11.63)
Degree of cell pleomorphism	
No evident	1 (2.33)
Low	22 (51.16)
Moderate	12 (27.91)
High	8 (18.60)
Necrosis index	
No evident	17 (39.53)
<50%	15 (34.88)
>50%	11 (25.58)
Mitotic index	
No evident	3 (6.98)
Low	13 (30.23)
Moderate	18 (41.86)
High	9 (20.93)

Legend: no.: number of cases; %: percentages.

3.5. Relationship between Antigens Expression and Overall Survival of Patients. No correlation between both CEA and ior C2 antigens overexpression and the overall survival of NSCLC patients was observed ($P = 0.1772$ and $P = 0.5043$; logrank test, resp.) (Figures 3 and 4). However, CEA-negative patients displayed higher overall survival rates as compared with positive ones (69.74 versus 58.26 months).

4. Discussion

Despite the recent advances in lung cancer therapy, this disease still has a very poor prognosis, representing one of the leading causes of cancer-related mortality worldwide [1]. For that reason, the identification of prognostic factors, including tumor markers, clinicopathological indicators and genetic alterations, is of the utmost importance for patients with lung cancer [3].

The overexpression of CEA has been reported in 30%–70% of patients with NSCLC and was most frequently observed in patients with adenocarcinoma and advanced stage carcinoma [20]. In this study, we showed the tissue reactivity of ior cea1 Mab, a murine IgG1 highly specific raised against carcinoembryonic antigen, in about the half of lung cancer sections evaluated. The pattern of staining with this Mab was finely granular and was localized in both, cell membrane and cytoplasm of malignant epithelial cells. A similar immunohistochemical result using a different anti-CEA Mab was previously described [10]. Moreover, the *in vivo* tissue reactivity of ior cea1 Mab labelled with 99mTc was demonstrated in both primary and metastatic colorectal carcinoma by the radioimmunoscintigrafic technique [16, 17, 21].

Several studies have shown that high CEA levels are a potential marker of poor prognosis in NSCLC regardless of treatment [6, 20]. Meanwhile, the determination of which amount of tissue CEA expression in lung tumors has clinical and/or biological relevance still remains unclear [10]. We obtained no differences between CEA tissue expression and overall survival of patients. Although, patients with high CEA expression displayed lower overall survival rates as compared with negative ones. In this way, the potential prognostic value of ior cea1 Mab reactivity in NSCLC should be evaluated using a higher number of cases.

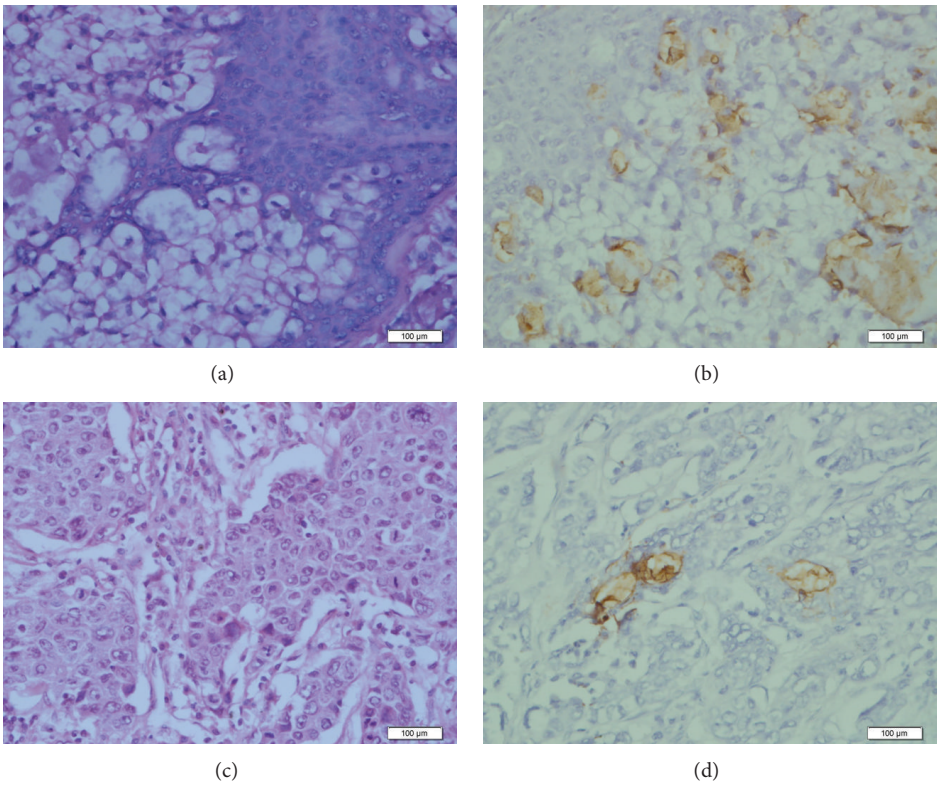
It is known that CEA is a cell surface adhesion protein related with the cell-to-cell adhesion [22], blocking cell differentiation effects and cellular transformation [23]. Additionally, the antiapoptotic effects of CEA expression in cancer cells have been demonstrated [24].

The overexpression of CEA has been considered to be directly related to the tumor aggressive behavior [8, 25]. Here, we found that high levels of CEA expression correlate with

Table 3: Immunohistochemical expression of CEA.

Histopathological type	H-score		
	0	1	2
	Number of cases (%)	Number of cases (%)	Number of cases (%)
SCLC	0	1/2 (50.00)	1/2 (50.00)
NSCLC			
Squamous cell carcinoma	5/14 (35.71)	6/14 (42.86)	3/14 (21.43)
Adenocarcinoma	9/14 (64.29)	4/14 (28.57)	1/14 (7.14)
Large cell carcinoma	6/9 (66.67)	2/9 (22.22)	1/9 (11.11)
Other	2/4 (50.00)	2/4 (50.00)	0

Legend: SCLC: small cell-lung cancer; NSCLC: non-small cell lung cancer; %: percentages; 0: negative; 1: (6%–50%) and 2: (more than 50%).



(a) (b)

(c) (d)

Figure 2: Hematoxylin and eosin staining of lung adenocarcinoma (a) and mucoepidermoid carcinoma (c) sections. Note: malignant cells exhibiting a moderate degree of cell pleomorphism and high mitotic activity index, respectively. (b) and (d) represent tissue detection of C2 antigen. Note the moderate reactivity of ior C5 Mab mainly located in cell secretion and cell clusters, respectively. White bar = 100 μm.

an increase in tumor cell proliferation measured by means of mitotic activity index. In previous studies, the expression of CEA in the proliferative phase of the human colonocytes cell cycle has been reported to be limited [26]. Nevertheless, induced constitutive expression of CEA at all phases of the growth cycle, as in most of colorectal carcinomas [27], has been related to the appearance of dramatic tumorigenic effects [26].

Previously, it was demonstrated that CEACAM6, a closely related family member of CEA, is able to induce lung cells proliferation, probably by interfering with the contact-inhibiting signal, leading to undifferentiated anchorage-independent cell growth [28]. In the present work, we obtained a correlation between CEA expression and the degree of cell pleomorphism measured by the evaluation of some cytomorphologic characteristics. Deregulated over-expression of CEA in human cells that remain potential division and have not yet differentiated can decrease the cellular differentiation, block cellular polarization, and distort normal cellular architecture both *in vitro* and *in vivo* [26].

In previous studies, little differences in the biological behavior of NSCLC in older and younger individuals have been reported [29]. In this way, molecular phenotypic studies have been suggested to investigate this controversial topic [30]. Here, we found that younger patients exhibited high

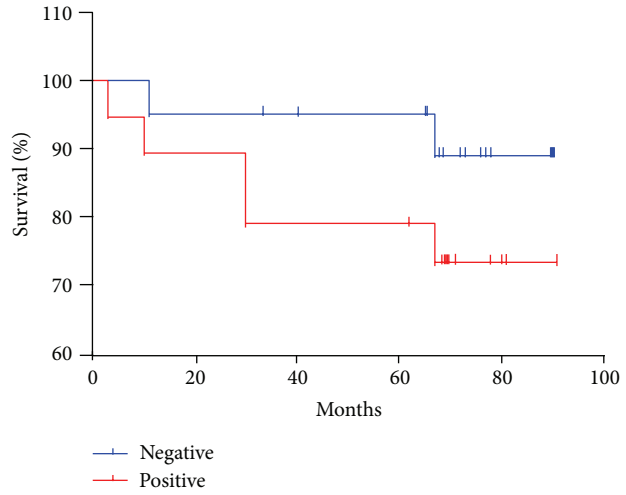


Figure 3: Kaplan-Meier estimate of overall survival among NSCLC patients showing different levels of tissue CEA expression (P = 0.1772; logrank test).

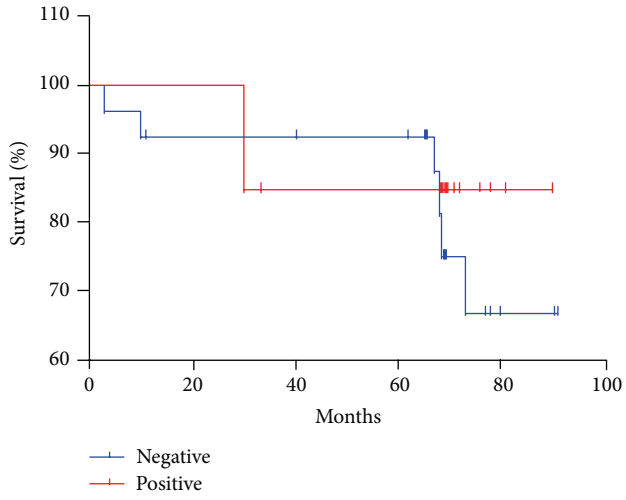


Figure 4: Kaplan-Meier estimate of overall survival among NSCLC patients showing different levels of tissue expression of ior C2 antigen (P = 0.5043; logrank test).

level of tissue CEA expression as compared with older patients. These results are in agreement with the suggestion that younger NSCLC patients generally display a more accelerated growth and progress than that in older patients [31]. However, NSCLC had been considered a similar entity in older and younger individuals, since the same clinicopathological prognostic factors were valid in both age groups [29, 30].

Our results seem to be in agreement with the important role of tissue CEA expression in lung tumor biology, although no correlation of CEA expression and some pathological features such as tumor size, tumor state, and recurrence was evidenced.

On the other hand, the ior C5 Mab is a murine IgG1 that recognizes the TAA ior C2. This antigen is a cell surface O-linked glycoprotein carbohydrate chain very limited in most normal tissues and heterogeneously expressed in a variety of human malignancies such as ovary, breast, uterus, and lung tumors, although it was mostly evidenced in colorectal tumors [12, 32]. In primary and metastatic colorectal carcinomas as well as in ovarian adenocarcinomas, these results were confirmed by immunoscintigraphy technique with ior C5 Mab labeled with 99mTc-labeled [13–17, 33].

In lung tumors, the expression of ior C2 antigen was only restricted to adenocarcinomas [12, 32]. In the present work, the tissue reactivity of ior C5 Mab was detected in about the 35% of lung carcinomas, not taking into account the histopathological classification. Curiously, the staining with this Mab was mostly evidenced in SCLC. The pattern of recognition was finely granular and was located in both epithelial cells and its secretion, similar to that described in [12, 32].

In spite of the expression of the ior C2 antigen in tumors that has been demonstrated, to our knowledge the clinical and/or biological significance in lung cancer still remains unclear. Unfortunately, in this study we obtained no correlation between ior C2 antigen expression and the overall survival of patients neither with the rest of the clinicopathological features assessed. In this way, a higher series of lung tumors must be evaluated in order to explore the role of ior C2 antigen in these malignancies, as well as its potential use in diagnosis, prognosis, and/or immunotherapy.

5. Conclusions

In summary, we reported the tissue expression of CEA antigen by means of ior cea1 Mab, in both SCLC and NSCLC. The correlation of CEA expression with age of patient, high mitotic activity index, and the degree of cell pleomorphism was also demonstrated. Our results seem to be in agreement with the role of CEA expression in tumor cell proliferation as well as in the inhibition of cell polarization and the distortion of tissue architecture. Additionally, we showed the immunohistochemical expression of ior C2 antigen, a cell surface O-linked glycoprotein carbohydrate chain, in the same system. However, no correlation between ior C2 antigen expression and the overall survival of patients neither with the rest of the clinicopathological features was obtained. In this way, the significance of ior C2 antigen for lung tumors biological behavior still remains unclear.

Conflict of Interests

The authors report no conflict of interests.

Authors' Contribution

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

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