Estrogen-Receptor-Related Protein p29 in Primary Nonsmall Cell Lung Carcinoma

Pathologic and Prognostic Correlations

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BACKGROUND. Estrogen-dependent intracellular processes are important in the growth regulation of normal tissue and may play a role in the regulation of malignancies. Utilization of estrogen receptor assays in breast carcinoma is well established, but the role of such evaluation in other cancers largely is unknown. In this study, immunohistochemical expression of estrogen receptor (ER) and the ER-related protein p29 was correlated with survival of patients with nonsmall cell carcinoma of the lung.

METHODS. All patients with a tissue diagnosis of primary nonsmall cell bronchogenic carcinoma diagnosed over a 6-year period at the Medical Center Hospital of Vermont were reviewed. Assays for p29 and ER using a streptavidin-biotin immunoperoxidase method were performed on each tumor. Results were correlated with clinical data, including survival.

RESULTS. Of 111 tumors examined, 109 (98%) were positive for p29 whereas none of the tumors reacted with ER (ER1D5). The relation between p29 expression and survival time was different for men and women. A statistically significant negative relation for women was observed; this relation was most pronounced in patients with Stage I and II tumors. A positive but not statistically significant relation was observed for men.

CONCLUSIONS. The ER-related protein p29 commonly is expressed in nonsmall cell carcinomas of the lung. The relation between p29 and survival time is different for males and females, suggesting the presence of gender specific factors that may influence tumor growth and overall patient survival, especially in patients with early stage lung carcinoma. ***Cancer* 1998;82:1495–500.**
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KEYWORDS: lung, bronchogenic carcinoma, nonsmall cell carcinoma, estrogen receptor, estrogen receptor-related protein, p29, immunohistochemistry, survival analysis.

Estrogen receptor (ER) and its related intracellular proteins commonly are known to be present in cells of the breast and endometrium, but also have been identified in diverse normal and neoplastic nonreproductive tissues.[1–16] ER is believed to mediate growth and maturation of normal tissue through a complex interaction with nuclear regulatory proteins that initiate cell division. The receptor's precise role in the development and biologic behavior of reproductive malignancies and other cancers remains largely unknown. Although ER has been demonstrated in primary bronchogenic carcinomas by methods involving both tissue pulverization[7,17–21] and immunohistochemistry,[6,8,9,22,23] other recent immunohistochemical studies have refuted the presence of ER in bronchogenic carcinomas.[24,25]

Expression of ER has been shown to be independently related to favorable prognosis in breast carcinoma[26] and endometrial carcinoma[27] and to poor prognosis in gastric carcinoma.[28] ER in lung carcinoma may[29] or may not[22] have prognostic value.

We attempted to determine whether ER was present in nonsmall cell lung carcinomas, and to correlate its expression with survival. Archival tissue from 111 patients with primary nonsmall cell lung carcinoma was evaluated retrospectively employing immunohistochemical analysis. To gain detection sensitivity, two antibodies were employed, one directed against ER and one directed against an ER-related protein (p29). Only patients who had undergone surgical resection or biopsy were evaluated.

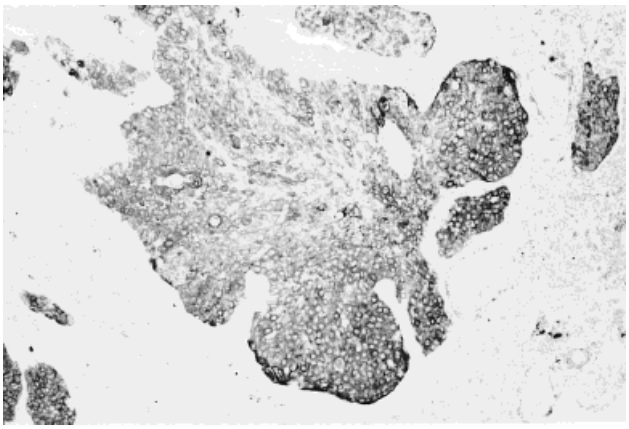

FIGURE 1. Squamous cell carcinoma immunoreactive with p29. A rim-like peripheral accentuation of staining was observed in some tumors (immunoperoxidase method, original magnification ×25).

MATERIALS AND METHODS

Lung Carcinoma Cases

Glass slides and paraffin embedded tissue blocks were retrieved from all primary nonsmall cell bronchogenic carcinomas diagnosed at the Medical Center Hospital of Vermont during a 6-year period from 1977 to 1982. The interval for study was chosen to allow a minimum of 10 years of follow-up for survival assessment. This included only patients in whom tissue was obtained by bronchoscopic biopsy or surgical resection. Corresponding clinical data were obtained from the Medical Center Hospital of Vermont Cancer Registry. Cases were excluded for incomplete staging,[30] incomplete follow-up information, insufficient tissue, or the possibility that the lesion was not primary in origin. All original glass slides were reviewed microscopically and reclassified by histologic type[31] and grade by a single pathologist (D.L.W.). Discrepancies from the original diagnosis were arbitrated by a second pathologist (K.O.L.). One representative paraffin embedded tissue block from each case was selected for immunohistochemical studies.

Immunohistochemistry

Antibodies were localized in tissue sections using a commercially available streptavidin-biotin immunoperoxidase method according to instructions provided by the manufacturer (BioGenix, San Ramon, CA). Immunohistochemical assays were performed on sections of formalin fixed tissue employing a robotic automated immunohistochemistry system (Biotek, Santa Barbara, CA). Heat-induced (microwave) antigen retrieval in citrate buffer was used in all assays. Immunostaining for ER protein was performed using a commercially available monoclonal mouse antibody specific for ER that is known to detect ER in formalin fixed, paraffin embedded tissues (ER1D5; Immunotech, Westbrook, ME). Immunostaining for the ER-related protein p29 was performed using a commercially available monoclonal mouse antibody directed against this protein (BioGenix). Positive tissue controls appropriate to each antibody were used. Immunoglobulin G2A monoclonal antibody without defined specificity and buffer alone were used as antibody and method controls, respectively. Diaminobenzidine (DAB) tetrahydrochloride (Polysciences, Warrington, PA) was used as the chromogen at a concentration of 0.5 mg/mL in TRIS buffer (pH 7.6) in the presence of hydrogen peroxide (H_2O_2) (25 uL of 3% H_2O_2/5 mL DAB). Sections were counterstained lightly with Harris' hematoxylin for morphologic orientation, dehydrated, and coverslipped with mounting medium. Counterstain was omitted for photomicroscopy.

Staining Analysis

All preparations were reviewed and graded by a single surgical pathologist who was blinded to the clinical details of the case. Immunohistochemical staining for ER and p29 was graded using the HSCORE method, in which intensity of staining was scored from 0 to 3 and multiplied by the percentage of stained cells in a field. The final specimen score was an averaged value of several field scores within the tumor and was expressed on a scale from 0 to 300.

Statistical Analysis

Relations between p29 score and other patient characteristics were assessed using Pearson's correlation for continuous variables and analysis of variance for categoric variables. Gender differences were analyzed by Student's t tests and chi-square tests. Cox regression analysis was used to analyze the relation between p29

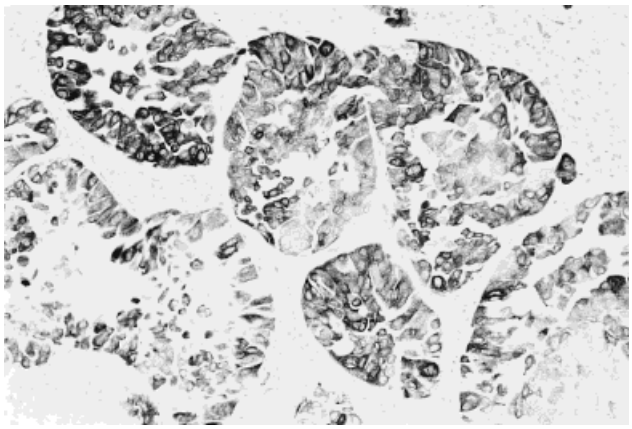

FIGURE 2. Adenocarcinoma immunoreactive with p29. Patchy intense staining commonly was observed (immunoperoxidase method, original magnification ×50).

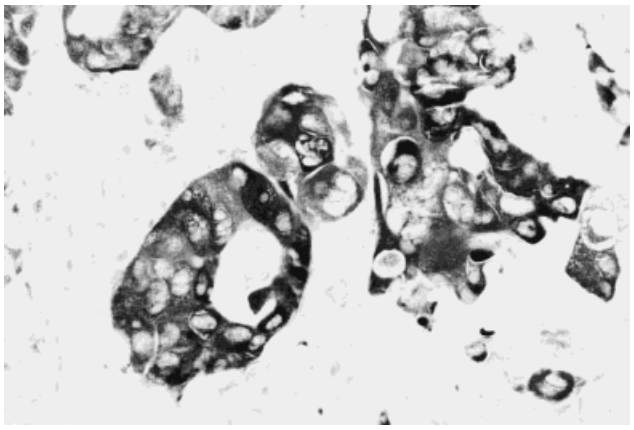

FIGURE 3. Adenocarcinoma immunoreactive with p29. A higher magnification of Figure 2 shows intense cell specific cytoplasmic staining (immunoperoxidase method, original magnification ×100).

score and survival time and to examine the effects of covariates on this relationship.

RESULTS

The Surgical Pathology archives held tissue from 153 patients diagnosed with bronchogenic carcinoma between 1977 and 1982. Of these available specimens, 16 cases were missing adequate clinical data or complete staging information, 18 specimens (bronchoscopic biopsies) were of insufficient size to study, 2 tumors were not convincingly of bronchogenic origin, and 6 specimens were small cell carcinoma. Table 1 shows the characteristics of the 111 cases that met all entry criteria of the study. The majority of specimens (76%) came from men. Age and stage were distributed similarly for men and women. The majority of men (55%) had squamous cell tumors, whereas women had a higher proportion of adenocarcinoma (59%).

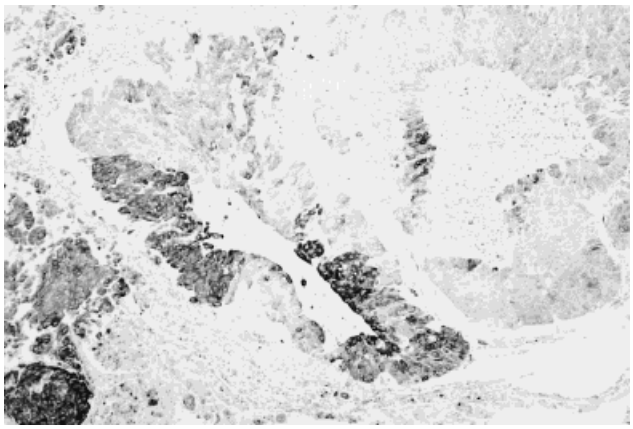

FIGURE 4. Large cell undifferentiated carcinoma immunoreactive with p29. Very focal but distinctive tumor staining occasionally was observed (immunoperoxidase method, original magnification ×25).

p29 immunoreactivity was expressed in nearly all cases (98%). None of the cases was immunoreactive for ER. Table 2 shows mean scores for p29 by cell type and gender. Overall, squamous cell carcinoma had significantly higher p29 scores than adenocarcinoma or undifferentiated large cell carcinoma. Within each histologic type, the scores for men and women did not differ significantly. There were no significant relations between p29 score, age, histologic type, and histologic grade. The seven subjects with Stage IIIB tumors were all men and had significantly lower p29 scores than did subjects with tumors of other stages.

The length of survival was not significantly related to p29 score when data from both men and women were analyzed together, but there was a significant interaction between the effects of gender and p29 score. For women, there was a significant negative relationship between p29 score and survival time ($P = 0.046$). In men, there was a nonsignificant positive relation between p29 score and survival time ($P = 0.125$). The observed difference between the regression coefficients for men and women was highly significant ($P < 0.001$). Inclusion of age, tumor type, histologic grade, or stage in the survival model did not alter these results; p29 score remained a significant predictor for women, but not for men. Age and histologic grade were not related to survival for either men or women. Tumor stage was significantly related to survival in men ($P < 0.001$), with men with Stage IIIA tumors having the shortest survival. Stage was not significantly associated with length of survival in women. However, when only Stages I and II were included in the survival analysis, the inverse relation between p29 score and

TABLE 1
Population Characteristics

Gender	Male	84 (76%)		
	Female	27 (24%)		
Age (yrs)			Male	Female
	Median (range)		61 (45–78)	59 (44–72)
	Mean (±SD)		61 (± 8.5)	59 (± 7.5)
Histologic type		All subjects	Male	Female
Squamous		53 (48%)	46 (55%)	7 (26%)
Adeno		42 (38%)	26 (31%)	16 (59%)
Undifferentiated		16 (14%)	12 (14%)	4 (15%)
Histologic grade		Grade 1 (3%)	Grade 2 (51%)	Grade 3 (46%)
Squamous		1	29	23
Adeno		3	27	12
Undifferentiated		0	0	16
Stage		Male		Female
I		39 (46%)		15 (56%)
II		15 (18%)		3 (11%)
IIIA		16 (19%)		6 (22%)
IIIB		7 (8%)		0 (0%)
IV		7 (8%)		3 (11%)

SD: standard deviation; Adeno: adenocarcinoma.

TABLE 2
Immunoreactive Staining Results Grouped by Histologic Cell Type and Gender

Cell type	Gender (no.)	p29 immunoreactivity score[a]
Squamous	Male (46)	199.89 ± 69.5
	Female (7)	225.70 ± 61.5
Adeno	Male (26)	133.96 ± 77.32
	Female (16)	180.00 ± 82.04
Undifferentiated	Male (12)	148.42 ± 88.96
	Female (4)	147.50 ± 34.03

Adeno: adenocarcinoma.
[a] Arbitrary units ± standard deviation.

length of survival in women was more highly significant (P = 0.003). Tumor type was not significantly related to survival time in men, but in women, undifferentiated large cell carcinomas were associated with shorter survival (P = 0.04).

DISCUSSION

This study identified a relation between the ER-related protein p29 and clinical outcome in women with non-small cell bronchogenic lung carcinoma. Examination of 111 tumors of various histologic types revealed that p29 expression, measured by immunohistochemical techniques, was present in nearly all tumors studied (98%) and the intensity of immunoreaction within women's tumors correlated inversely with the length of survival. Expression of p29 staining in carcinomas from men was unrelated to length of survival. We believe this is the first observed gender-dependent difference in the behavior of bronchogenic carcinoma related to a protein postulated to have a role in estrogen-dependent signaling mechanisms.

The *P* value of 0.046 for the inverse relation between p29 and survival in women may be considered marginal by some. However, the relation for men was in the opposite direction and although the regression coefficient was not significantly different from zero for men, its difference from the coefficient for women was highly significant ($P < 0.001$). This finding suggests a gender specific difference in the role of p29 that warrants further study.

Our findings are in keeping with those of Yang,[29] who studied 80 cases of lung carcinoma in women and found that 5-year survival rates for ER negative patients were significantly higher than for ER positive patients (72.5% vs. 8.8%, respectively). This study did not include a comparison with male patients. One other study has examined survival in relation to the presence of ER in lung carcinoma from both men and women and found no correlation, but this study followed 64 patients for only 3 years.[22]

The exact relation between p29, a 29-kilodalton serine phosphoprotein, and ER has not been elucidated. p29 is the ER-related protein correlating most closely with the presence of ER in breast carcinoma.[32,33,34] It does not itself bind steroids but has been shown to form complexes with cytosolic ER, is quantitatively and qualitatively related to concentrations of ER in breast carcinomas, and has been shown to predict response to hormone therapy.[35] Furthermore, p29 is down-regulated whereas ER remains unchanged in human placental tissue and decidua after treatment with mifepristone (RU 38,489) and a prostaglandin E1 analogue, suggesting a functional interdependence.[36]

It is possible that the relation between p29 expression and lung carcinoma behavior observed in this study is dependent on estrogen-dependent interactions with growth control receptors. The apparent stimulatory and inhibitory effects of estrogen have been invoked to explain the gender differences in the epidemiology of various tumors.[7,37] There is evidence suggesting that exogenous estrogens increase the risk of lung carcinoma in women[38] and in rats.[39] The manipulation of estrogen levels has in fact been shown to affect the behavior of a number of tumor types.[35,40,41]

A proportional increase in the number of young women affected by lung carcinoma has been observed in recent years and the prognosis appears to be worse in these individuals.[42] The identification of hormonal

influences affecting lung tumor behavior may be important in this group. If estrogen is mitogenic in lung carcinoma with ERs, this may explain the poorer prognosis in female patients with higher p29 tumor expression in this study.

None of the lung carcinomas in this study expressed ER (ER1D5). The current study was performed using archival paraffin embedded tissue specimens that were at least 10 years old. Although it is unlikely that false-positive immunostaining reactions would occur as a result of this, it is possible that diminished reactivity for ER using ER1D5 may reflect declining antigenicity related to processing and storage.

Our study demonstrated that p29 commonly is present in nonsmall cell lung carcinoma and is related to the length of survival in female patients. Our findings also suggest that estrogen-dependent mechanisms are involved that may have implications related to the prognosis and treatment of lung carcinoma.

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