MUC4 Expression in Non–Small Cell Lung Carcinomas

Relationship to Tumor Histology and Patient Survival

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● ***Context.*—Mucin 4 (MUC4) is a high-molecular-weight membrane–bound glycoprotein that is expressed in the foregut before epithelial differentiation. It is found in normal adult airway epithelium, non–small cell lung carcinoma (NSCLC) and in other human malignancies independent of mucus secretion. Although its tissue distribution has been studied, its utility in predicting prognosis in NSCLC is unknown.**

***Objective.*—To evaluate the relationship between MUC4 overexpression and long-term survival in patients with NSCLC.**

***Design.*—Immunohistochemical staining for MUC4 was performed on formalin-fixed, paraffin-embedded tissue samples from 343 cases of NSCLC arranged in a high-density tissue microarray. Information about long-term survival and tumor stage was collected for all patients. Semiquantitative assessment of MUC4 staining was correlated with survival (Kaplan-Meier analysis).**

***Results.*—MUC4 was frequently expressed in adenocarcinomas (151/187 [81%]), squamous cell carcinomas (69/88 [78%]), adenosquamous carcinomas (6/8 [75%]), and large cell carcinomas (33/60 [55%]). High levels of expression (combined score, 2+/3+) for MUC4 were more characteristic of adenocarcinomas (126/187 [68%]) and adenosquamous carcinomas (6/8 [75%]) than of squamous cell carcinomas (46/88 [52%]) and large cell carcinomas (17/60 [28%]) ($P < .001$). In patients with stage I and II adenocarcinoma, there was a trend toward longer patient survival with higher levels of MUC4 immunoreactivity compared with lower levels ($P = .11$).**

***Conclusion.*—MUC4 expression is common in pulmonary adenocarcinomas and may indicate a more favorable prognosis in early-stage adenocarcinomas.**

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Mucin (MUC) is a high-molecular-weight membrane–bound glycoprotein with extensive O-glycosidic–linked oligosaccharides[1,2] that was cloned from tracheobronchial mucosa complementary DNA by Porchet et al.[3] The 8 chemical subtypes of mucin are distinguished from each other by different chromosomal location and different genetic encoding.[4] MUC2, MUC5AC, MUC5B, and MUC6 map to 11p15.5 and encode secretory gel-forming mucins, while MUC1, MUC3, MUC4, and MUC7 are scattered on different chromosomes and encode membrane-bound or secreted mucins. MUC4 is generally expressed in the foregut before epithelial differentiation and is found in normal adult airway epithelium, non–small cell lung carcinoma (NSCLC) and other human malignancies independent of mucin secretion status.[5–10] Northern hybridization and in situ hybridization analyses have shown that levels of MUC4 messenger RNA expression are elevated in lung carcinoma cells compared with cells in normal lung tissues.[11–14]

The distribution and immunogenicity of MUC4 in normal and neoplastic tissues have been studied, and the clinical outcome of other organ malignancies with overexpression for MUC4 has been reported.[5,15–17] In the literature, MUC4 overexpression is associated with more aggressiveness and increased metastases in breast cancer, extrahepatic bile duct carcinoma, and cholangiocarcinoma.[9,17–19] Conversely, however, improved patient survival was associated with MUC4 expression in ovarian cancer, mucoepidermoid carcinoma of the salivary glands, and squamous cell carcinoma of the upper aerodigestive tract.[5,16,20]

The relationship between MUC4 expression and survival in NSCLC has not been determined. The current study builds on the previous in vitro work to determine the relationship between MUC4 expression, tumor histologic findings, and long-term survival, using high-density tissue microarray samples from 343 patients.

MATERIALS AND METHODS

Institutional review board approval of research protocols for this project was obtained through The Methodist Hospital Research Institute.

Construction of High-Density Tissue Microarrays

High-density tissue microarrays were constructed from archival formalin-fixed, paraffin-embedded samples of carcinomas obtained from 343 patients with primary NSCLCs. The archival specimens were originally received between 1974 and 1991 at The Methodist Hospital, Houston, Tex. Cases with a diagnosis of

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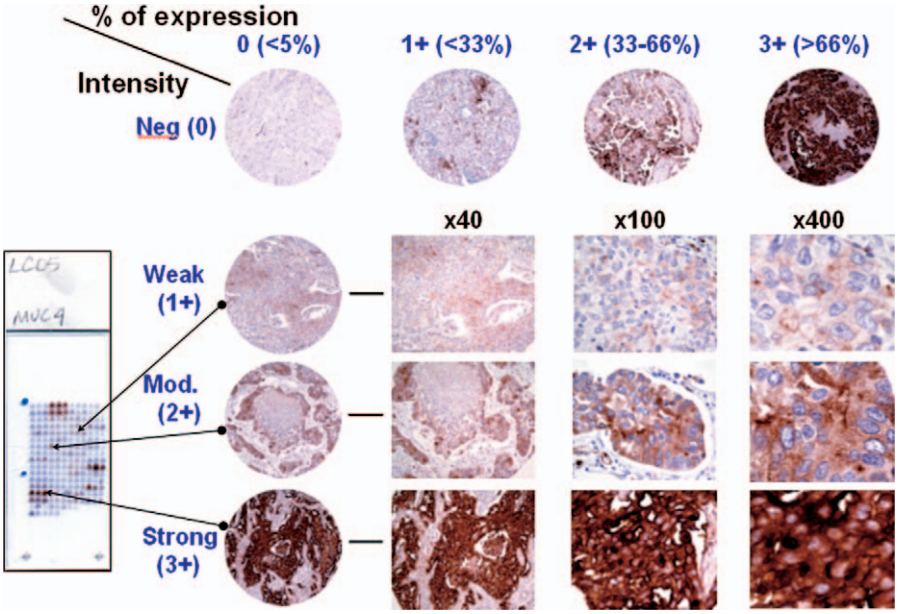

Figure 1. *Scoring of positive expression for mucin 4 immunoreactivity by using high-throughput tissue microarray in non–small cell lung carcinoma (n = 343).*

small cell carcinoma, mixed non–small cell and small cell lung carcinoma, giant cell carcinoma, mucoepidermoid carcinoma, sarcomatoid carcinoma, and basaloid carcinoma were excluded. Histopathologic classification of all selected cases was performed according to current World Health Organization criteria.[21] For each sample, areas rich in viable tumor cells were identified by light microscopic examination of hematoxylin-eosin–stained sections and selected for use in the tissue microarrays. Three cores measuring 0.1 cm in diameter were taken from the donor paraffin tissue blocks of each case and were arranged in a recipient paraffin tissue array block by using a Manual Tissue Arrayer (MTA-1, Beecher Instruments Inc, Sun Prairie, Wis).

Clinical and Staging Data

Retrospective chart review was performed to obtain clinical data, including patient age, sex, smoking history, and survival status. Tumor stage was assigned by using the international staging system for lung cancer published by Mountain.[22]

Immunohistochemistry

Recut sections 4 μm thick were obtained from each tissue microarray block and placed in a 60°C oven overnight. Sections were then dewaxed in xylene for 10 minutes and rehydrated through graded ethanols to water. Endogenous peroxidases were blocked with a solution of 3% H_2O_2. The sections were steamed for 25 minutes in citrate-buffered saline (pH 6.0) and then treated with mouse monoclonal anti-MUC4 (1:750, Zymed Laboratories Inc, San Francisco, Calif) antibody. Detection was performed by using the EnVision+ Labeled Polymer System (DakoCytomation, Carpinteria, Calif). Nonneoplastic lung tissues were used as positive control specimens, and negative control specimens were prepared by using the identical staining protocol except for substitution of Universal Negative Mouse Control (DakoCytomation) for the primary antibody. MUC4 expression was assessed in a blinded fashion without knowledge of the patient's identity and clinical history. In general, a cytoplasmic and membranous staining pattern was observed.

Extensiveness and intensity of staining in tumor cells were each assessed for each sample by using a semiquantitative scale. Extensiveness of MUC4 staining in the tumors was scored as follows: 0 (<5% immunoreactive), 1+ (<33% immunoreactive), 2+ (33%–66% immunoreactive), and 3+ (>66% immunoreactive). MUC4 staining intensity was graded by using the following scale: 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong). Then, for each tumor, a combined score was calculated by adding the scores for extensiveness and intensity, using the following scale: 0 = score 0, 1+ = score 1 to 2, 2+ = score 3 to 4, 3+ = score 5 to 6 (Figure 1).

Table 1. Clinicopathologic Characteristics

Characteristic	Patients, No. (%)
Sex (n = 343)	
Male	207 (60)
Female	136 (40)
Smoking history (n = 328)	
Current smoker	285 (87)
Former smoker	23 (7)
Nonsmoker	20 (6)
Histologic type (n = 343)	
Adenocarcinoma	187 (55)
Squamous cell carcinoma	89 (26)
Large cell carcinoma	59 (17)
Adenosquamous carcinoma	8 (2)
Pathologic stage (n = 343)	
Stage I	223 (65)
Stage II	57 (17)
Stage III	42 (12)
Stage IV	21 (6)

Statistical Analysis

Statistical analyses were performed by using Microsoft Excel WinStat (Microsoft Corp, Redmond, Wash) and SPSS version 12 (SPSS Inc, Chicago, Ill). The χ^2 test was used to evaluate for associations between MUC4 expression and clinicopathologic variables. Kaplan-Meier analysis was performed to investigate a potential relationship between MUC4 expression and patient survival, using the log-rank test (Cox-Mantel) for statistical significance. Univariate and multivariate analyses of potential prognostic factors were done by using Cox proportional hazards modeling. A *P* value less than .05 was considered to indicate a statistically significant difference.

RESULTS

Clinicopathologic Characteristics

The patient population consisted of 207 (60%) men and 136 (40%) women (Table 1). The mean patient age at the time of diagnosis was 63 (range, 29–90 years). Most pa-

Table 2. Mucin 4 (MUC4) Immunoreactivity in Non–Small Cell Lung Carcinomas (n = 337)

MUC4 Expression	No. of Patients (%)
Extensiveness	
0 (<5%)	102 (30)
1+ (5%–32%)	82 (24)
2+ (33%–66%)	52 (16)
3+ (>66%)	101 (30)
Intensity of expression	
0 (negative)	102 (30)
1+ (weak)	47 (14)
2+ (moderate)	114 (34)
3+ (strong)	74 (22)
Combined score*	
0 (negative)	84 (24)
1+ (1–2)	64 (19)
2+ (3–4)	90 (26)
3+ (5–6)	105 (31)
Histologic type	
Adenocarcinoma (n = 187)	151 (81)
Squamous cell carcinoma (n = 88)	69 (78)
Large cell carcinoma (n = 60)	33 (55)
Adenosquamous carcinoma (n = 8)	6 (75)

* Combination of extensiveness and intensity of expression. 0 indicates negative; 1+, 1 to 2; 2+, 3 to 4; 3+, 5 to 6.

tients (87%) were current smokers at the time of diagnosis, 7% were ex-smokers, and 6% had never smoked. The most common tumor cell type was adenocarcinoma, followed by squamous cell carcinoma, large cell carcinoma, and adenosquamous carcinoma. Of the 187 adenocarcinomas, 45 (24%) cases were classified as adenocarcinoma with bronchioloalveolar carcinoma pattern (26 women and 19 men). All pathologic stages were represented, with the largest group of patients assigned to stage I.

Expression of MUC4 Protein in NSCLC

Of the 343 cases, 337 had sufficient tissue in the microarray slides to analyze. In 6 cases, tumor representation was judged to be inadequate. Individual scores for MUC4 staining extensiveness and intensity, and combined scores, are shown in Table 2, with information about staining frequencies for each histologic type of NSCLC. Among NSCLCs, adenocarcinomas had the highest frequency of staining for MUC4. In cases of adenocarcinoma with moderate or strong MUC4 expression (combined score, 2+/3+) (n = 126), 41 cases (33%) showed predominantly cytoplasmic pattern, 11 cases (9%) showed a membranous pattern, and 74 cases (58%) showed cytoplasmic and membranous staining. The cases of adenocarcinoma with tubular and papillary type showed strong membranous expression for MUC4 on the luminal and free surface, but weaker expression in the cytoplasm of tumor cells (Figure 2, A). The luminal contents also stained strongly for MUC4. In cases of squamous cell carcinoma, MUC4 was positively expressed in more well-differentiated tumor cells centrally located in tumor nests, in squamous pearls, and in necrotic areas of the tumor nests, and was weakly expressed or negative in peripheral portions and less differentiated tumor cells in the tumor nests (Figure 2, B). In cases of adenosquamous carcinoma, MUC4 expression was primarily seen in the areas of adenocarcinoma (Figure 2, C). In 45 cases of adenocarcinoma with bronchioloalveolar carcinoma pattern, the MUC4 immunoreactivity was seen both in mucinous (Figure 2, D) and nonmucinous cells (Figure 2, E). Interestingly, in contrast to bronchioloalveolar carcinoma, foci of atypical adenomatous hyperplasia did not show MUC4 expression (Figure 2, F). In nonneoplastic lung parenchyma, MUC4 expression was evident in bronchiolar epithelial cells and alveolar capillary endothelial cells, while alveolar epithelial cells, proliferated bronchiolar reserve cells, and foci of squamous metaplasia did not stain (Figure 2, G).

Statistical Analysis of MUC4 Expression in NSCLC

MUC4 expression showed no statistically significant relationship to patient sex, age at diagnosis, smoking status, or tumor stage. However, MUC4 expression was significantly correlated with histologic type. Of the 105 cases with combined score 3+ MUC4 expression, adenocarcinoma was the most highly represented histologic type (83/187 [44%]), followed by adenosquamous carcinoma (3/8 [38%]), squamous cell carcinoma (12/88 [13%]), and large cell carcinoma (7/60 [10%]). Of the 195 cases with combined score 2+/3+ expression for MUC4, adenocarcinoma accounted for 126 (67%) of 187 cases, compared with 46 (52%) of 88 for squamous cell carcinoma and 17 (17%) of 60 for large cell carcinoma ($P < .001$) (Table 3). In the patient group as a whole, survival analysis revealed no significant association between elevated MUC4 expression and survival (Figure 3, A). After controlling for tumor stage, we did not find any significant correlation between elevated MUC4 expression (combined score 0/1+ vs 2+/3+) and patient survival in all patients with stage I or II NSCLC ($P = .33$; Figure 3, B). However, we did observe a strong trend ($P = .11$) toward longer patient survival in patients with stage I or II adenocarcinoma and combined score 2+/3+ MUC4 expression (Figure 3, C). The latter patients had a mean 5-year survival rate of 70.8%, compared with 60.5% for patients with 0/1+ MUC4 expression.

Multivariate analysis was performed to identify the factors that significantly affected survival time for patients with stage I or II adenocarcinoma, with patient age, sex, smoking, MUC4 expression, and stage as covariates. As expected, we observed a significant relationship between tumor stage and survival for patients with stage I or II adenocarcinoma ($P < .001$). There was no significant correlation between MUC4 expression (combined score 2+/3+ vs 0/1+) and survival for patients with stage I or II squamous cell carcinoma ($P = .47$; Figure 3, D) and large cell carcinoma ($P = .82$).

COMMENT

The results of this study indicate that MUC4 is widely expressed by NSCLC. Expression varies among the histologic types of NSCLCs; adenocarcinomas express MUC4 more frequently than do squamous cell carcinomas and large cell carcinomas. The results support previously reported studies showing that MUC4 expression depends on histologic type and differentiation grade of the tumor cells.[12,23] Furthermore, the intensity and localization of MUC4 expression vary with specific architectural and cytologic features. Adenocarcinomas with tubular and papillary features show strong membranous expression on the luminal and free surfaces, but less strong expression in the cytoplasm of tumor cells. This distribution of staining correlates with findings of recent nucleotide sequencing studies that have shown that MUC4 contains a transmem-

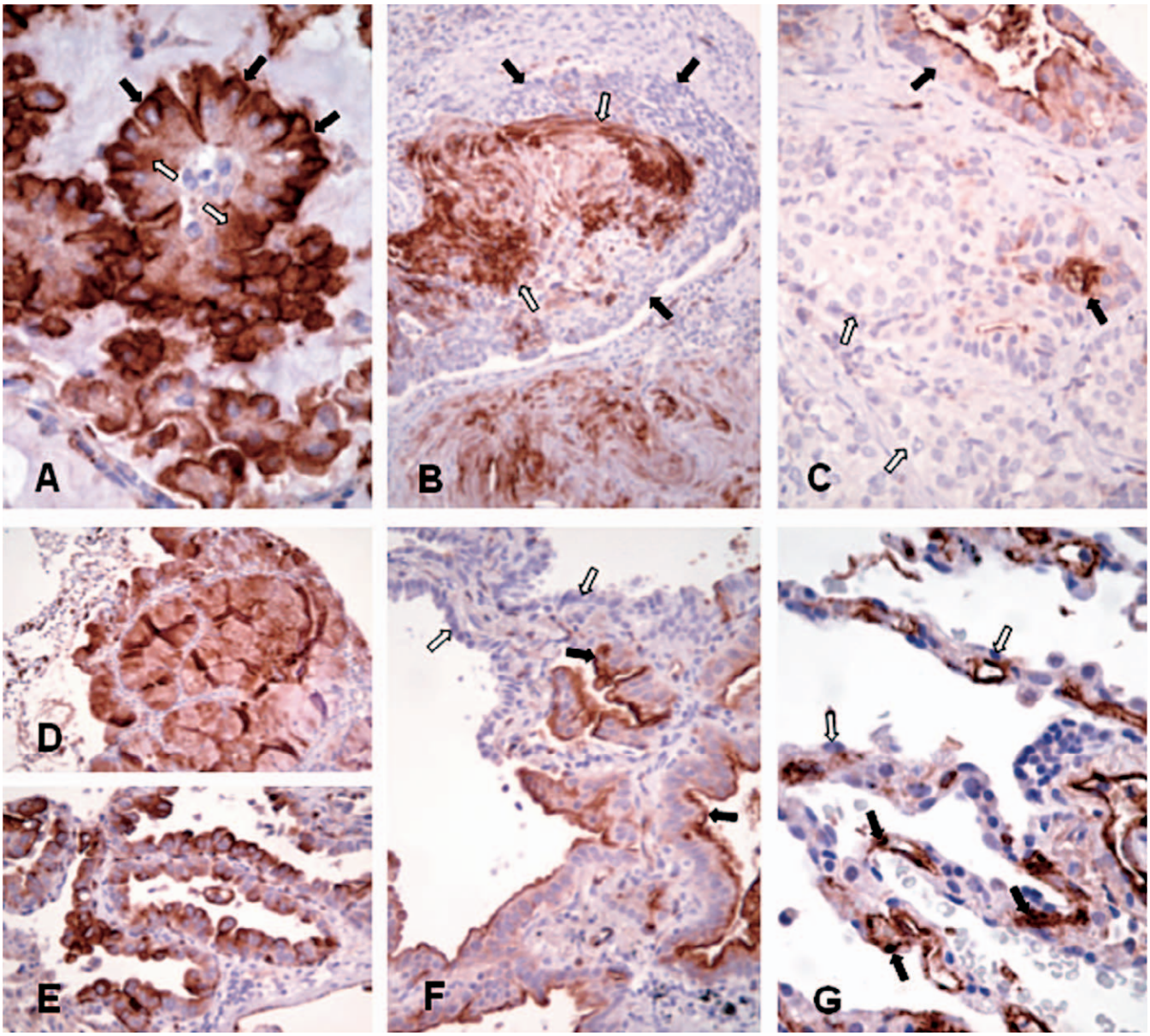

Figure 2. *Staining patterns for mucin 4 (MUC4) vary among different histologic types of non–small cell lung carcinoma. A, An adenocarcinoma of papillary type shows strong membranous expression on the free surface (black arrows) and moderate expression in the cytoplasm (white arrows) of the tumor cells (original magnification ×400). B, A squamous cell carcinoma demonstrates strong MUC4 expression in the cells in the center of a tumor nest and in the keratinaceous material (white arrows), while the peripheral portion of the tumor nest does not stain (black arrows) (original magnification ×100). C, In case of adenosquamous carcinoma, the glandular structure of adenocarcinoma showed positive expression (black arrows), but foci of squamous cell carcinoma showed negativity for MUC4 (white arrows) (original magnification ×200). D and E, In this adenocarcinoma with a bronchioloalveolar carcinoma pattern, MUC4 immunoreactivity is seen in both the mucinous (D) and nonmucinous (E) cell types (original magnifications ×100 [D] and ×200 [E]). F, A bronchioalveolar carcinoma demonstrates clear-cut MUC4 expression (black arrows), while adjacent atypical pneumocytes show no expression of MUC4 (white arrows) (original magnification ×200). G, In the nonneoplastic lung parenchyma, alveolar capillary endothelial cells express MUC4 (black arrows), and alveolar pneumocytes are negative (white arrows) (original magnification ×200).*

brane domain.[24] In squamous cell carcinomas, MUC4 expression is increased in the central areas of tumor cell nests and in squamous pearls, but is reduced or absent in the peripheral, less well differentiated tumor cells. A study of mucin gene expression during differentiation of cultured human airway epithelia revealed upregulation of MUC4 in cultures of well-differentiated cells compared with poorly differentiated cells.[15] The functional role of MUC4 in cell signaling implicates that MUC4 plays a role in cell differentiation rather than cell proliferation in the normal mucus-secreting goblet cells, the stratified squamous epithelial cells, and malignant epithelial tumor cells.[5,15]

Mucin is a major component of the extracellular mucus blanket that protects and lubricates mammalian epithelial cells.[2,25] Mucins are high-molecular-mass glycoconjugates (154 to >7000 kd) with hundreds of oligosaccharide chains in O-glycosidic linkages to a protein backbone.[25] MUC4 is a heterodimeric glycoprotein complex that consists of a mucin subunit, MUC4α, tightly bound to a trans-

Table 3. Comparison of Mucin 4 Expression Between Each Combined Score and Groupings of Combined Scores in Non–Small Cell Lung Carcinomas (n = 343)*

	Combined Score, No. (%)†					Groupings, No. (%)		
	0	1+	2+	3+	*P*	0/1+	2+/3+	*P*
ADC (n = 187)	36 (20)	25 (13)	43 (23)	83 (44)	<.001	61 (33)	126 (67)	<.001
SqC (n = 88)	19 (22)	23 (26)	34 (39)	12 (13)		42 (48)	46 (52)	
LCC (n = 60)	27 (45)	16 (27)	10 (18)	7 (10)		43 (72)	17 (28)	
ADSqC (n = 8)	2 (25)	0 (0)	3 (38)	3 (38)		2 (25)	6 (75)	

* ADC indicates adenocarcinoma; SqC, squamous cell carcinoma; LCC, large cell carcinoma; and ADSqC, adenosquamous carcinoma.
† Combination of extensiveness and intensity of expression. 0 indicates negative; 1+, 1 to 2; 2+, 3 to 4; 3+, 5 to 6.

membrane subunit, MUC4β.[5,25] In the normal state, MUC4 probably contributes to a steric barrier at the apical surface of epithelial cells, serving a protective function as well as functioning as an important regulatory molecule.[25]

MUC4 expression is seen in many normal epithelia, including stratified squamous mucosa of the oral cavity and upper aerodigestive tract, major and minor salivary gland acini and ductal epithelium, lacrimal gland, larynx and trachea, lung, stomach, intestine, uterus, cervix, mammary gland, ovary, kidney, ependymal epithelium of the brain, and corneal and conjunctival epithelium.[6,7] MUC4 expression has been reported in many carcinomas, including squamous cell carcinoma of the upper aerodigestive tract and carcinomas of pancreatic, salivary, breast, lung, gastric, colon, biliary tract, and ovarian origin.[5–9,15] MUC4 expression in many tumors exhibits strong associations with cytodifferentiation patterns that often reflect patterns of developmental mucin expression.[10,26,27] The expression of the MUC4 protein on tumor cells correlates well with the expression of its messenger RNA.[11] Northern blot analysis reveals that messenger RNA expression of MUC4 is greater in lung carcinoma cells compared with normal epithelial cells.[11] Seregni et al[13] reported that MUC4 messenger RNA levels were elevated in 17 of 18 patients with lung carcinoma compared with levels in the normal counterparts.

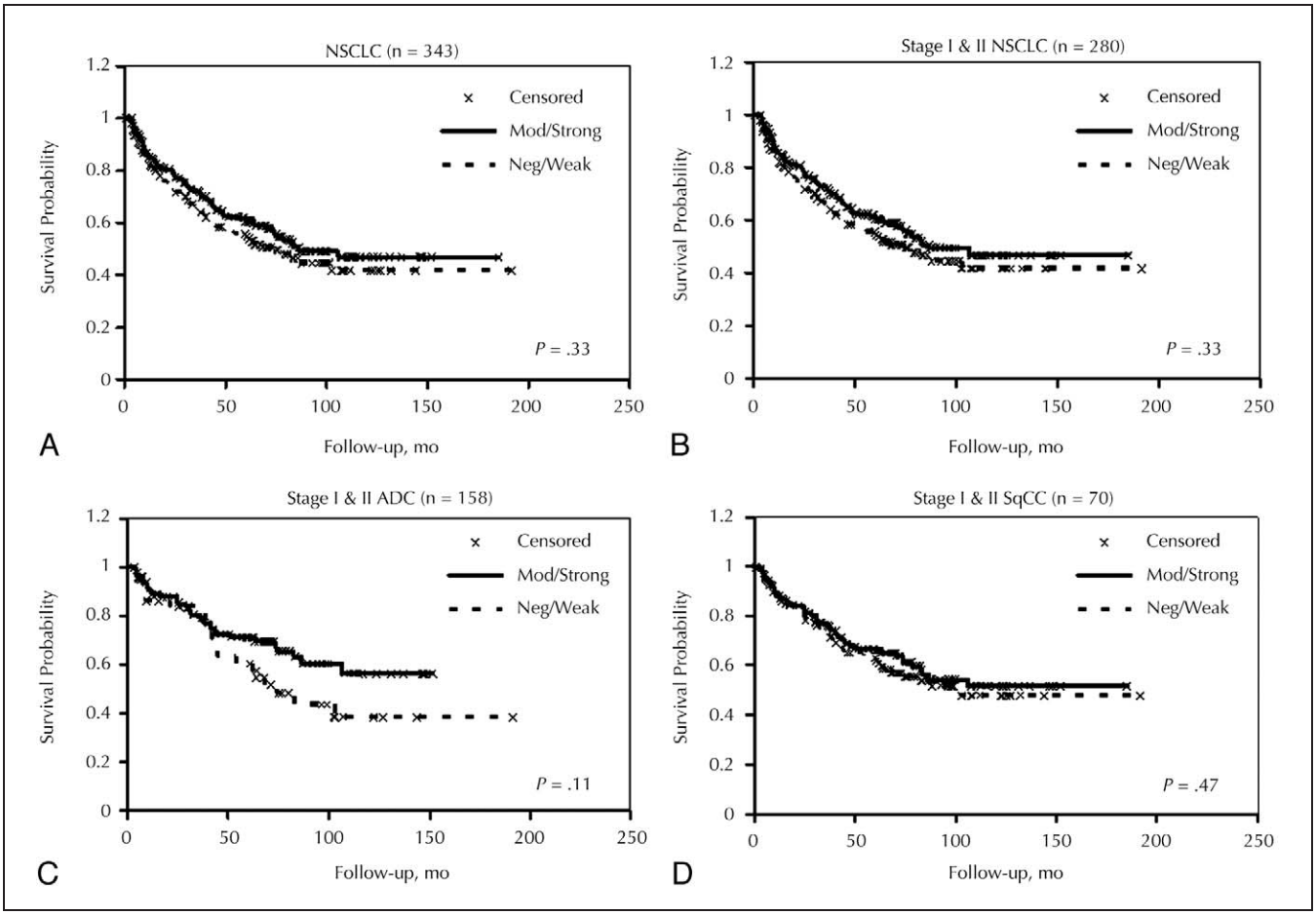

Figure 3. *Kaplan-Meier analysis (log-rank test) reveals no significant association between elevated mucin 4 (MUC4) expression (combined score 2+/3+) and patient survival in all patients with non–small cell lung carcinoma (NSCLC) (*P *= .33) (A), patients with stage I and II NSCLC (*P *= .33) (B), or patients with stage I and II squamous cell carcinoma (SqCC) (*P *= .47) (D). But there is a trend toward improved survival in association with increased MUC4 expression (2+/3+) in patients with stage I and II adenocarcinoma (ADC) (*P *= .11) (C).*

Although a trend associating MUC4 expression with well-differentiated squamous cell carcinoma has been suggested, little information is available correlating MUC4 expression patterns with clinical outcomes in NSCLC.[15] In this study, increased MUC4 expression showed an association with improved survival in stage I and II adenocarcinomas. The association between MUC4 overexpression and favorable clinical outcome has also been reported in cases of squamous cell carcinoma in the upper aerodigestive tract.[5] In the literature, MUC4 expression appears to be associated with more aggressiveness and increased metastases in breast cancer, extrahepatic bile duct carcinoma, pancreatic cancer, and cholangiocarcinoma.[9,17–19,28] On the other hand, an association between MUC4 expression and improved patient survival is also reported in ovarian cancer, mucoepidermoid carcinoma of the salivary glands, and squamous cell carcinoma of the upper aerodigestive tract.[5,16,20] These divergent findings suggest that the relationship between MUC4 expression and tumor behavior appears to differ depending on the organs.

In conclusion, MUC4 protein expression appears to be common among NSCLCs, particularly adenocarcinomas. Varying patterns of expression are associated with specific histologic characteristics of the neoplasms. Although the association between MUC4 expression and survival was not significant for NSCLCs as a group, MUC4 expression was associated with a trend toward improved survival in early-stage adenocarcinomas.

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