

Expression of Syndecan-1 and Expression of Epidermal Growth Factor Receptor Are Associated with Survival in Patients with Nonsmall Cell Lung Carcinoma

Lori Shah, M.D.¹
 Kristin L. Walter, M.D.¹
 Alain C. Borczuk, M.D.²
 Steven M. Kawut, M.D.^{1,3}
 Joshua R. Sonett, M.D.⁴
 Lyall A. Gorenstein, M.D.⁴
 Mark E. Ginsburg, M.D.⁴
 Kenneth M. Steinglass, M.D.⁴
 Charles A. Powell, M.D.^{1,5}

¹ Department of Medicine, Columbia University College of Physicians & Surgeons, New York, New York.

² Department of Pathology, Columbia University College of Physicians & Surgeons, New York, New York.

³ Department of Epidemiology, Joseph L. Mailman School of Public Health, New York, New York.

⁴ Department of Surgery, Columbia University College of Physicians & Surgeons, New York, New York.

⁵ Herbert Irving Comprehensive Cancer Center; Columbia University College of Physicians & Surgeons, New York, New York.

Supported by Grant CRTG00058 from the American Cancer Society, by Grant ES00354 from the National Institutes of Health, and by the Herbert and Florence Irving Scholar Fund.

Address for reprints: Charles A. Powell, M.D., Department of Medicine, Columbia University College of Physicians & Surgeons, 630 West 168th Street, Box 91, New York, NY 10032; Fax: (212) 305-0089; E-mail: cap6@columbia.edu

Received February 29, 2004; revision received June 24, 2004; accepted June 24, 2004.

BACKGROUND. Recently, the authors identified molecular signatures and pathways associated with nonsmall cell lung carcinoma histology and lung development. They hypothesized that genetic classifiers of histology would provide insight into lung tumorigenesis and would be associated with clinical outcome when evaluated in a broader set of specimens.

METHODS. Associations between patient survival and immunostaining for 11 representative histologic classifiers (epidermal growth factor receptor [EGFR], CDK4, syndecan-1, singed-like, TTF-1, keratin 5, HDAC2, docking protein 1, integrin α 3, P63, and cyclin D1) were examined using a tissue microarray constructed from nonsmall cell lung carcinoma specimens.

RESULTS. Sixty-three tumors were examined, including 43 adenocarcinomas, 11 large cell carcinomas, and 9 squamous cell carcinomas. Sixty-three percent of tumors were clinical Stage I lesions, and 37% were Stage II–III lesions. In a multivariate analysis that controlled for age, gender, and race, syndecan-1 expression was found to be associated with a significant reduction in the risk of death (hazard ratio, 0.31 [95% confidence interval, 0.18–0.87]; $P < 0.05$). Multivariate analysis also indicated that EGFR expression was associated with a significant reduced risk of death.

CONCLUSIONS. The authors demonstrated that expression of either of the nonsmall cell lung carcinoma subtype classifiers syndecan-1 and EGFR was associated with a 30% reduction in the risk of death, with this reduction being independent of histology and other confounders. The results of the current study suggest that loss of expression of these histologic classifiers is associated with biologic aggressiveness in lung tumors and with poor outcome for patients with such tumors. If their significance can be validated prospectively, these biomarkers may be used to guide therapeutic planning for patients with nonsmall cell lung carcinoma. *Cancer* 2004; **101**:1632–8. © 2004 American Cancer Society.

KEYWORDS: Syndecan-1, epidermal growth factor receptor, lung carcinoma, prognosis, nonsmall cell lung carcinoma.

Lung carcinoma is the leading cause of malignancy-related mortality in the United States, with 164,440 deaths expected in 2004.¹ Despite innovations in diagnostic testing, surgical techniques, and the development of therapeutic agents, the 5-year survival rate for patients with lung carcinoma has remained at approximately 14% throughout the past 3 decades. Factors that contribute to this relatively low survival rate include the small proportion of patients (20–

25%) who present with resectable disease.² However, even among patients with resected Stage I lung carcinoma, up to 30% will die of disease within 5 years. Data from systemic therapy trials in early-stage lung carcinoma indicate that neoadjuvant chemotherapy combined with radiation therapy³ and adjuvant chemotherapy⁴ may provide a survival benefit for a small proportion of patients. It is likely that the targeting of individuals with the highest risk of disease-related death will enhance the benefit of these novel therapeutic approaches.

In the past decade, extensive research has been directed toward the discovery of biologic markers of tumor aggressiveness, responses to various chemotherapeutic regimens, and risk of recurrence or metastases.⁵⁻⁷ Currently, no biologic markers are routinely used in the clinical management of patients with lung carcinoma; however, several studies have identified molecular markers that provide insight into lung tumorigenesis and tumor progression.

Recently, we identified gene expression profiles and molecular pathways associated with nonsmall cell lung carcinoma (NSCLC) histology and lung development.⁸ Genes that are functionally related to later stages of lung maturation were expressed by adenocarcinomas, whereas genes that were related to earlier stages of lung maturation were expressed by undifferentiated large cell carcinomas. Because the large cell histologic type is associated with a poorer prognosis compared with other NSCLC types,⁹ we hypothesized that gene classifiers of histology would provide insight into lung tumorigenesis and would be associated with clinical outcome. Using a lung tumor tissue microarray (TMA), we examined the association between patient survival and expression of representative histologic subtype gene classifiers.

MATERIALS AND METHODS

Study Design and Patient Population

We performed a retrospective cohort study of patients with NSCLC to assess the association between protein markers and survival. Clinical data were acquired from the medical records and from treating physicians. The primary outcome was time to death. The date of death was obtained from physician records or from the Social Security Death Index (<http://ssdi.genealogy.rootsweb.com>). All procedures were approved by the Columbia University Medical Center Institutional Review Board (New York, NY).

Tissue Analysis

For each patient, tissue pathology reports were reviewed, and paraffin blocks were marked in areas that

were representative of the histologic subtype of the tumor. Lung tumor TMAs were constructed using cores from lung carcinoma specimens that were acquired at surgical resection. Each TMA consisted of 2 pairs of cylinders (diameter, 1 mm), with each pair selected from a different area of the tumor. Thus, four cores were available for each tumor. TMA blocks were sectioned at a thickness of 5 μ m, dewaxed in xylene, rehydrated through a graded ethanol series, and washed into phosphate-buffered saline. Antigen retrieval was achieved by heat treatment for 40 minutes in 10 mM citrate buffer, pH 6.0. Before staining, endogenous peroxidase activity was quenched.

TMA sections underwent immunohistochemical staining with 11 different commercially available antibodies against proteins that were previously found to be associated with histologic tumor subtypes.⁸ Review of sections was performed using uniform criteria by a pathologist (A.C.B.) who was blinded to clinical outcomes. Each antibody staining sample was scored as *negative* (score 0); *low-positive, with multifocal or diffuse faint staining* (score 1); or *high-positive, with multifocal or diffuse strong staining* (score 2). Scoring was based on nuclear staining alone for TTF-1, P63, cyclin D1, CDK4, and HDAC2 and on cytoplasmic and membranous staining for keratin 5, integrin α 3, docking protein 1, syndecan-1, and singed-like. For epidermal growth factor receptor (EGFR), only membranous staining was scored. Immunostaining was analyzed using ranked levels (0, 1, and 2) and also as being present or absent. Because there was no significant difference in the results obtained using either of these two methods of analysis, the data are presented with classification of immunostaining as being present or absent.

Antibodies against TTF-1, syndecan-1, cyclin D1, keratin 5, singed-like, and P63 were obtained from DAKO (Carpinteria, CA); antibodies against integrin α 3, docking protein 1, and CDK4 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA); antibodies against HDAC2 were obtained from Abcam (Cambridge, MA); and antibodies against EGFR were obtained from Zymed (San Francisco, CA). The clones and dilutions used in the immunohistochemical analysis have been reported previously.⁸

Statistical Analysis

Continuous variables were reported as mean values \pm standard deviations, and frequencies with 95% confidence intervals (95% CIs) were reported for all categorical variables. Univariate analyses were performed using Cox proportional hazards regression.¹⁰ We assessed the association of protein expression status

TABLE 1
Characteristics of Study Patients

Characteristic	All (n = 63)	Adenocarcinoma (n = 43)	Large cell (n = 11)	Squamous cell (n = 9)
Male gender	32	22	5	5
Age (yrs) ^a	68 ± 11	67 ± 11	66 ± 10	75 ± 7
"Race"/ethnicity				
White	56	38	10	8
Black	4	2	1	1
Hispanic	3	3	0	0
Stage ^b				
I	40	29	6	5
II	13	5	5	3
III	10	9	0	1

^a Mean ± standard deviation.

^b American Joint Committee on Cancer Pathologic TNM classification system, 1997.

with the risk of death, expressed as the hazard ratio (HR). We next constructed multivariable regression models for each protein and included potential confounding variables, such as gender, age, tumor stage, and histologic subtype, which have been linked to survival in patients with lung carcinoma. Covariates that changed the unadjusted HR of protein expression by 15% were considered to be confounders and were retained in the model. The proportional hazards assumption was confirmed for protein expression using tests based on weighted residuals.¹¹ *P* values < 0.05 were considered indicative of statistical significance. Statistical analyses were performed using STATA software (Version 7.0; STATA Corporation, College Station, TX).

RESULTS

The original study cohort included 78 patients. Because we wished to focus our analysis on the most common nonsmall cell histologic subtypes, three patients with carcinoid, two patients with bronchioloalveolar carcinoma, one patient with small cell carcinoma, and one patient with blastoma were excluded. Eight additional patients were excluded either because their TMA cores contained no malignant tissue or because immunostaining was technically inadequate. Therefore, the final cohort consisted of 63 patients. Table 1 summarizes demographic and clinical data. There were no significant differences in age, gender, disease stage, race, or survival between the patients who were excluded for technical reasons and the final cohort (data not shown).

According to histologic subtype, 43 tumors (68%) were adenocarcinomas, 11 (18%) were large cell carcinomas, and 9 (14%) were squamous cell carcinomas.

Among the 63 patients, there were 22 deaths (35%), and the overall median survival was 3.7 years. For the entire cohort, the 1-year survival rate was 92% (95% CI, 82–96%), and the 3-year survival rate was 54% (95% CI, 39–67%). Patients with Stage I disease had a 1-year survival rate of 89% (95% CI, 74–96%), and patients with Stage II–III disease had a 1-year survival rate of 96% (95% CI, 73–99%); the corresponding 3-year survival rates were 60% (95% CI, 41–75%) and 43% (95% CI, 20–64%), respectively. There appeared to be an increased risk of death for patients with higher-stage tumors (Stage II–III vs. Stage I: HR, 1.8 [95% CI, 0.85–4.1%]; *P* = 0.12). There were no significant differences in survival according to histologic subtype or gender (data not shown).

Supervised clustering of gene expression previously identified cyclin D1, docking protein 1, integrin α 3, syndecan-1, and TTF-1 as adenocarcinoma subtype classifiers; CDK4 and HDAC2 were identified as classifiers for large cell carcinoma; and EGFR, keratin 5, P63, and singed-like were identified as classifiers for squamous cell carcinoma.⁸ Examination of immunostaining in a larger set of tumors indicated that protein expression was associated with gene expression for most histologic subtype classifiers but was less specific in the cases of HDAC2, syndecan-1, and docking protein 1.

Univariate analysis indicated that the presence of EGFR staining was significantly associated with a reduced risk of death (HR, 0.33 [95% CI, 0.13–0.79]; *P* = 0.013) (Table 2). Other proteins, including syndecan-1 and CDK4, exhibited a possible association with the risk of death. Because clinical variables associated with outcome, such as age, gender, race (white vs. nonwhite), tumor stage (Stage I vs. Stage II–III), and histology, may have confounded these findings, multivariate analyses incorporating these variables were performed for each protein (Table 3). The inclusion of age, gender, and race in the multivariate model significantly altered the HR associated with syndecan-1 expression, indicating that these variables were confounders. After the sequential inclusion of these 3 covariates in the final model, syndecan-1 expression was associated with a significant reduction in the risk of death (HR, 0.31 [95% CI, 0.18–0.87]; *P* = 0.025). Because patients were selected on the basis of clinical disease stage and protein markers were selected on the basis of previously documented associations with histologic tumor subtype, we examined the impact of including clinical stage and histologic subtype in the model. We determined that inclusion of these co-

TABLE 2
Univariate Analysis of Protein Expression by Histologic Subtype and Risk of Death

Protein	No. of tumors expressing protein			HR (95% CI)	P value
	Adenocarcinoma (n = 43)	Large cell carcinoma (n = 11)	Squamous cell carcinoma (n = 9)		
EGFR	77 (33)	54 (6)	100 (9)	0.33 (0.13–0.79)	0.013
CDK4	39 (17)	82 (9)	33 (3)	1.9 (0.87–4.3)	0.11
Syndecan-1	77 (33)	54 (6)	89 (8)	0.54 (0.23–1.2)	0.14
Singed-like	60 (26)	91 (10)	100 (9)	0.67 (0.30–1.5)	0.35
TTF-1	74 (32)	45 (5)	0 (0)	0.70 (0.32–1.5)	0.38
Keratin 5	12 (5)	18 (2)	100 (9)	0.74 (0.28–2.0)	0.54
HDAC2	65 (28)	100 (11)	100 (9)	1.3 (0.47–3.4)	0.64
Docking protein 1	65 (28)	64 (7)	67 (6)	0.89 (0.38–2.1)	0.79
Integrin α 3	39 (17)	0 (0)	11 (1)	0.89 (0.37–2.1)	0.79
P63	23 (10)	18 (2)	100 (9)	0.95 (0.40–2.3)	0.91
Cyclin D1	65 (28)	36 (4)	44 (4)	0.97 (0.44–2.2)	0.95

HR: hazard ratio; 95% CI: 95% confidence interval.

TABLE 3
Risk of Death According to Syndecan-1 and Epidermal Growth Factor Receptor Protein Expression

Protein	HR (95% CI)	P value
Syndecan-1		
Unadjusted	0.54 (0.23–1.2)	0.14
Adjusted for ^a :		
Age	0.45 (0.19–1.06)	0.07
Age, gender	0.39 (0.16–0.96)	0.04
Age, gender, race ^b	0.31 (0.18–0.87)	0.025
Age, gender, race, clinical stage, histology	0.31 (0.11–0.86)	0.024
EGFR		
Unadjusted	0.33 (0.13–0.79)	0.013
Adjusted for ^a :		
Age	0.26 (0.10–0.66)	0.005
Age, clinical stage ^b	0.29 (0.11–0.74)	0.01
Age, clinical stage, gender, race, histology	0.28 (0.11–0.77)	0.014

HR: hazard ratio; 95% CI: 95% confidence interval; EGFR: epidermal growth factor receptor.

^a Race: white versus nonwhite; clinical stage: Stage I versus Stages II and III.

^b Final multivariate model.

variates in the model did not alter the results (Table 3).

Similarly, the association between EGFR expression and the risk of death was confounded by age and tumor stage, with the calculated HR decreasing to 0.29 (95% CI, 0.11–0.74; $P = 0.01$) in the multivariate model (Table 3). Additional adjustments for gender, race, and histology did not change these results significantly. None of the other proteins had significant confounders. We also repeated the analysis controlling for individual stage (IA, IB, IIA, IIB, etc.) rather than using

the stage groupings (I vs. II–III) described above. This additional analysis did not change our findings.

DISCUSSION

Previously, we used gene expression profiling to identify genes associated with histologic subtype in NSCLC.⁸ In the current study, we found that patients with tumor protein expression of syndecan-1 or EGFR had a significant risk of death compared to patients without protein expression; independent of histology, tumor stage, and demographic characteristics.

Syndecan-1, also known as CD138, is the best-characterized member of the syndecan family, which consists of four closely related proteins. In adults, syndecan-1 is present in most cell types, and abundant expression is found on the basolateral membrane of lung epithelial cells. The biologic effects of syndecan-1 are mediated primarily by heparan sulphate glycosaminoglycans, which bind extracellular matrix proteins (collagen Types I, III, and IV; fibronectin; tenascin; thrombospondin; and amphoterin) and members of the fibroblast growth factor family. Syndecan-1 regulates cell growth and differentiation in part by modulating the interactions of growth factors with cellular receptors.¹²

The loss of syndecan-1 expression is associated with tumor cell growth and invasiveness. Syndecan-1-null murine mammary gland epithelial cells lose normal epithelial morphology and become invasive, acquiring anchorage-independent growth.¹³ Ectopic expression of syndecan-1 in tumorigenic mouse epithelial cells restores normal epithelial phenotype and growth characteristics.¹⁴ Analysis of human carci-

noma tissues has revealed lower levels of syndecan-1 immunostaining in less differentiated, more invasive foci.¹⁵⁻¹⁷ These data suggest that syndecan-1 is associated with biologic aggressiveness in human tumors and that increased expression is potentially associated with improved patient outcomes.

In patients with squamous cell carcinomas of the head and neck, increased syndecan-1 expression is associated with improved recurrence-free and overall survival.¹⁸ Syndecan-1 expression also is associated with improved survival in patients with malignant pleural mesothelioma.¹⁹ Recent studies have examined syndecan-1 expression in human lung carcinoma. In univariate analyses, Anttonen et al. found that increased expression of syndecan-1 was associated with improved survival in patients with resected squamous cell lung carcinoma.²⁰ However, syndecan-1 expression was not identified as an independent prognostic factor on multivariate analysis. Toyoshima et al. examined syndecan-1 expression in 97 NSCLC specimens and in 18 small cell lung carcinoma specimens and found no significant difference in survival according to syndecan-1 expression levels.²¹ Univariate analysis also did not reveal any correlation between syndecan-1 expression and parameters such as gender, age, pathologic type, and pathologic stage.

It is noteworthy that some authors have demonstrated an association between increased serum syndecan-1 levels and decreased survival for patients with nonsmall cell carcinoma as well as patients with small cell carcinoma. However, serum syndecan-1 levels were not found to be correlated with syndecan-1 expression levels in tissue.²² Epithelial cell-bound syndecan-1 can be cleaved by proteins associated with tumor invasiveness, such as matrix metalloproteinase-7, suggesting that serum syndecan-1 levels may serve as a biomarker of tumor invasion.²³

Although syndecan-1 expression is a favorable prognostic factor in many human malignancies, to our knowledge, the current study is the first to indicate that increased syndecan-1 expression is associated with improved survival in patients with lung carcinoma, with this association persisting even after adjustment for multiple confounding variables. It is biologically plausible that syndecan-1 expression is associated with increased survival due to its role in cell adhesion, invasion, and the modulation of growth factors. Additional studies focusing on syndecan-1 expression and cleavage, as well as on the coexpression of matrix metalloproteinases, may provide further insight into the role of syndecan-1 in lung carcinogenesis.

We also found an association between EGFR ex-

pression and improved survival. EGFR is a transmembrane protein that is expressed on the surface of many human epidermal and mesenchymal cells. Receptor activation is associated with the initiation of multiple signal transduction pathways, such as the signaling cascade involving Ras/microtubule-associated protein kinase and Src and the signal transducers and activators of transcription pathway. Increased expression of EGFR and its signaling pathways has been detected in a high percentage of tumors in the lung, breast, head and neck, colon, prostate, and esophagus. In vitro studies suggest that increased levels of EGFR in tumors lead to an increase in EGFR ligand-binding sites as well as an increase in initiation sites for signal transduction.²⁴

Although laboratory and clinical studies suggest that aberrant EGFR signaling is important in the development and progression of various human tumors, the prognostic significance of EGFR expression in NSCLC remains controversial. A recent metaanalysis²⁵ reviewed studies examining the relation between EGFR expression in NSCLC and patient survival. Of the 16 eligible studies, 12 found that EGFR expression had no significant impact on survival, 3 found that increased EGFR expression was associated with poorer survival, and 1 found that EGFR expression was associated with improved survival. The heterogeneity of these results may be attributable to differences in study design, including differences in antibody sources, immunostaining methods, and cutoff levels for defining positive EGFR expression. In addition, adjustment for potential confounders was not performed in any of those 16 studies. More recently, Hirsch et al.²⁶ reported a significant correlation between EGFR expression and histologic subtype in NSCLC, with the highest expression levels being observed in squamous cell carcinoma and the lowest expression levels being observed in large cell carcinoma. On univariate analysis, EGFR expression was not significantly associated with survival. However, when considered in combination with gene amplification, reduced EGFR expression was associated with poorer survival. It is noteworthy that EGFR expression was more abundant in tumors with well differentiated histology, suggesting that loss of EGFR expression may serve as a biomarker of tumor dedifferentiation. The frequently observed correlation between dedifferentiation and tumor invasion²⁷ suggests a potential mechanism underlying the association between EGFR staining and survival in the current cohort. Recent research has indicated that somatic *EGFR* mutations in lung carcinoma are associated

with an enhanced clinical response to treatment with gefitinib (Iressa; AstraZeneca Pharmaceuticals, Wilmington, DE), a tyrosine kinase inhibitor that targets EGFR.^{28,29} This finding suggests that in selected patients, EGFR activity may be directly associated with improved patient outcome. Although tumor EGFR activation may have been a significant factor in the current cohort, the immunohistochemical procedures that we used were unable to ascertain EGFR activation status.

Other limitations of the current study included possible selection bias in a relatively small cohort, as the study population included only patients with operable, resected tumors (excluding Stage IV carcinoma). Differential misclassification of protein expression (i.e., erroneous classification of protein expression in association with patient outcomes) was unlikely, because the pathologist was blinded to all clinical information. Misclassification of patient outcomes also was unlikely, as we used multiple strategies, including a computerized death registry search, to follow patients. Although the finding of an association between protein expression and patient outcome does not clearly establish causality, the association between developmentally regulated genes and outcomes in patients with lung carcinoma may provide insight into lung carcinogenesis and may lead to the development of novel therapeutic agents. Our findings, therefore, warrant validation in biopsy samples obtained from a broader spectrum of tumors.

In conclusion, we examined the immunohistochemical expression of 11 biomarkers produced by genes that were previously identified as being significant classifiers of lung carcinoma histology. Our objective was to determine whether any of these developmentally regulated gene products were associated with patient survival. On multivariate analysis, we found that positive immunohistochemical staining for syndecan-1 and positive staining for EGFR both were significantly associated with increased survival for patients with lung carcinoma. The identification and validation of clinically significant biomarkers could lead to the individualization of lung carcinoma treatment approaches on the basis of clinically relevant molecular tumor characteristics as well as clinical disease stage.

REFERENCES

- Jemal A, Tiwari RC, Murray T, et al. Cancer statistics, 2004. *CA Cancer J Clin.* 2004;54:8–29.
- Datta D, Lahiri B. Preoperative evaluation of patients undergoing lung resection surgery. *Chest.* 2003;123:2096–2103.
- Pisters KM, Ginsberg RJ, Giroux DJ, et al. Induction chemotherapy before surgery for early-stage lung cancer: a novel approach. Bimodality Lung Oncology Team. *J Thorac Cardiovasc Surg.* 2000;119:429–439.
- Arriagada R, Bergman B, Dunant A, Le Chevalier T, Pignon JP, Vansteenkiste J. Cisplatin-based adjuvant chemotherapy in patients with completely resected non-small-cell lung cancer. *N Engl J Med.* 2004;350:351–360.
- Bhattacharjee A, Richards WG, Staunton J, et al. Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. *Proc Natl Acad Sci U S A.* 2001;98:13790–13795.
- Yanagisawa K, Shyr Y, Xu BJ, et al. Proteomic patterns of tumour subsets in non-small-cell lung cancer. *Lancet.* 2003;362:433–439.
- Beer DG, Kardias SL, Huang CC, et al. Gene-expression profiles predict survival of patients with lung adenocarcinoma. *Nat Med.* 2002;8:816–824.
- Borcuzak AC, Gorenstein L, Walter KL, Assaad AA, Wang L, Powell CA. Non-small-cell lung cancer molecular signatures recapitulate lung developmental pathways. *Am J Pathol.* 2003;163:1949–1960.
- Takei H, Asamura H, Maeshima A, et al. Large cell neuroendocrine carcinoma of the lung: a clinicopathologic study of eighty-seven cases. *J Thorac Cardiovasc Surg.* 2002;124:285–292.
- Cox DR. Regression models and life tables (with discussion). *J R Stat Soc.* 1972;34:187–220.
- Grambsch PM, Therneau TM. Proportional hazards tests and diagnostics based on weighted residuals. *Biometrika.* 1994;81:515–526.
- Couchman JR. Syndecans: proteoglycan regulators of cell-surface microdomains? *Nat Rev Mol Cell Biol.* 2003;4:926–937.
- Kato M, Saunders S, Nguyen H, Bernfield M. Loss of cell surface syndecan-1 causes epithelia to transform into anchorage-independent mesenchyme-like cells. *Mol Biol Cell.* 1995;6:559–576.
- Leppa S, Mali M, Miettinen HM, Jalkanen M. Syndecan expression regulates cell morphology and growth of mouse mammary epithelial tumor cells. *Proc Natl Acad Sci U S A.* 1992;89:932–936.
- Mukunyadzi P, Sanderson RD, Fan CY, Smoller BR. The level of syndecan-1 expression is a distinguishing feature in behavior between keratoacanthoma and invasive cutaneous squamous cell carcinoma. *Mod Pathol.* 2002;15:45–49.
- Fujiya M, Watari J, Ashida T, et al. Reduced expression of syndecan-1 affects metastatic potential and clinical outcome in patients with colorectal cancer. *Jpn J Cancer Res.* 2001;92:1074–1081.
- Matsumoto A, Ono M, Fujimoto Y, Gallo RL, Bernfield M, Kohgo Y. Reduced expression of syndecan-1 in human hepatocellular carcinoma with high metastatic potential. *Int J Cancer.* 1997;74:482–491.
- Inki P, Joensuu H, Grenman R, Klemi P, Jalkanen M. Association between syndecan-1 expression and clinical outcome in squamous cell carcinoma of the head and neck. *Br J Cancer.* 1994;70:319–323.
- Kumar-Singh S, Jacobs W, Dhaene K, et al. Syndecan-1 expression in malignant mesothelioma: correlation with cell differentiation, WT1 expression, and clinical outcome. *J Pathol.* 1998;186:300–305.

20. Anttonen A, Heikkila P, Kajanti M, Jalkanen M, Joensuu H. High syndecan-1 expression is associated with favourable outcome in squamous cell lung carcinoma treated with radical surgery. *Lung Cancer*. 2001;32:297-305.
21. Toyoshima E, Ohsaki Y, Nishigaki Y, Fujimoto Y, Kohgo Y, Kikuchi K. Expression of syndecan-1 is common in human lung cancers independent of expression of epidermal growth factor receptor. *Lung Cancer*. 2001;31:193-202.
22. Joensuu H, Anttonen A, Eriksson M, et al. Soluble syndecan-1 and serum basic fibroblast growth factor are new prognostic factors in lung cancer. *Cancer Res*. 2002;62:5210-5217.
23. Shapiro SD. Immunology: mobilizing the army. *Nature*. 2003;421:223-224.
24. Herbst RS, Bunn PA Jr. Targeting the epidermal growth factor receptor in non-small cell lung cancer. *Clin Cancer Res*. 2003;9:5813-5824.
25. Meert AP, Martin B, Delmotte P, et al. The role of EGF-R expression on patient survival in lung cancer: a systematic review with meta-analysis. *Eur Respir J*. 2002;20:975-981.
26. Hirsch FR, Varella-Garcia M, Bunn PA Jr., et al. Epidermal growth factor receptor in non-small-cell lung carcinomas: correlation between gene copy number and protein expression and impact on prognosis. *J Clin Oncol*. 2003;21:3798-3807.
27. Fish EM, Molitoris BA. Alterations in epithelial polarity and the pathogenesis of disease states. *N Engl J Med*. 1994;330:1580-1588.
28. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med*. 2004;350:2129-2139.
29. Paez JG, Janne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science*. 2004;304:1497-1500.