The Mesenchymal State Predicts Poor Disease-Free Survival in Resectable Non-Small Cell Lung Cancer

CrossMark

Kunal Mehta, BSE,* Erika Moravcikova, PhD,* David McFall, AB, James D. Luketich, MD, Arjun Pennathur, MD, Albert D. Donnenberg, PhD,* and Vera S. Donnenberg, PhD, FCP*

Departments of Cardiothoracic Surgery and Medicine, School of Medicine, University of Pittsburgh, Pittsburgh; Hillman Cancer Center, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania; and Department of Molecular Biology, Princeton University, Princeton, New Jersey

Background. The epithelial-mesenchymal transition (EMT) is thought to contribute to the overall invasiveness of malignant cells. Expression of cluster of differentiation (CD) 44 and CD90 mark the mesenchymal state in multiple epithelial malignancies. Their role in lung cancer remains unclear, however. This study evaluated the prognostic significance of CD44 and CD90 coexpression in patients with resectable primary non-small cell lung cancer (NSCLC).

Methods. This was a nonconcurrent cohort study of patients with resectable NSCLC, stratified by the degree of expression of CD44/CD90 double-positive cells in their primary tumor. Flow cytometry was used for immunophenotyping of freshly isolated disaggregated tumor. We analyzed the relationship between expression of CD44/CD90 and relapse-free survival.

Results. We evaluated 37 patients (18 men; median age, 70 years) with NSCLC. For this group, the geometric mean proportion of cells coexpressing CD44/CD90 was 0.52%. Expression of CD44/CD90 was significantly elevated (24.4%, geometric mean) in 6 patients. The median relapse-free survival for patients with high CD44/CD90 coexpression was 7.7 months (95% confidence interval, 4.2 to 11.7) compared with 40 months (95% confidence interval, 18.2 to 77.8) for the group with low CD44/CD90 coexpression ($p = 0.00006$ by Mantel log-rank test). The assessment of risk based upon CD44/CD90 expression status was not correlated with pathologic staging ($p = 0.073$ by χ^2).

Conclusions. High expression of CD44 and CD90 was associated with significantly reduced relapse-free survival in NSCLC patients. These results suggest that CD44 and CD90 may be important markers of tumor progression in NSCLC.

(Ann Thorac Surg 2017;104:321–8)
© 2017 by The Society of Thoracic Surgeons

Non-small cell lung cancer (NSCLC) refers to a heterogeneous group of primary lung cancers and frequently carries a poor prognosis. The 5-year survival rates for NSCLC patients range from 61% for those with stage IA disease to 1% in stage IV disease [1, 2]. Given the poor outcomes despite aggressive treatment, markers reflective of the biologic behavior of the tumor could prove useful in patient management.

The epithelial to mesenchymal transition (EMT) process has been demonstrated in a number of epithelial cancers and is thought to contribute to the overall invasiveness of malignant cells, conferring motility and drug resistance [3]. Cluster of differentiation (CD) 44 and CD90 have been proposed as cancer stem cell (CSC) markers [4] and are also closely associated with the mesenchymal state in malignancies of the head and neck [5–7], breast [8–10], colon [11, 12], and prostate [13]; however, their role in lung cancer remains to be defined.

This study evaluated the prognostic importance of CD44 and CD90 coexpression in patients with resectable primary NSCLC cancer. We used flow cytometry to quantify CD44/CD90 expression on cytokeratin^{+} cells because it can be used to identify specific cell-types within heterogeneous samples and measure the expression of multiple markers on single cells, even among rare cell types.

Patients and Methods

Patient Samples

Tissue specimens from 80 patients with NSCLC were obtained under protocols approved by the University of Pittsburgh Institutional Review Board. Written informed

Accepted for publication Jan 25, 2017.

*Dr Moravcikova and Kunal Mehta contributed equally to this work and Drs Albert Donnenberg and Vera Donnenberg are cosenior authors.

Address correspondence to Dr Donnenberg, Hillman Cancer Center, Research Pavilion Ste 2.42, 5117 Centre Ave, Pittsburgh PA 15232; email: donnenbergvs@upmc.edu.

The Supplemental Table and Figure can be viewed in the online version of this article [http://dx.doi.org/10.1016/j.athoracsur.2017.01.091] on http://www.annalsthoracicsurgery.org.

© 2017 by The Society of Thoracic Surgeons
Published by Elsevier Inc.

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2017.01.091

Abbreviations and Acronyms

AJCC	=	American Joint Committee on Cancer
CD	=	cluster of differentiation
cDNA	=	complementary DNA
CI	=	confidence interval
CSC	=	cancer stem cell
C_T	=	threshold cycle
CTK	=	cytokeratin
ECD	=	energy coupled dye (also known as phycoerythrin-texas red)
EMT	=	epithelial to mesenchymal transition
EpCAM	=	epithelial cell adhesion molecule
KRT19	=	keratin 19
MPE	=	malignant pleural effusion
NSCLC	=	non-small cell lung cancer
PCR	=	polymerase chain reaction
PE	=	phycoerythrin
PBS	=	phosphate-buffered saline
RT	=	reverse transcriptase

consent was obtained from all patients who chose to provide identifiable data (No. 99-053). All other samples were collected as medical waste (No. 0503126). Specimens were collected at the time of surgical resection of the tumor or therapeutic drainage of pleural effusions (n = 44). Primary tumor (n = 62) and adjacent normal lung samples (n = 48) were immersed immediately in sterile heparinized culture medium and transported to the laboratory on ice.

Of 62 patients with primary NSCLC studied, long-term follow-up was available for 38, and detailed staging data were available for 37. The entire workflow for tissue collection and preparation, flow cytometry, immunohistologic staining, and molecular analysis has been outlined [14] and is summarized in Supplemental Figure S1.

Flow Cytometry and Analysis

Our methodology for tissue disaggregation and flow cytometry on lung tumors and normal lung was performed as previously described in detail [15–17].

The strategies used to address sources of artifact encountered in disaggregated lung tissue have been described previously [16]. In addition to tumorigenic cells, tumor samples contain stromal, reactive, and immune cells [4, 18, 19]. We used markers to identify and eliminate nontumor cells during data analysis. Antibodies specific for each marker are conjugated to a different fluorochrome (eg, anticytokeratin-fluorescein) and reacted with the cells. The fluorescent DNA intercalator 4,6-diamidino-2-phenylindole dihydrochloride was used to quantify DNA in each cell. Cell-bound fluorochromes are distinguished from each other by their absorption and emission spectra. Cells belonging to individual cell types (eg, cytokeratin$^+$ tumor cells) are recognized by the presence of fluorescein, and the absence of other fluorochromes used to identify hematopoietic and mesothelial cells (defined as CD45 or CD14 or CD33 or CD235a positive). Analysis of CD44 and CD90 expression was confined to the cytokeratin-positive epithelial component of tumor and adjacent tissues [10, 17, 20]. Following this strategy, the denominator for all determinations in this study was nonhematopoietic, nonmesothelial, singlet cells with at least 2N DNA.

Histology and Immunostaining

NSCLC tumor samples were fixed in 10% neutral buffered formalin (Sigma-Aldrich, St. Louis, MO; Cat. No. F5554) for 24 hours and embedded in paraffin. Serial sections (4 μm) were cut from embedded tissues, heated at 60°C for 2 hours, deparaffinized with three washes in Histo-Clear (National Diagnostics, Atlanta, GA; Cat No. HS-200), rehydrated by successive washing in absolute ethanol, 90% ethanol, 75% ethanol, and deionized water, and rinsed twice with phosphate-buffered saline (PBS) at pH 7.4. Replicate slides were stained with hematoxylin, followed by eosin. For immunofluorescence, antigen retrieval was performed at 125°C for 20 minutes using a ethylenediaminetetraacetic acid buffer (DAKO, Glostrup, Denmark; Cat. No. S1699) at pH 6.0. To reduce nonspecific antibody binding, tissue sections were incubated in a blocking solution (1% bovine serum albumin, 5% goat serum, 0.05% Tween 20 in PBS) for 1 hour.

Immunofluorescent staining was performed using rabbit anti-human CD90 (1:100; Abcam, Cambridge, MA; Cat. No. Ab133350). Rabbit serum (DAKO, Cat. No. IR600;) was used in parallel as a negative control. Primary antibody and control were incubated for 2 hours at room temperature in humid chamber. Tissue sections were washed twice with PBS before the application of goat anti-rabbit-biotin (1:500; DAKO, Cat. No. E0432). Tissue sections were washed twice using PBS and incubated with streptavidin-cyanine 3 (1:500; Sigma-Aldrich, Cat. No. 6402) for 60 minutes. Sections were washed twice again and incubated with 4,6-diamidino-2-phenylindole dihydrochloride (ThermoFisher Scientific, Molecular Probes, Waltham, MA Cat. No. D21490) for 5 minutes. Slides were mounted in Prolong Gold anti-fade reagent (Invitrogen, Carlsbad, CA; Cat. No. P36934). An epifluorescence microscope (Nikon 90i; Nikon, Melville, NY) was used to document immunofluorescent staining.

RNA Isolation and Quantitative Real-Time Reverse Transcriptase Polymerase Chain Reaction Analysis

Total RNA from 3×10^6 cells was isolated using QIAGEN RNeasy Plus mini kit (QIAGEN, Germantown, MD; Cat no. 74134,) according to manufacturer's instructions. The RNA concentration was measured by NanoDrop Spectrophotometer ND-1000 (NanoDrop Technologies, Wilmington, DE). The transcriptional expression of CD40, CD90, TWIST, SNAIL, SLUG, EPCAM, E-CADHERIN, and β-actin genes was quantified by a two-step quantitative polymerase chain reaction (PCR). Total RNA (1 μg) was transcribed to complementary (c)DNA using the cDNA Affinity Script kit (Agilent Technologies, Santa Clara, CA;

Download English Version:

https://daneshyari.com/en/article/5596879

Download Persian Version:

https://daneshyari.com/article/5596879

Daneshyari.com