Featured Article

Osteopontin But Not Osteonectin Messenger RNA Expression Is a Prognostic Marker in Curatively Resected Non-Small Cell Lung Cancer

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Abstract

Purpose: The purpose of this study was to better define the role of osteopontin (OPN) and osteonectin [also known as secreted protein acidic and rich in cysteine (SPARC)] in lung tumorigenesis by comparing the expressions of these genes in lung tumor tissue and matched normal tissue and by determining the prognostic significance of the gene expressions.

Experimental Design: Quantitative real-time reverse transcription-PCR was used to analyze OPN and SPARC mRNA expression in normal lung tissue and matching tumor samples from 82 patients with non-small cell lung cancer. Gene expression data for each patient were matched to survival data.

Results: The overall median mRNA expression level of OPN was about 20-fold higher in tumor tissues than in matching normal lung tissues (P < 0.001), whereas SPARC gene expression was not significantly different in both tissue types. Forty of 82 patients had high (≥4.1) intratumoral OPN expression, and 15 of 82 patients had high (≥15.5) SPARC expression. High OPN expression in the tumor tissue was associated with inferior survival (P = 0.014), whereas high SPARC expression showed a trend toward longer survival (P = 0.095). The impact of high OPN and low SPARC expression on patient survival was additive (P = 0.001).

Conclusions: The large increase in OPN expression in tumors compared with normal tissue and its association with survival suggest a role for OPN in lung tumorigenesis.

Introduction

Lung cancer is one of the most common malignancies in the world and is the leading cause of cancer-related deaths for both men and women in the United States. For the year 2003, the American Cancer Society estimated about 171,900 new lung cancer cases and approximately 157,200 deaths due to lung cancer (1). Despite improvements in the detection and treatment of lung cancer in the past two decades, the 5-year survival rate remains <15% (2). New treatment strategies based on molecular classification of each patient’s tumor may provide further improvements in outcome for patients with lung cancer.

Osteopontin (OPN), also called secreted phosphoprotein 1, and secreted protein acidic and rich in cysteine (SPARC) belong to a group of bone matrix-associated factors that mediate cell-matrix interactions but do not serve primarily structural roles (3, 4). Both proteins are normally expressed when tissue undergoes events that affect changes in cell-matrix or cell-cell interaction such as tissue renewal or remodeling and embryogenic development (5). The deposition of hydroxyapatite in bone tissue requires the coordinated expression of a number of molecules including OPN and SPARC in cells of osteoblastic lineage (6).

OPN has been clearly implicated in the process of carcinogenesis. Enhanced OPN expression correlates with tumorigenic transformation in a variety of stromal and epithelial cell lines (7–11). OPN-null cell lines displayed decreased survival at clonal density compared with OPN-producing lines (12). Transfection of cDNA for OPN into benign mammary epithelial cells induces metastasis in rodent models (9, 11, 13), whereas specific suppression of OPN mRNA results in a reduction of tumorigenicity and metastatic ability (14, 15). In humans, OPN protein or gene expression levels have been shown to increase in several cancer types, suggesting a role in tumor progression (16–21). OPN protein is increased in the blood of patients with lung, prostate, and breast cancer (22), and plasma OPN levels correlate to the clinical outcome in patients with head and neck cancer (23). A correlation of OPN protein staining with worse patient survival has recently been indicated for breast cancer (19) and colon cancer (17). Overexpression of OPN mRNA has been associated with worse prognosis in ovarian cancer (24), colon cancer (17), and gastric cancer (25). In lung cancer, OPN has been detected in primary tumors by immunohistochemistry and Northern blotting (16, 20, 26), but the impact of OPN on the patient outcome remains unclear.

The relationship of SPARC expression with cancer progression is not as clear as that of OPN. Some observations even seem to suggest a tumor suppressor role. In human melanoma cell lines, SPARC protein overexpression is associated with invasive behavior (27), and suppression of SPARC expression by antisense RNA abrogates the tumorigenicity of these cells (28). On the other hand, SPARC protein expression in ovarian cancer cells is inversely correlated with the degree of malig-
nancy, and overexpression induces apoptosis in cancer cells (29). Transfection of SPARC cDNA into an ovarian carcinoma cell line reduces its growth rate in culture and its ability to induce tumors in rodents (30). Similarly, transfection of SPARC cDNA into breast cancer cell lines inhibits cancer cell proliferation by slowing the progression to S phase and does not stimulate cell migration (31). In vivo, SPARC protein or RNA is overexpressed in esophageal cancer (32), breast cancer (18), hepatocellular cancer (33), bladder cancer (34), and malignant melanomas (35), but high SPARC levels were associated with worse survival only in bladder cancer (34) and malignant melanomas (35). The role of SPARC in lung cancer has not yet been explored.

Only one previous study has tried to compare the prognostic role of both OPN and SPARC simultaneously in the same system (breast cancer), and that result came out to be negative for both genes (18). In the present study, to delineate the roles of OPN and SPARC in non-small cell lung cancer (NSCLC), we determined (a) gene expressions of OPN and SPARC in tumors compared with the normal tissue from which the tumors arose and (b) the prognostic value of the quantitative gene expression values of OPN and SPARC. We measured mRNA expression levels in surgically removed tumor specimens and adjacent nonmalignant tissue from 82 patients with curatively resected NSCLC. In contrast to previous studies, which used semiquantitative methods such as immunohistochemical staining techniques or Northern blotting to assess protein or gene expression levels, we performed quantitative reverse transcription-PCR (QRT-PCR) analysis, which is a sensitive, precise and reproducible methodology that gives nonsubjective numerical data for the amount of expressed mRNA and has the advantage of yielding a wider variability in gene expression levels than semiquantitative methods. The results we obtained are consistent with a major role for OPN in NSCLC tumorigenesis, whereas SPARC has, at best, only a marginal role.

Materials and Methods

Patients and Specimens. Included in this study were tumor specimens and paired normal lung tissues from patients with NSCLC that were available from a previous prospective tissue procurement trial of 103 consecutive patients with completely resected (R0 resection) NSCLC (36). For 82 of these patients, tumor plus corresponding normal tissue was available for gene analysis. There were 63 (77%) men and 19 (23%) women, with a median age of 62.7 years (range, 34–82 years). Thirty-eight (46.3%) patients had squamous cell carcinomas, 29 (35.4%) had adenocarcinomas, and 15 (18.3%) had large cell carcinomas. The primary tumors were graded histopathologically as well differentiated (G1, 1 patient), moderately differentiated (G2, 18 patients), and poorly differentiated (G3, 63 patients). Tumor staging was performed according to the International Union Against Cancer (UICC) tumor-node-metastasis (TNM) classification (37): 41 patients (50.0%) had stage I tumors; 17 patients (20.7%) had stage II tumors; and 24 patients (29.3%) had stage IIIa tumors. All 82 patients underwent thoracic surgery. Tumors were radically removed by lobectomy (n = 53), bilobectomy (n = 10), pneumonectomy (n = 10), or extended pneumonectomy (n = 9), including mediastinal lymphadenectomy. Patients with histopathological stage IIIa tumors received postoperative radiotherapy. Informed consent was obtained from all patients. The median follow-up was 86 months (range, 63–105 months), and no patient was lost to follow-up. Thirty patients developed metastases during the follow-up. Nine patients had lung metastases, 8 had bone metastases, 1 had liver metastasis, 11 had brain metastases, 5 had pleural metastases, 2 had metastases in the adrenal glands, and 1 had distant lymphatic metastasis. Tissue for gene expression analysis was obtained intraoperatively after lung resection and before mediastinal lymphadenectomy. Samples were collected from patients operated on between May 9, 1989 and October 19, 1992. Patients were followed-up until December 31, 1997 (each survivor was followed for at least 5 years). Data and tissue collection were in accordance with the regulations of the local ethic committee.

The tissues were immediately frozen in liquid nitrogen and stored at −80°C. Tissues were analyzed from the following two locations: (a) tumor; and (b) uninvolved lung tissue taken from the maximum distance to the tumor. Frozen sections (6 μm) were taken from blocks of tumor tissue, and starting with the first section, every fifth section was routinely stained with H&E and evaluated histopathologically. Sections were pooled for analysis from areas estimated to have at least 75% malignant cells.

RNA Isolation. Total RNA was isolated by a single-step guanidinium isothiocyanate method using the Quick Prep Micro mRNA Purification Kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ) according to the manufacturer’s instructions. After RNA isolation, cDNA was prepared from each sample as described previously (38).

QRT-PCR Quantification. Relative cDNA quantitations for OPN, SPARC, and an internal reference gene (β-actin) were done using a fluorescence-based real-time detection method [ABI PRISM 7900 Sequence Detection System (TaqMan); Perkin-Elmer Applied Biosystems, Foster City, CA] as described previously (38–40). The PCR reaction mixture consisted of 1.2 μM each of the primers; 200 nM probe; 0.03 unit/μl AmpliTaq Gold Polymerase; 200 μM each of dATP, dCTP, dGTP, and dTTP; 3.5 mM MgCl2; and 1× TaqMan Buffer A, which contains a reference dye, to a final volume of 25 μl (all reagents were from Perkin-Elmer Applied Biosystems). Cycling conditions were 50°C for 10 s and 95°C for 10 min, followed by 46 cycles at 95°C for 15 s and 60°C for 1 min. Colon, liver, and lung RNAs (all from Stratagene, La Jolla, CA) were used as control calibrators on each plate. Primers and probe sequences used are listed in Table 1. For each sample, parallel QRT-PCR reactions were performed for the gene of interest and the β-actin reference gene to normalize for input cDNA. The ratio between the values obtained provided relative gene expression levels for the gene locus investigated.

Statistical Analysis. QRT-PCR analyses yield values that are expressed as ratios between two absolute measurements: the gene of interest; and the internal reference gene β-actin. The Mann-Whitney t test and Kruskal-Wallis test were used to evaluate the associations between the continuous test variable gene expression and patients’ clinicopathological variables [Mann-Whitney t test for dichotomous variables (sex, smokers versus nonsmokers, metastasis versus no metastasis); Kruskal-
Wallis test for nondichotomous variables (age, tumor stage, lymph node metastases, UICC stage, grading, and histology). The \( t \) test for paired samples was applied to compare the gene expression levels in NSCLC tissue and normal lung tissue. The association between gene expression and survival time was evaluated by categorizing gene expression values at optimal cut points. The maximal \( \chi^2 \) method of Miller and Siegmund (41) and Halpern (42) was adapted to determine which gene expression (optimal cut point) best segregated patients into poor and good prognosis subgroups. Survival curves were estimated according to the Kaplan-Meier method from the date of primary tumor surgery to the time of death due to tumor progression. The difference in survival curves was examined by means of the log-rank test. The Brookmeyer-Crowley method was used to calculate the 95% confidence interval (CI) for the median period of survival, and Pearson’s correlation coefficient was used to screen for linear relation between the gene expression levels of \textit{SPARC} and \textit{OPN}. Cox’s proportional hazards modeling of factors that were significant in univariate analysis was performed to identify which factors might have a significant influence on survival. Statistical significance was defined as \( P < 0.05 \). SPSS Version 10.0 software (SPSS, Inc., Chicago, IL) was used for all statistical analyses. All \( P \) s were two-sided.

**Results**

\textit{OPN} mRNA Expression in Nonmalignant and Tumor Tissues from NSCLC Patients. \textit{OPN} mRNA expression was detectable by real-time QRT-PCR in 76 of 82 (92.6%) tumor specimens and 48 of 82 (58.5%) nonmalignant lung specimens. \textit{OPN} expression was higher in tumor tissue than in the matched normal tissue in 77 of 82 (93.9%) individual cases. The overall median mRNA expression level of \textit{OPN} was about 20-fold higher in tumor tissues (13.9; 95% CI, 0.0–100.1) compared with matching normal lung tissues (0.7; 95% CI, 0.0–9.2; Fig. 1; \( P < 0.001 \), paired samples \( t \) test). There were no significant associations between \textit{OPN} levels and the dichoto-

### Table 1  PCR primer and probe sequences of the analyzed genes.

<table>
<thead>
<tr>
<th>Primer and probe sequences</th>
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<tbody>
<tr>
<td><strong>Osteopontin</strong></td>
</tr>
</tbody>
</table>
| Forward primer | 5'-ATCACCTGTGCCATACCA-3'  
| Reverse primer | 5'-CCACAGATCTGGGTATTTTG-3'  
| Probe | 6FAM-5'-TAAAGCTGCTTTTCCTAGAATCT-3'-TAMRA*  
| **Osteonectin** |  
| Forward primer | 5'-TCTTGGCTAGACGGCATGC-3'  
| Reverse primer | 5'-AGACTGGGTGAGGAGGT-3'  
| Probe | 6FAM-5'-CGCTGACGCAGACCCATGGAC-3'-TAMRA  
| \( \beta \)-Actin |  
| Forward primer | 5'-TGACGGCGGCTACAGCCT-3'  
| Reverse primer | 5'-TCTTTTATGTCAGCAGGATT-3'  
| Probe | 6FAM-5'-ACCACAGGGCGAGCAGG-3'-TAMRA  

* FAM, 6-carboxyfluorescein; TAMRA, 6-carboxytetramethylrhodamine.

Fig. 1  Box and whisker plot of relative osteopontin mRNA expression levels for non-small cell lung cancer tissue and normal lung tissue. The boxes show the 25th and 75th percentile (interquartile) ranges. Median values are shown as a horizontal black bar within each box. The whiskers show levels outside the 50% confidence interval and within the 85% confidence interval.

Fig. 2  Box and whisker plot of relative secreted protein acidic and rich in cysteine mRNA expression levels for non-small cell lung cancer tissue and normal lung tissue. The boxes show the 25th and 75th percentile (interquartile) ranges. Median values are shown as a horizontal black bar within each box. The whiskers show levels outside the 50% confidence interval and within the 85% confidence interval.
mous variables sex ($P = 0.186$), smoking ($P = 0.393$), or development of metastases ($P = 0.476$, all from Mann-Whitney $t$ test). There were also no significant associations between $OPN$ expression and any of the following factors: age ($P = 0.463$); tumor stages ($P = 0.686$); prevalence of lymph node metastases ($P = 0.762$); UICC stage ($P = 0.492$); tumor cell types ($P = 0.656$); or grades of differentiation ($P = 0.537$, all from Kruskal-Wallis test).

**SPARC mRNA Expression in Nonmalignant and Tumor Tissue.** $SPARC$ mRNA expression could be detected in 79 of 82 (96.3%) tumor specimens and in 69 of 82 (84.1%) nonmalignant lung specimens. $SPARC$ gene expression was higher in tumor tissue than in the matched normal tissue in 50 of 82 (61.0%) individual cases. The overall median mRNA expression level of $SPARC$ was not significantly different in tumor tissues (5.86; 95% CI, 0.0–105.3 months) compared with matching normal lung tissues (6.60; 95% CI, 0.0–22.41 months). The effects of various clinical variables and $OPN$ and $SPARC$ expression status on the median and 5-year survival rates are summarized in Table 2.

A relative $OPN$ gene expression level of 4.1 segregated the patients best into groups with better and worse outcome ($P = 0.014$, log-rank test; Fig. 3). Forty patients had high ($\geq 4.1$) $OPN$ gene expression levels, and 42 patients had low ($< 4.1$) $OPN$ gene expression levels. The median survival for patients with high $OPN$ gene expression was 27.67 months (95% CI, 19.61–35.73 months), whereas the median survival for those with an $OPN$ level of $< 4.1$ was not reached (Fig. 3). For $SPARC$, the largest segregation between survival times was reached with a relative expression level of 15.5 (5-year survival rates are summarized in Table 2).

### Table 2 Association between the median $OPN^a$ and $SPARC$ mRNA expression in tumor tissue and clinicopathological variables

<table>
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<tr>
<th>Variable</th>
<th>OPN</th>
<th>$P^c$</th>
<th>Median (Range)</th>
<th>$SPARC$</th>
<th>$P^c$</th>
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<tbody>
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<td></td>
<td>n</td>
<td></td>
<td></td>
<td></td>
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<td>M</td>
<td>64</td>
<td>0.186</td>
<td>4.65 (0.00–225.21)</td>
<td>6.59 (0.01–44.22)</td>
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<tr>
<td>Yes</td>
<td>75</td>
<td>0.393</td>
<td>4.34 (0.00–225.21)</td>
<td>6.31 (0.00–44.20)</td>
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<td>3.33 (0.02–11.97)</td>
<td>7.20 (0.02–40.17)</td>
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<td>pT</td>
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<td>$pT_1$</td>
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<td>10.68 (0.35–40.17)</td>
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<td>$pT_2$</td>
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<td>5.45 (0.00–44.20)</td>
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<td>$pT_3$</td>
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<td>4.70 (0.01–20.87)</td>
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<tr>
<td>pN</td>
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<td>6.73 (0.02–44.20)</td>
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<td>$pN_2$</td>
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<td>4.25 (0.00–100.44)</td>
<td>6.83 (0.00–15.79)</td>
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</table>

$^a$ $OPN$, osteopontin; SSC, squamous cell carcinoma; AC, adenocarcinoma; LC, large cell carcinoma; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; UICC, International Union Against Cancer; $pT$, tumor infiltration; $pN$, lymph node status.

$^b$ Number of patients.

$^c$ $Ps$ according to Mann-Whitney $t$ test, Kruskal-Wallis test.
The Pearson correlation coefficient revealed no statistically significant linear correlation between the gene expression levels of OPN and SPARC (Pearson correlation coefficient, 0.028; \( p = 0.805 \); Fig. 5). This allowed us to combine both genes to analyze whether the combination of both genes improved the strength of significance in patient survival.

High expression levels of both OPN and SPARC were found in 9 of 82 (10.98%) patients. Thirty-seven (45.12%) patients showed a low expression status for OPN and SPARC, whereas 31 (37.80%) showed a high expression for OPN only, and 5 (6.10%) patients displayed a high expression for SPARC only. The median survival was not reached in the groups that showed either low OPN and/or high SPARC expression, compared with 22.03 months (95% CI, 13.23–30.83 months; \( p = 0.001 \); log-rank test, Fig. 6) in the high SPARC and low OPN coexpression group. Univariate analysis displayed high OPN and low SPARC coexpression as a significant unfavorable prognostic factor (Table 3). In a multivariate analysis of prognostic factors (Table 4, Models A and B), high OPN/low SPARC

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**Fig. 3** Estimated probability of survival of curatively resected non-small cell lung cancer patients versus osteopontin (OPN) expression in non-small cell lung cancer. The median survival was not reached in the group that showed low OPN expression, compared with 27.67 months (95% confidence interval, 19.61–35.73; \( p = 0.014 \)) in the group with high OPN gene expression.

**Fig. 4** Estimated probability of survival of curatively resected non-small cell lung cancer patients versus secreted protein acidic and rich in cysteine (SPARC) expression in non-small cell lung cancer. The median survival was not reached in the group that showed high SPARC expression, compared with 44.07 months (95% confidence interval, 11.68–76.46; \( p = 0.095 \)) in the group with low SPARC gene expression.

**Fig. 5** Scatter plot presenting intratumoral secreted protein acidic and rich in cysteine (SPARC) gene expression versus intratumoral osteopontin (OPN) gene expression. There is no correlation between SPARC and osteopontin gene expression [correlation coefficient, 0.028; \( p = 0.805 \) (Pearson correlation coefficient)].

**Fig. 6** Estimated probability of survival of curatively resected non-small cell lung cancer patients versus combined patterns of osteopontin (OPN) and secreted protein acidic and rich in cysteine (SPARC) coexpression in non-small cell lung cancer. The median survival was not reached in the group that showed low OPN and/or high SPARC gene expression, compared with 22.03 months (95% confidence interval, 13.23–30.83; \( p < 0.001 \)) in the group with high OPN and low SPARC gene expression.
coexpression was a significant unfavorable prognostic factor, as were advanced pN stage and tumor stage (UICC stage, pT stage).

Discussion

In this study, we analyzed the mRNA expression of OPN and SPARC in 82 tumor specimens and corresponding nontumor tissue from patients with NSCLC. The few existing studies investigating OPN in NSCLC used immunohistochemical staining or Northern blot analysis to analyze OPN expression levels. Whereas these methods allow the dichotomous differentiation of positive and negative OPN expressions, the real-time QRT-PCR method used in this study provides accurate and reproducible quantitation of gene copies and has a large range of results (39, 40). This capability permits a more precise delineation of the difference in gene expressions between tumor and normal tissue and of numerical cutoff values for better or worse prognosis. Previous studies of OPN expression in NSCLC reported variations in frequencies of tumors scored positive for OPN expression. Overexpression of OPN protein, defined as immunopositive staining, was reported in 21–88% in adenocarcinoma (16, 20, 26), in 31–100% in squamous cell carcinoma (16, 20, 26), and in 100% in lung cancer (16). In our study, we found a high (i.e., ≥4.1) intratumoral OPN expression status in 48.8% of NSCLC patients analyzed, without significant differences between histological subgroups. OPN gene expression in corresponding normal lung tissue was either not analyzed (20, 26) or not detectable by Northern blot analysis (16) in previous studies, whereas in this study, 58.5% of all normal lung tissues showed detectable expression of OPN mRNA, which emphasizes the high sensitivity of the QRT-PCR used in this study. With regard to the relationship of OPN to patient outcome, one previous study reported that patients with UICC stage I lung cancer and

Table 3 Survival in NSCLC<sup>a</sup> based on clinical and molecular parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>5-yr Survival probability</th>
<th>Median survival (mo)</th>
<th>95% CI</th>
<th>P&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
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<tbody>
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<td>Total no. of patients</td>
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<td>0.49 ± 0.06</td>
<td>51.37</td>
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<td>Age at diagnosis (yrs)</td>
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<tr>
<td>&lt;60</td>
<td>33</td>
<td>0.52 ± 0.09</td>
<td>NR</td>
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<td>60–69</td>
<td>28</td>
<td>0.46 ± 0.09</td>
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<td>≥70</td>
<td>21</td>
<td>0.48 ± 0.11</td>
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<td>0.50 ± 0.63</td>
<td>63.9</td>
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<td>Female</td>
<td>18</td>
<td>0.44 ± 0.12</td>
<td>44.07 ± 11.6</td>
<td>21.33–66.81</td>
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<tr>
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<td>75</td>
<td>0.49 ± 0.06</td>
<td>63.90</td>
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<td>No</td>
<td>7</td>
<td>0.43 ± 0.19</td>
<td>12.60 ± 2.92</td>
<td>6.88–18.32</td>
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<td>pT&lt;sub&gt;1&lt;/sub&gt;</td>
<td>18</td>
<td>0.67 ± 0.11</td>
<td>NR</td>
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<td>pT&lt;sub&gt;2&lt;/sub&gt;</td>
<td>52</td>
<td>0.48 ± 0.07</td>
<td>51.37</td>
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<td>pT&lt;sub&gt;3&lt;/sub&gt;</td>
<td>12</td>
<td>0.25 ± 0.13</td>
<td>26.43 ± 6.79</td>
<td>13.12–39.74</td>
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<td>45</td>
<td>0.69 ± 0.07</td>
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<td>pN&lt;sub&gt;1&lt;/sub&gt;</td>
<td>23</td>
<td>0.39 ± 0.10</td>
<td>33.67 ± 3.97</td>
<td>25.89–41.45</td>
<td>0.000</td>
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<td>pN&lt;sub&gt;2&lt;/sub&gt;</td>
<td>14</td>
<td>0.00 ± 0.00</td>
<td>16.70 ± 3.80</td>
<td>9.26–24.14</td>
<td>0.026</td>
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<td>UICC stage</td>
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<tr>
<td>I</td>
<td>40</td>
<td>0.70 ± 0.07</td>
<td>NR</td>
<td></td>
<td>&lt;0.001</td>
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<tr>
<td>II</td>
<td>18</td>
<td>0.52 ± 0.12</td>
<td>NR</td>
<td></td>
<td></td>
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<tr>
<td>IIIa</td>
<td>24</td>
<td>0.13 ± 0.07</td>
<td>18.83 ± 4.46</td>
<td>10.09–27.57</td>
<td>0.991</td>
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<tr>
<td>WD-MD&lt;sup&gt;c&lt;/sup&gt;</td>
<td>19</td>
<td>0.47 ± 0.11</td>
<td>46.77</td>
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<td>PD</td>
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<td>63.90</td>
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<td>Squamous cell</td>
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<td>0.55 ± 0.04</td>
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<td>Adenocarcinoma</td>
<td>27</td>
<td>0.37 ± 0.09</td>
<td>44.07 ± 11.34</td>
<td>21.85–66.29</td>
<td>0.004</td>
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<td>Large cell</td>
<td>15</td>
<td>0.53 ± 0.13</td>
<td>NR</td>
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<td>OPN (tumor)</td>
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<tr>
<td>&lt;4.06</td>
<td>42</td>
<td>0.59 ± 0.08</td>
<td>NR</td>
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<td>0.014</td>
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<tr>
<td>≥4.06</td>
<td>40</td>
<td>0.38 ± 0.08</td>
<td>27.67 ± 4.11</td>
<td>19.61–35.73</td>
<td>0.095</td>
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<td>SPARC (tumor)</td>
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<tr>
<td>&lt;15.5</td>
<td>68</td>
<td>0.44 ± 0.06</td>
<td>44.07 ± 16.53</td>
<td>11.68–76.46</td>
<td>0.000</td>
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<tr>
<td>≥15.5</td>
<td>14</td>
<td>0.71 ± 0.12</td>
<td>NR</td>
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<td>Double marker (OPN/SPARC)</td>
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<tr>
<td>High/high</td>
<td>9</td>
<td>0.78 ± 0.14</td>
<td>NR</td>
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<tr>
<td>High/low</td>
<td>31</td>
<td>0.26 ± 0.08</td>
<td>22.03 ± 4.49</td>
<td>13.23–30.83</td>
<td>0.001</td>
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<tr>
<td>Low/low</td>
<td>5</td>
<td>0.60 ± 0.22</td>
<td>NR</td>
<td></td>
<td></td>
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<tr>
<td>Low/high</td>
<td>37</td>
<td>0.59 ± 0.08</td>
<td>NR</td>
<td></td>
<td></td>
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</table>

<sup>a</sup> NSCLC, non-small cell lung cancer; CI, confidence interval; WD, well differentiated; MD, moderately differentiated; PD, poorly differentiated; OPN, osteopontin; UICC, International Union Against Cancer; NR, not reached, cannot be calculated.

<sup>b</sup> Based on median survival (log-rank test).

<sup>c</sup> Only one patient with grade 1.
positive protein staining had a significantly higher postoperative relapse rate (26). Another study classified patients into two categories, “dead” and “alive” (although without specifying either the cause of death or survival time), and found that the group of dead patients had stronger protein staining for OPN in the cancer specimen than did the group of patients who were still alive (16). These studies suggest the hypothesis that high OPN gene expression would be an unfavorable factor in NSCLC, which was supported by our data. We were able to show that high OPN gene expression is a significantly unfavorable prognostic factor for the survival of patients with NSCLC (P = 0.014).

A number of previous studies in cell lines and animal models suggested that enhanced OPN expression indicated a high metastatic potential of the tumor (9, 11, 13–15). Until now, this issue has not been addressed in studies in humans. In our investigation, there was no significant correlation between enhanced OPN gene expression and the incidence of metastases.

Because of the apparently related biochemical roles of OPN and SPARC in cell-matrix interactions, at the outset of this study we considered it possible that both proteins might also have parallel or similar roles in carcinogenesis. Just like OPN, SPARC is found to be overexpressed (on a pre- or posttranscriptional level) in many cancer types, including malignant melanomas (35), breast cancer (18, 43), esophageal cancer (32), hepatocellular cancer (33), bladder cancer (34), and gliomas (44). In view of these previous studies, it was somewhat surprising to find no significant difference in the expression level of SPARC in noncancerous lung tissue compared with NSCLC tissue. This observation suggests that, in contrast to OPN, SPARC does not have a strong role in promoting NSCLC carcinogenesis.

Previous studies suggest that the effects of SPARC on tumor development and behavior may be cell or tumor type specific. Whereas SPARC protein overexpression was significantly associated with risk of progression, incidence of distant metastases, and survival in thin cutaneous malignant melanomas (35), and mRNA expression was significantly correlated with worse prognosis in patients with bladder cancer (34), this correlation could not be found in previous investigations for breast cancer (18) and esophageal cancer (32). Our results are similar to the latter two studies in that the prognostic value for SPARC gene expression did not reach statistical significance. Interestingly, in the present study, patients with high intratumoral SPARC gene expression levels showed a trend toward longer survival than patients with low expression, which is more consistent with SPARC being a tumor suppressor than a tumor promoter. This observation probably explains why SPARC does not become overexpressed in NSCLC compared with normal tissue and may reflect the growth-inhibitory and apoptosis-inducing effects of SPARC observed in breast and ovarian cancer cell lines overexpressing SPARC (29, 31, 45).

OPN and SPARC were both found by immunohistochemistry to be strongly expressed in in situ and invasive ductal carcinoma of the breast, and both expressions were elevated to a similar extent in lymph node-positive cancer compared with lymph node-negative cancer (32). Although this observation would suggest a coregulation of OPN and SPARC in breast cancer, our data show no correlation between the expressions of these genes in NSCLC, i.e., some tumors with high OPN had low SPARC and vice versa. Consistent with independent regulation of OPN and SPARC, we found that even though SPARC expression, as a prognostic factor by itself, did not reach statistical significance, a combination of high OPN and low SPARC gene expression characterizes patients with low survival rates better than each factor taken separately. Interestingly, a small group of patients with high OPN and high SPARC gene expression had the highest 5-year survival rates of all combination subgroups. This observation could be due to the small sample size. Another explanation could be that OPN was the dominant factor for prognosis and that SPARC gene expression levels become of prognostic importance only if the expression of OPN is high.

Because OPN is highly expressed in several cancer types but is expressed only at a low level in normal tissue, it may have a direct role in tumor development and thus could be a candidate target for cancer therapy. Past research has discovered several methods to modulate OPN and its main receptors on tumor cells. These methods include inhibition of gene expression on the levels of gene transcription and RNA message, blocking of OPN protein with antibodies (46–49), and targeting the OPN receptor by cytotoxic and immunotherapeutic strategies (50–52). The high OPN gene expression measured in NSCLC in this study suggests a potential utility of such therapeutic approaches in the therapy of patients with NSCLC.

In summary, we have demonstrated intra-individual heterogeneity in OPN and SPARC gene expression of NSCLCs and identified high OPN gene expression and high OPN/low SPARC coexpression as unfavorable prognostic factors in NSCLCs. These two genes may have a value in distinguishing tumors with more aggressive behavior and may be helpful in selecting patients who will benefit from intensive adjuvant or neoadjuvant treatment.

### Table 4 Cox proportional hazard regression models

<table>
<thead>
<tr>
<th>Model</th>
<th>Parameter</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P</th>
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<tr>
<td>A</td>
<td>Stage</td>
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<tr>
<td>I/IIa</td>
<td>0.186</td>
<td>0.08–0.38</td>
<td>&lt;0.001</td>
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<tr>
<td>II/IIIa</td>
<td>0.359</td>
<td>0.16–0.79</td>
<td>0.011</td>
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<tr>
<td>Double marker</td>
<td>0.307</td>
<td>0.16–0.57</td>
<td>&lt;0.001</td>
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</tr>
<tr>
<td>B</td>
<td>pT</td>
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<tr>
<td>pT1/pT3</td>
<td>0.161</td>
<td>0.05–0.51</td>
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<td>pT1/pT2</td>
<td>0.480</td>
<td>0.21–1.01</td>
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<tr>
<td>pN1</td>
<td>0.119</td>
<td>0.05–0.27</td>
<td>&lt;0.001</td>
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<tr>
<td>pN1/pN2</td>
<td>0.232</td>
<td>0.10–0.52</td>
<td>&lt;0.001</td>
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<tr>
<td>Double marker</td>
<td>0.293</td>
<td>0.15–0.56</td>
<td>&lt;0.001</td>
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</table>

* CI, confidence interval for hazard ratio.
* Parameter section: e.g., stage I/IIa means stage I compared with stage IIIa.
* Osteopontin/secreted protein acidic and rich in cysteine gene coexpressions (high/low).

### References


Non-Small Cell Lung Cancer Expression Is a Prognostic Marker in Curatively Resected Osteopontin But Not Osteonectin Messenger RNA

Sylke Schneider, JiMin Yochim, Jan Brabender, et al.


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