Introduction: Osteopontin (OPN) is a multifunctional protein with an important but poorly understood role in non-small cell lung cancer (NSCLC) pathogenesis. Moreover, the role of the three known mRNA isoforms (OPNa, OPNb, and OPNc) has not been reported. We hypothesize that OPN isoforms play different roles in determining the metastatic potential of NSCLC.

Methods: We amplified mRNA for each OPN isoform in NSCLC tumors and matched normal lung. The functional impact of each isoform was evaluated by transfecting cDNA plasmids specific to each isoform into NSCLC cell lines and comparing behavior to empty vector controls in scratch closure, cell proliferation, soft-agar colony formation, and Matrigel invasion assays. Gene array was used to evaluate differences in downstream targets and was compared with a panel of controls. Expression arrays revealed an increase in EMT with OPNa overexpression but not OPNc overexpression. Differences were validated by quantitative reverse transcriptase-polymerase chain reaction.

Conclusions: Overexpression of the individual OPN isoforms in NSCLC results in divergent functional phenotypes. OPNa produced an aggressive phenotype, whereas OPNb produced a more indolent phenotype. Exon 4, which is transcribed in OPNa but absent in OPNc, may be central to this phenomenon and could serve as a target for isoform-specific inhibition of OPN in NSCLC.

Key Words: Osteopontin, Non-small cell lung cancer, mRNA isoforms, Tumor progression, Epithelial-mesenchymal transition.

Osteopontin (OPN) is a multifunctional protein identified in a remarkable range of normal and pathologic contexts. It plays a key role in the mediation of immune cell recruitment, wound healing, and tissue remodeling. OPN’s diverse biologic functions involve cell adhesion, migration, and invasion, which are associated with its two highly preserved central binding domains, the arginine-glycine-aspartic acid (RGD) and serine-valine-valine-tyrosine-glycine-leucine-arginine [SVVYGLR] sequences. OPN is a ligand for the αvβ3 integrins and mediates adhesive cell-matrix interactions and signal transduction pathways through binding at these locations.

The importance of OPN in tumor dissemination is highlighted in gene transfer experiments, where transfection of OPN increases the malignant phenotype and OPN knock out with antisense oligonucleotides decreases malignant potential. Structural heterogeneity may be responsible for varying functions of OPN from different cellular sources. Alternative gene splicing may provide an explanation for its diversity. Three distinct OPN cDNA isoforms have been identified in humans, but little is known about their function. Osteopontin-a (OPNa) represents the full-length cDNA, whereas osteopontin-b (OPNb) has a deletion at exon 5, and osteopontin-c (OPNc) has a deletion at exon 4 (Figure 1).

OPN is overexpressed by immunohistochemistry in non-small cell lung cancer (NSCLC) tumors compared with surrounding normal lung. NSCLC cell lines, which overexpress OPN, have increased in vitro migration and invasion. Elevated tumor OPN levels are also associated with increased stage, lymph node involvement and poor long-term survival. These data indicate an important role for OPN in determining the metastatic potential in NSCLC; however, reports of OPN expression in clinical NSCLC samples and tumor models have not reported which isoforms are expressed. We hypothesize that the individual isoforms are not equally expressed in NSCLC tumors and have significantly different roles in determining the metastatic potential in NSCLC through alteration in downstream gene targets.
MATERIALS AND METHODS

Tumor Specimens and RNA Isolation
We evaluated mRNA expression of the OPN isoforms in 43 resected NSCLC tumors and matched normal lung. Tissue specimens were collected from consenting patients undergoing resection of NSCLC at Wayne State University from 1998 to 2005, with institutional review board approval. The specimens were transferred to New York University School of Medicine under a cooperative materials transfer agreement and the NYU Institutional Review Board granted approval for use of deidentified specimens for biomarker discoveries. Tumor and normal tissues were homogenized, and mRNA was isolated using RNEasy mini kit from Qiagen (QIAGEN, Valencia, CA).

NSCLC Cell Lines
NSCLC cell lines, H358, A549, and H460, were obtained from American Type Tissue Collection (Manassas, VA) and maintained in culture in Dulbecco’s Modified Eagle Medium (DMEM) (Invitrogen, Grand Island, NY) with 10% fetal bovine serum (FBS) (Invitrogen, Grand Island, NY) at 37° and 5% CO2. Prior evaluation had determined that H358 had minimal native OPN mRNA expression and protein secretion, whereas A549 and H460 had high native mRNA expression and protein secretion of OPNa and OPNb and high protein secretion.12

Reverse Transcriptase-Polymerase Chain Reaction Primers
Reverse transcriptase-polymerase chain reaction (RT-PCR) primers were designed based on the three NCBI GenBank translated exons, whereas OPNa has a deletion of exon 5, OPNb has a deletion of exon 4. The PCR primers amplify the region of variability between the three isoforms shown by the arrows. The isoforms can be discriminated in a single reaction RT-PCR localized to that region.

FIGURE 1. Three human cDNA splice variants of osteopontin (OPN) and GenBank accession numbers. OPNa has six translated exons, whereas OPNb has a deletion of exon 5, and OPNc has a deletion of exon 4. The PCR primers amplify the region of variability between the three isoforms shown by the arrows. The isoforms can be discriminated in a single-reaction RT-PCR localized to that region.

OPN Isoform Expression Constructs and Transfection
pCMV2-based plasmid constructs expressing OPNa (NM_001040058), OPNb (NM_00058), OPNc (NM_001040060), and empty vector for control (Origene, Rockville, MD) were complexed with lipofectamine 2000 (Invitrogen, Carlsbad, CA) for transfection into NSCLC cell lines. Proof of transfection was evaluated by RT-PCR from a pooled population of cells. Pooled populations of transfected cells were used in scratch closure, cell proliferation, soft-agar colony formation, and Matrigel invasion assays. Comparisons were made between cells transfected with each isoform and empty cytomegalovirus plasmid vector. Assays were performed in triplicate, and the data were collected independently and expressed as mean ± SE. A two-sided Student’s t test was used to evaluate data, and differences were regarded as significant at p ≤ 0.05.

Scratch Closure Assay
Two × 105 transfected cells were plated in 3 ml of serum-free DMEM in six-well plates. A scratch was made through the bottom of the plate using a 1-ml sterile pipette tip 24 hours later. Plates were washed with phosphate buffered saline (PBS) and the undersides of plates were marked. The scratch width was measured at six locations. Plates were incubated for 24 hours and scratch width measured at the

TABLE 1. Primers Used for Semiquantitative PCR Analysis

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5′-3′)</th>
<th>Reverse (5′-3′)</th>
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<tr>
<td>Osteopontin</td>
<td>TTGCAATGTAGTATTTGCCTTCTGGT</td>
<td>GTCAATGGAGTCTGGCTCTG</td>
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<td>PPIA</td>
<td>TCTGAGCACCTGAGAGGAAAGG</td>
<td>GGAAGAACATGGAACCCAAAGG</td>
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<tr>
<td>Snail-2</td>
<td>CATGCCATTTGGAGCCTAAA</td>
<td>GCGATGAGGGCAAGAAAGG</td>
</tr>
<tr>
<td>TGF receptor-b-1</td>
<td>ACCTGCAACCCGGTGGTCTCT</td>
<td>CCGTATGTAACCCGTTGAT</td>
</tr>
<tr>
<td>N-Cadherin</td>
<td>GCGCTGCTGAGGCTTCTGG</td>
<td>ACTGCCAATTTGCAGCATTTTC</td>
</tr>
<tr>
<td>Vimentin</td>
<td>CGAAAAAACACCTGGAATCTTT</td>
<td>TGGTGAACCCACATTTATG</td>
</tr>
<tr>
<td>Desmopakin</td>
<td>CGTATGACCAACCGCAAGAC</td>
<td>GGGCAAAACATCTCAATG</td>
</tr>
<tr>
<td>Cytokeratin-20</td>
<td>TGAAGGACTGGAAGGCTAGCAG</td>
<td>CAGCCTCACCCCTCAGTTG</td>
</tr>
<tr>
<td>E-Cadherin</td>
<td>CAGGTCCTCCTTTGGCTCTG</td>
<td>GGCCTAGCAAGAAGATGGA</td>
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</table>

PPIA, peptidal prolyl isomerase A; TGF, transforming growth factor.
same six points. Closure was calculated as a percent of initial scratch width.

**Cell Proliferation Assay**

Three $10^3$ transfected cells in 100 μl of DMEM/10% FBS were plated in 96-well flat bottom plates in six replicates and incubated for 48 hours. Twenty microliters of CellTiter-Blue reagent (Promega, Madison, WI) was added to each well and incubated for 1 hour. Optical density was measured at 560/590A using the Universal Reader Victor3 (PerkinElmer, Waltham, MA) and compared with standard curve derived for each cell line.

**Soft-Agar Colony Formation**

Base agars were made by combing equal volumes of 1% agarose and 2 × DMEM/10% FBS, both at 40°. Warm base agar (1.5 ml) was plated into six-well plates and allowed to polymerize. The top agar was produced by combing equal volumes $5 \times 10^3$ transfected cells/ml in 1 × DMEM/10% FBS with warm 0.7% agarose. Two milliliters of the agarose cell mixture was placed on top of each base coat. Plates were incubated at 37°, 5% CO2. At 21 days, colonies were stained with 0.5 ml of Crystal violet and counted under a dissecting microscope.

**Matrigel Invasion Assay**

The BD Biocoat Matrigel invasion chambers were used in accordance with the manufacturer’s protocol (BD Sciences, Franklin Lakes, NJ). The $1 \times 10^5$ transfected cells were seeded onto 8-M pore polycarbonate membrane inserts coated with a thin layer Matrigel basement membrane matrix diluted 1:100 in PBS. Plates were incubated for 48 hours. Cells that migrated through the Matrigel to the lower surface were fixed and stained with Giemsa solution and counted under microscope at 10× amplification. Ten fields from each membrane were counted.

**Gene Expression Analysis**

mRNA extracted from the pooled population of transfected cells used in the functional assays was analyzed using Affymetrix U133 plus-2 chips (Affymetrix Inc., Santa Clara, CA). Each probe was scanned twice (Hewlett-Packard Gene Array Scanner), the images overlaid, and the average intensities of each probe compiled and converted into expression file format using Affymetrix Gene Chip Operating System. We evaluated gene expression profiles in the A549 cell line with overexpression of OPNa and OPNc isoforms and empty vector controls in duplicate. Raw GeneChip data were normalized at the probe level using the robust multichip average algorithm13 and filtered using Genespring 7.2 (Aligent Technologies, Palo Alto, CA). The differentially abundant mRNA between experimental cell lines and controls was filtered using an intersection of t test results with p value of 0.05 and false discovery rate set at 5%. Array results were compared against a panel of common downstream markers for epithelial-mesenchymal transition (EMT) to correlate functional results with downstream gene pathways.14 RT-PCR was performed using parameters described above to validate expression of Snail-2, TGFβ1-r, N-cadherin, vimentin, desmoplakin, cytokeratin-20, and e-cadherin. Primers are listed in Table 1.

**OPN Silencing with sh-RNA**

sh-RNA plasmid 5′-GACCTGCCAGCAACCGAAG-3′ (Geneocopoeia, Rockville, MD) was complexed with lipofectamine 2000 (Invitrogen, Carlsbad, CA) for transfection into NSCLC cell lines. Proof of knock down was evaluated by RT-PCR from a pooled population of cells.

**Real-Time Quantitative PCR Analysis**

The semiquantitative expression levels were validated with quantitative PCR in four targets: TGFβ1-r, N-cadherin,

<table>
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<th>Gene</th>
<th>Forward (5′-3′)</th>
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<td>CCTACATTGGAAGCCTGGAAC</td>
<td>CCGGGTTATGCTGGTTGTA</td>
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<td>N-Cadherin</td>
<td>CTGGAGACATTGGGGACTTC</td>
<td>AGTCATATGGTGGAGCTGTGG</td>
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<tr>
<td>Desmoplakin</td>
<td>GGAAATTGAGAAATTCCAAAAGC</td>
<td>GCCCTCCTCTTTCTGGATAC</td>
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<tr>
<td>Cytokeratin-20</td>
<td>TCAGTTAAGCACCCTGGGAGA</td>
<td>CGACCTTGCCATCACCTACT</td>
</tr>
</tbody>
</table>

**FIGURE 2.** Osteopontin (OPN) mRNA expression from non-small cell lung cancer (NSCLC) specimens (T) and paired normal lung (N) using primers which amplify each of the three OPN isoforms. OPN was overexpressed in 39 of 43 (90.7%) of NSCLC specimens compared with matched normal lung and in all but specimen 2 shown here. OPNa is the dominant isoform expressed in all the tumor samples; OPNc was detected in normal samples but in none of the tumor tissues.
FIGURE 3. Bar graft depiction of results from functional assays with overexpression of the individual osteopontin (OPN) isoforms in non-small cell lung cancer (NSCLC) cell lines. H358 (low native OPN expression) and A549 and H460 (high native OPN expression), transfected with empty vector for control or the individual OPN isoforms; stars denote significant differences between experimental cell lines and controls. A, Scratch closure: overexpression of OPNa caused significant increase in percent closure at 24 hours compared with controls. OPNb overexpression had a less significant increase, and OPNc had no impact compared with controls. B, Cell proliferation assay: OPNa overexpression resulted in a significant increase in cell numbers at 24 hours in all cell lines, OPNb overexpression had no impact compared with controls, and OPNc resulted in a significant decrease in cell number compared with controls. C, Soft-agar colony formation assay: OPNa overexpression resulted in a significant increase in the size and numbers of colonies at 21 days in all cell lines, OPNb overexpression had no impact compared with controls in A549 but resulted in a significant increase in H358 and a significant decrease in H460 cell lines, whereas OPNc overexpression resulted in a significant decrease in size and number of colonies in all cell lines compared with controls. D, Matrigel invasion assay: OPNa overexpression resulted in a significant increase in invasion at 48 hours in all cell lines compared with controls, OPNb overexpression had no impact compared with controls in any of the cell lines, whereas OPNc overexpression resulted in a significant decrease in invasion in all cell lines compared with controls. E, Matrigel invasion assay normalized to cell number due to proliferation differences: OPNa expression significantly increased invasion at 48 hours in all cell lines even after normalization for increased rates of proliferation, whereas OPNb had no impact and OPNc significantly decreases invasion despite normalization for decreased proliferation compared with controls.
desmoplakin, and cytokeratin-20. Super Script III first strand synthesis super mix (Invitrogen, Carlsbad, CA) was used to synthesize first strand cDNA. PCR was performed using 7900HT Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA). Samples were assayed in 10-μl reaction mixture containing 2-μl cDNA, 0.5 μl of 5 μM gene-specific primers (Table 2), 5 μl of 2 X SYBR green master mixes, and 2 μl of molecular grade H₂O. Beta actin was used as a normalization control. The amplifications were performed for 40 cycles with annealing at 60°C for 1 minute. Samples were run in triplicates including negative controls. Relative quantification (2ΔΔC_T method) was performed to determine the change in gene expression levels.15

RESULTS

OPN mRNA Expression is Increased in 91% NSCLC Tumors

OPN was overexpressed in the NSCLC tumors compared with the matched normal lung in 39 of 43 pairs (90.7%), and the dominant isoform expressed in all tumor samples was OPNa (Figure 2). OPNc expression was detected in normal lung but not in any of the tumor samples. The OPNa PCR products from NSCLC tumor specimens were sequenced and match the OPNa NCBI GenBank sequence NM_001040060.

Functional Heterogeneity with Individual OPN Isoform Overexpression in NSCLC Cell Lines

In all three cell lines, OPNa overexpression significantly increased activity in scratch closure and proliferation assays at 24 hours. OPNa overexpression significantly increased the size and number of colonies in soft agar and significantly increased the rate of invasion through Matrigel compared with controls (Figures 3 A–D). Overexpression of OPNb produced a less significant modulation of function compared with OPNa in all assays and all cell lines (Figures 3 A–D). OPNc overexpression had no impact compared with control in scratch closure (Figure 3A) but significantly decreased activity in proliferation, soft-agar colony formation, and Matrigel invasion compared with controls (Figures 3B, C). When Matrigel invasion results were normalized to differential rates of proliferation, the differences remained significant (Figure 3E).

OPNa Overexpression Induces EMT

We examined gene expression data from the A549 cell line with OPNa and OPNc overexpression compared with cytomegalovirus plasmid controls and compared it with a stan-
standard panel of makers associated with EMT.\textsuperscript{16} \textit{OPNa} overexpression resulted in a relative increase in expression of matrix metalloproteinase (MMP)-2, Snail-1, Snail-2, TGF\(\beta\)-r, MMP-9, N-cadherin, ILK, vimentin, SOX-8, and SOX-9, and a relative decrease in the expression of cytokeratin 18 and 20, desmoplakin, and E-cadherin, all consistent with activation of EMT pathways (Figure 4A). We noted no significant change in expression of Twist, goosecoid, FoxC-2, Sox 10, or MMP-3. \textit{OPNc} overexpression resulted in relative increase in expression of MMP-2, MMP-9, and Snail-1 but a relative decrease in Snail-2, TGF\(\beta\)-r, N-cadherin, ILK, vimentin, Sox-8, Sox-9, and fibronectin. Cytokeratin 18 and occludin were downregulated, but cytokeratin 20 and desmoplakin were upregulated, overall a far less consistent picture for EMT activation with \textit{OPNc} overexpression. Relative differences in expression of the markers between \textit{OPNa} and \textit{OPNc} overexpressing cell lines and controls were analyzed by semiquantitative RT-PCR and were similar across all three cell lines (Figure 5).

Changes in expression of four markers were validated by quantitative PCR. Computed tomography (CT)-

<table>
<thead>
<tr>
<th>marker</th>
<th>expression with EMT</th>
<th>H358</th>
<th>A549</th>
<th>H460</th>
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<tbody>
<tr>
<td>Snail 2</td>
<td>increase</td>
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<td>O O O</td>
<td>c a b c</td>
</tr>
<tr>
<td>TGF(\beta)-r</td>
<td>increase</td>
<td>p O P P</td>
<td>O O O</td>
<td>c a b c</td>
</tr>
<tr>
<td>N-cadherin</td>
<td>increase</td>
<td>p O P P</td>
<td>O O O</td>
<td>c a b c</td>
</tr>
<tr>
<td>Vimentin</td>
<td>increase</td>
<td>p O P P</td>
<td>O O O</td>
<td>c a b c</td>
</tr>
<tr>
<td>Desmoplakin</td>
<td>decrease</td>
<td>m N N N</td>
<td>m N N N</td>
<td>m N N N</td>
</tr>
<tr>
<td>Cytokeratin 20</td>
<td>decrease</td>
<td>m N N N</td>
<td>m N N N</td>
<td>m N N N</td>
</tr>
<tr>
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<td>decrease</td>
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<tr>
<td>PPIA</td>
<td>control</td>
<td>m N N N</td>
<td>m N N N</td>
<td>m N N N</td>
</tr>
</tbody>
</table>

FIGURE 5. Semiquantitative PCR analysis of differences in relative expression in series of epithelial-mesenchymal transition (EMT) markers discovered in gene arrays. Expression changes are validated in three non-small cell lung cancer (NSCLC) cell lines, each with overexpression of the individual osteopontin (OPN) isoforms. \textit{OPNa} overexpression resulted in downstream changes consistent with activation of EMT pathways, demonstrated here with relative increase in expression of Snail-2, TGF\(\beta\)-r, N-cadherin, and vimentin and downregulation in desmoplakin, e-cadherin, and cytokeratin-20 in all three cell lines. The pattern of expression resulting from overexpression of \textit{OPNb} and \textit{OPNc} are not reflective of EMT pathways.

FIGURE 6. Quantitative PCR results validating differential in expression of TGF\(\beta\)-r and N-cadherin. A, Bar graphs depict relative changes in mRNA levels of TGF\(\beta\)-r and N-cadherin normalized to controls. Overexpression of osteopontin \textit{OPNa} resulted in a significant increase in mRNA levels of both markers in each non-small cell lung cancer (NSCLC) cell line, whereas \textit{OPNc} overexpression resulted in significant decrease in the mRNA levels in each cell line. B, sh-RNA inhibition of native OPN expression had minimal impact on mRNA level of TGF\(\beta\)-r and N-cadherin in H358, but significantly decreased the levels in A549 and H460.
values for TGFb1-r and N-cadherin were higher in H358 controls than in H460 and A549, and were significantly decreased in all three cell lines with the overexpression of OPNa and increased with overexpression of OPNc, consistent with gene array expression data (Figure 6A). Baseline CT-values for desmoplakin and cytokeratin-20 were lower in the H358 cell line than in H460 and A549, and were significantly increased with OPNa expression and decreased with OPNc expression across all cell lines (Figure 7A).

Inhibition of native OPN expression by sh-RNA resulted in minimal changes in mRNA levels of TGFb1-r, N-cadherin, and desmoplakin in H358, the cell line with minimal native OPNa expression, but resulted in a significant decrease in TGFb1-r and N-cadherin mRNA levels and significantly increased desmoplakin and cytokeratin-20 in the A549 and H460 cell lines, each with high native OPNa expression (Figures 6B and 7B).

**DISCUSSION**

Lung cancer is the world’s leading cause of cancer death. A better understanding of the molecular pathogenesis is necessary to detect new biomarkers, develop new treatment strategies, and improve survival. OPN is a ubiquitous protein whose functions center on cell migration and invasion. Hu et al.\(^9\) noted an association between OPN overexpression and aggressive NSCLC phenotypes with increased in vitro cell invasion. The data presented here are unique because it identifies a single-putative isoform, which is uniquely overexpressed and tied to malignant behavior. OPNa, the full length isoform, produced a more aggressive phenotype with increased migration, proliferation, anchorage independent growth, and invasion when overexpressed. In contrast, OPNc, which is defined by the deletion of exon 4, confers the opposite effect when overexpressed in NSCLC cell lines, decreasing proliferation, anchorage independent growth, and invasion compared with controls. Importantly, OPNa is the isoform preferentially upregulated in NSCLC tumors (Figure 2) and cell lines.\(^12\) This rate of overexpression is consistent with others who have noted increased OPN mRNA expression in 80% of NSCLC tumors compared with normal lung\(^17\) and recognize OPN as one of the most consistently overexpressed genes in NSCLC cDNA libraries.\(^18\)

Heterogeneous expression of the OPN isoforms has been documented in human glioma and breast cancer cells.\(^8,19\) In breast cancer, OPNc is a unique marker for malignant tissue, which is not seen in normal breast tissue, and correlates with tumor grade.\(^20\) A varied role for the isoforms is identified in breast cancer, where in vitro experiments demonstrate enhanced anchorage independent growth and a lack of calcium-dependant aggregation and cell adhesion with the incorporation of OPNc but not OPNa.\(^21\) This supports the hypothesis that the isoforms have differing functions in malignancy.
This work also correlates the functional findings with gene array data where an increase in EMT markers was noted with OPNa overexpression but not OPNc. EMT enables benign tumor cells the capacity to infiltrate surrounding tissue and ultimately metastasize to distant sites and is implicated in the conversion of early tumors to invasive malignancies. The molecular basis of EMT involves changes in the expression, distribution, and function of multiple proteins including integrins, vimentin, MMPs, and cadherins.

The cadherins are a family of calcium-dependent cell adhesion molecules critical to the development and maintenance of epithelial architecture. E-cadherin maintains cell to cell interactions, and its loss is a hallmark for metastatic progression of solid tumors. Conversely, N-cadherin stimulates tumor invasiveness and progression by providing tumor cells the ability to permeate tissues. It promotes adhesive interactions with host stroma and endothelium that facilitates tumor dissemination. Overexpression of OPNa in NSCLC cell lines resulted in a classic switch in cadherin class: loss of E-cadherin and gain of N-cadherin, which has been closely linked to both EMT and tumor progression. Knockdown of endogenous OPNa by sh-RNA reversed the cadherin switch, increasing expression of E-cadherin and decreasing N-cadherin. Overexpression of OPNc did not produce the same classic downstream pattern consistent with increased EMT and malignant potential.

The observations that OPNa overexpression in NSCLC increased malignant behavior in vitro and EMT pathways compared with controls coupled with the finding of a decrease in the same properties with OPNc overexpression is fortuitous because the only difference between OPNa and OPNc is the transcription of exon 4, a 27-amino acid sequence in the amino terminus of the protein. Although two centrally preserved integrin binding domains (RGD and SSVVGLR) of OPN is essential to its activity, these data implicate exon 4 as an important regulator in the malignant potential of NSCLC. This region should contain the structure that dictates function relevant to at least some of the demonstrated malignant properties conferred by OPNa in NSCLC. The gene array data provide important information on downstream gene targets and molecular pathways, which are responsible for this differential function. Targeted small molecules and monoclonal antibody therapies are highly successful treatment programs in oncology, and the structural similarities between the isoforms may make OPN an ideal target for such treatment strategies in NSCLC.

ACKNOWLEDGMENTS

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