Epigenetic Inactivation of Laminin-5-encoding Genes in Lung Cancers

Ubaradka G. Sathyanarayana, Shinichi Toyooka, Asha Padar, Takashi Takahashi, Elizabeth Brambilla, John D. Minna, and Adi F. Gazdar

Hamon Center for Therapeutic Oncology Research [U. G. S., S. T., A. P., J. D. M., A. F. G.] and Departments of Pathology [U. G. S., A. F. G.] and Internal Medicine [J. D. M.], University of Texas Southwestern Medical Center, Dallas, Texas 75390-8593; Division of Molecular Oncology, Aichi Cancer Center, Nagoya 464-8681, Japan [T. T.]; and Laboratoire de Pathologie Cellulaire, Centre Hospitalier Regional Universitaire, Grenoble 38043, France [E. B.]

ABSTRACT
Purpose: We investigated the loss of expression of three laminin-5 (LN5)-encoding genes in lung cancer cell lines and elucidated the mechanism of inactivation of the genes in lung cancer cell lines and tumors.

Experimental Design: We examined the expression of LN5-encoding genes by reverse transcription-PCR in 49 lung cancer cell lines. To elucidate the mechanism of gene silencing, we treated expression-negative cell lines (two for each gene) with a demethylating agent and examined the restoration of expression by reverse transcription-PCR. We dissected out the methylation patterns of CpG sites unique to the promoter regions of LN5-encoding genes by bisulfite genomic sequencing of expression-negative cell lines. We designed methylation-specific primers and validated the methylation status of the promoter regions in lung cancer cell lines using methylation-specific PCR. We further studied the methylation patterns of primary non-small cell lung cancer [NSCLC (n = 36)], small cell lung cancer [SCLC (n = 26)], and carcinoids (n = 24) tumors.

Results: We observed frequent losses of expression in NSCLC (20–60%) and SCLC (65–86%) cell lines. Expression of one or more genes was lost in 90% of SCLC cell lines and 65% of NSCLC cell lines. Treatment of expression-negative cell lines with demethylating agent restored expression in all of the cases. Methylation of LN5-encoding genes was present more frequently in SCLC cell lines (60–80%) than in NSCLC cell lines (15–60%), and at least one gene was methylated in 95% of SCLC and 60% of NSCLC cell lines. The concordances between loss of expression and methylation in 40 lung cancer cell lines for the three genes (90–95%) were statistically significant. Methylation was more frequent in SCLC tumors (58–77%) than in NSCLC tumors (22–42%) and carcinoids (13–33%), and at least one gene was methylated in 92% of SCLC tumors, 47% of NSCLC tumors, and 33% of carcinoids.

Conclusions: Our results demonstrate frequent epigenetic inactivation of LN5-encoding genes in lung cancers, and these findings are of biological interest and are potentially of clinical importance.

INTRODUCTION

BMs are characteristic of multicellular organisms, and they are the first ECM component produced during embryogenesis (1). The BM is a thin (20–200-nm) carpet-like ECM structure that regulates cell attachment, differentiation, and growth. It is a flat structure separating the epithelial cells from the underlying stromal tissues and forms an important barrier to invasion. Epithelial cells must be attached both to appropriate ECM components and to other similar epithelial cells to survive, a process termed anchorage dependence. ECM consists of several molecules; two of the most important are LN5 (secreted by the overlying epithelial cells) and collagen 4 (secreted by the stromal cells). Laminins, which are the major component of BM, play a major role in anchorage dependence (2). Whereas in situ carcinomas are intraepithelial by definition, invasion and destruction of the BM is the earliest morphological feature of invasive carcinomas.

The epithelium and underlying stroma function as a unit and constantly communicate. Two-way signaling occurs via extracellular proteins (laminins) and their transmembrane receptors, the integrins. In the BM, especially at specialized attachment units known as hemidesmosomes, the crucial integrin is α,β, and its ligand is LN5. LN5 is a heterotrimeric protein member of the laminin family, and it consists of three polypeptide chains, A3, B3, and C2, which are the products of three different genes, LAMA3, LAMB3, and LAMC2 (3). The chains are assembled in a coiled cruciate-like structure, which is deposited in the BM. LN5 is specific to epithelium and is one of the pivotal hemidesmosomal proteins involved in the structural...
relationship between the epithelium and stroma. In addition to α6β4, it can bind to α5β1 and α6β1 integrins.

Multiple mechanisms of gene silencing including loss of heterozygosity, point mutations, homozygous deletions, and aberrant promoter methylation have been reported in tumors (4). Aberrant methylation of CpG-rich sites (CpG islands) was identified as an epigenetic mechanism for the transcriptional silencing of tumor suppressor genes in many cancer types, and the number of methylated genes in individual cancers is estimated to be very high (5–7). Down-regulation of LNS chains and hemidesmosomal component proteins has been reported in breast (8, 9), prostate (10), lung (11), colon (12), and other types of cancers. To investigate whether LN5-encoding genes are silenced by epigenetic phenomenon in lung cancers, we studied the methylation status of promoters of the three genes in lung cancer cell lines, tumors, and nonmalignant tissues. We report here that LAMA3, LAMB3, and LAMC2 genes are transcriptionally silenced by aberrant methylation of their respective promoters and that there is a high degree of concordance between loss of expression and methylation.

**MATERIALS AND METHODS**

**Cell Lines.** Human lung cancer cell lines (20 NSCLC lines and 29 SCLC lines) and B-lymphoblastoid cell lines (n = 2) were established by us (13, 14). Most NSCLC lines were established from primary tumors, and most SCLC lines were established from metastases. Cell cultures were grown in RPMI 1640 (Life Technologies, Inc., Rockville, MD) supplemented with 5% fetal bovine serum and incubated in 5% CO2 at 37°C.

**Clinical Samples.** Tumor samples from 36 primary NSCLCs (19 adenocarcinomas, 15 squamous cell carcinomas, 1 large cell carcinoma, and 1 adenosquamous carcinoma) and 21 corresponding nonmalignant lung tissues were obtained from surgical resections performed at the University of Texas Southwestern Medical Center and M. D. Anderson Cancer Center (Houston, TX). Tumor samples from 26 primary SCLCs and 24 bronchial carcinoids were obtained from surgical resections performed in the United States, France, and Japan. For gene expression studies, six nonmalignant tissue samples (four bronchial brushes, one airway epithelial cell culture, and two peripheral lung tissues) were obtained as far from the tumor tissue as possible. Epithelial cells from buccal swabs of 12 healthy non-smoking volunteers and peripheral blood lymphocytes from 14 healthy volunteers were also obtained. Appropriate institutional review board permission was obtained at participating centers, and written informed consent was obtained from all subjects. Tissues were stored at −80°C before testing.

**Expression of LAMA3, LAMB3, and LAMC2.** Expression of LN5-encoding genes was analyzed by the RT-PCR technique. Total RNA was extracted from 49 lung cancer cell lines, 4 bronchial brushes, 1 airway epithelial cell culture, and 2 peripheral lung tissues by using TRIzol reagent (Life Technologies, Inc.) following the manufacturer’s instructions. Two μg of total RNA treated with DNase I (1 unit; Life Technologies, Inc.) were reverse transcribed into cDNA using SuperScript II First-Strand Synthesis System (Life Technologies, Inc.) at 42°C for 52 min using oligo(dT) primer according to the manufacturer’s instructions. The resulting cDNA was subjected to PCR using primers and temperature conditions as described in Fig. 2. For each primer set, the sense and antisense primer pairs were located on different exons to avoid amplification of contaminating genomic DNA. The housekeeping gene β-actin was used as an internal control to confirm the success of the RT-PCR (15). PCR products were analyzed on 2% agarose gels stained with ethidium bromide.

**5-Aza-CdR Treatment.** Six tumor cell lines with loss of expression for one or more of the three genes were treated with the demethylating agent 5-aza-CdR (2 μg/ml) as described previously (16, 17).

**DNA Extraction.** Genomic DNA was extracted from cell lines, primary tumors, and nonmalignant cells by digestion with proteinase K (Life Technologies, Inc.) for 1 day at 50°C, followed by two extractions with phenol:chloroform (1:1; Ref. 18).

**Bisulfite Genomic Sequencing.** DNA was modified by sodium bisulfite as described previously (19). Modified DNA was stored at −80°C until use. Treatment of genomic DNA with sodium bisulfite converts unmethylated cytosines (but not methylated cytosines) to uracil, which is then converted to thymidine during subsequent PCR (19). To determine the methylation status of CpGs in the promoter regions of LAMA3, LAMB3, and LAMC2, primers specific to non-CpG regions were designed to perform PCR (see Fig. 1, A–C). The sequence information of cDNA (LAMA3, NM_000227; LAMB3, NM_000228; and LAMC2, NM_005562) and promoter region DNA was obtained from the University of California Santa Cruz genome browser. Using 2 μl of resuspended sodium bisulfite-treated DNA (from RT-PCR-positive and RT-PCR-negative cell lines; Fig. 1, A–C), PCR was performed in a 25-μl reaction using Hot star enzyme (Qiagen, Valencia, CA) as described in Fig. 1. PCR products were visualized on 2% agarose gels stained with ethidium bromide. The PCR products were gel purified, ethanol precipitated, and sequenced by the Applied Biosystems PRISM dye terminator cycle sequencing method from both ends by using the same primers used for amplification.

**MSP.** The MSP reaction uses an initial bisulfite reaction to modify the DNA (19) as described above. After bisulfite treatment, alleles that were originally methylated have DNA sequences different from those of their corresponding unmethylated alleles, and these differences can be used to design PCR primers that are specific for methylated or unmethylated alleles. Based on the bisulfite genomic sequencing information on methylation status of CpGs in the promoter regions of LAMA3, LAMB3, and LAMC2 (Fig. 1, A–C), we designed two sets of methylation-specific (i.e., unmodified by bisulfite treatment) and unmethylation-specific primers (i.e., modified by bisulfite to UpG) to amplify each region of interest. The primer sequences and temperature conditions are described in Fig. 2. p16 unmethylated primer was used as control in MSP to check the integrity of tissue-extracted bisulfite-treated DNA (19). DNA from peripheral blood lymphocytes (n = 14) and buccal swabs (n = 12) from healthy nonsmoking subjects was used as negative control for MSP assays. DNA from lymphocytes of healthy non-malignant donors was used as positive control for MSP assays.

Nucleotide sequencing of the 5' flanking regions of the genes LAMA3, LAMB3, and LAMC2 after sodium bisulfite treatment of genomic DNA of RT-PCR-positive (RT+) and RT-PCR-negative (RT-) lung and breast (HCC1143, HCC712, and HCC2157) cancer cell lines. **MSP** indicates the genomic DNA region sequenced. Numbers below the horizontal line are the positions (in bp) of the genomic DNA. Vertical lines indicate positions of CpG sites. **Open circles** indicate unmethylated CpGs, and **filled circles** indicate methylated CpGs. **MSP-F**, sense methylation-specific primer; **MSP-R**, antisense methylation-specific primer. A. **LAMA3**. There are 25 CpGs between the −1000 and +138 bp region in reference to translation start site (+1 ATG). Primers were used to amplify the bisulfite-treated DNA for sequencing [5'-GGTTGAATTTTTAGTTTATAGGTTT-3' (sense; −450 to −423 from ATG) and 5'-CTCTCAATCCACCCATTTA-3' (antisense; +244 to +222)]. Reactions were hot started at 95°C for 10 min. Temperature conditions for PCR were as follows: 35 cycles of 95°C for 30 s, 59°C for 30 s, and 72°C for 1 min; followed by 1 cycle of 72°C for 5 min. The O/E ratio of CpG was 0.5, and %G+C was 62 (20). B. **LAMB3**. There are 10 CpGs between −91 to +4 bp region with reference to transcription start site (+1 TSS). Primers were used to amplify the bisulfite-treated DNA for sequencing [5'-TTTTGGATTTTTATGTTGT-3' (sense; −297 to −273 from TSS) and 5'-CAAAACCAAAACATCCAAA-3' (antisense; +121 to +101)]. Reactions were hot started at 95°C for 10 min. Temperature conditions for PCR were as follows: 35 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 1 min; followed by 1 cycle of 72°C for 5 min. The O/E ratio of CpG was 0.5, and %G+C was 62 (20). C. **LAMC2**. There are 36 CpGs between the −1018 and +76 bp region with reference to translation start site (+1 ATG). Primers were used to amplify the bisulfite-treated DNA for sequencing [5'-TTAGTTAATATTTAGTTTATAGGTTT-3' (sense; −682 to −659 from ATG) and 5'-CACCCTCCCACTTACCCAATTTATTTT-3' (antisense; +82 to +62)]. Reactions were hot started at 95°C for 10 min. Temperature conditions for PCR were as follows: 35 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 1 min; followed by 1 cycle of 72°C for 5 min. The O/E ratio of CpG was 0.6, and %G+C was 62.

**Fig. 1** Nucleotide sequencing of the 5' region of LN5-encoding genes LAMA3, LAMB3, and LAMC2 after sodium bisulfite treatment of genomic DNA of RT-PCR-positive (RT+) and RT-PCR-negative (RT-) lung and breast (HCC1143, HCC712, and HCC2157) cancer cell lines. **Horizontal line** indicates the genomic DNA region sequenced. **Numbers below the horizontal line** are the positions (in bp) of the genomic DNA. **Vertical lines** indicate positions of CpG sites. **Open circles** indicate unmethylated CpGs, and **filled circles** indicate methylated CpGs. **MSP-F**, sense methylation-specific primer; **MSP-R**, antisense methylation-specific primer. A. **LAMA3**. There are 25 CpGs between the −1000 and +138 bp region in reference to translation start site (+1 ATG). Primers were used to amplify the bisulfite-treated DNA for sequencing [5'-GGTTGAATTTTTAGTTTATAGGTTT-3' (sense; −450 to −423 from ATG) and 5'-CTCTCAATCCACCCATTTA-3' (antisense; +244 to +222)]. Reactions were hot started at 95°C for 10 min. Temperature conditions for PCR were as follows: 35 cycles of 95°C for 30 s, 59°C for 30 s, and 72°C for 1 min; followed by 1 cycle of 72°C for 5 min. The O/E ratio of CpG was 0.5, and %G+C was 62 (20). B. **LAMB3**. There are 10 CpGs between −91 to +4 bp region with reference to transcription start site (+1 TSS). Primers were used to amplify the bisulfite-treated DNA for sequencing [5'-TTTTGGATTTTTATGTTGT-3' (sense; −297 to −273 from TSS) and 5'-CAAAACCAAAACATCCAAA-3' (antisense; +121 to +101)]. Reactions were hot started at 95°C for 10 min. Temperature conditions for PCR were as follows: 35 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 1 min; followed by 1 cycle of 72°C for 5 min. The O/E ratio of CpG was 0.5, and %G+C was 62 (20). C. **LAMC2**. There are 36 CpGs between the −1018 and +76 bp region with reference to translation start site (+1 ATG). Primers were used to amplify the bisulfite-treated DNA for sequencing [5'-TTAGTTAATATTTAGTTTATAGGTTT-3' (sense; −682 to −659 from ATG) and 5'-CACCCTCCCACTTACCCAATTTATTTT-3' (antisense; +82 to +62)]. Reactions were hot started at 95°C for 10 min. Temperature conditions for PCR were as follows: 35 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 1 min; followed by 1 cycle of 72°C for 5 min. The O/E ratio of CpG was 0.6, and %G+C was 62.

**RESULTS**

Expression of LN5-encoding Genes LAMA3, LAMB3, and LAMC2 in Lung Cancer Cell Lines and Nonmalignant Tissues. RT-PCR analysis revealed expression of LN5 genes in all control tissues (samples of bronchial brushes, airway epithelial cells, and peripheral lung tissues). However, expression was lost in 12 of 20 (60%), 4 of 20 (20%), and 5 of 20 (25%) NSCLC cell lines and in 25 of 29 (86%), 20 of 29 (69%), and 19 of 29 (65%) SCLC cell lines for LAMA3, LAMB3, and LAMC2 genes, respectively (Fig. 2, A–C). The differences in loss of expression between NSCLC and SCLC cell lines for all three of the genes were compared using χ² test, and they were statistically significant (P = 0.034 for LAMA3, P = 0.001 for LAMB3, and P = 0.002 for LAMC2). In NSCLC cell lines, 13 of 20 (65%) lost at least one of the three chains, whereas in SCLC cell lines, 26 of 29 (90%) lost at least one of the three chains (Fig. 3A). The above differences in chain loss between NSCLC and SCLC cell lines were statistically significant (P = 0.04). Mean chain loss in SCLC cell lines (mean ± SE, 2.24 ± 0.190) was statistically significant (P = 0.004) from mean chain loss in NSCLC cell lines (mean ± SE, 1.05 ± 0.223). All six cell lines tested (two for each gene) with loss of gene expression had expression restored after treatment with the demethylating agent 5-Aza-CdR (Fig. 3B).

Aberrant Promoter Methylation of LAMA3, LAMB3, and LAMC2 Genes in Lung Cancer Cell Lines, Lymphocytes, and Buccal Swabs. To examine the methylation patterns of the 5' region of LNS5 genes, we designed methylation-independent primers (to non-CpG sites) for each gene separately and did bisulfite genomic sequencing of DNA from RT-PCR-

Downloaded from clincancerres.aacrjournals.org on April 12, 2017. © 2003 American Association for Cancer Research.
negative and RT-PCR-positive cell lines as described in "Materials and Methods." The standard CpG value for a CpG island was \( \pm 0.6 \) (the O/E ratio and \%G+C was \( \pm 0.2 \)). LAMA3 and LAMB3 partially satisfied the standard CpG criteria, whereas LAMC2 fully satisfied the criteria (Fig. 1, A–C). Upon sequencing, we noted the CpG sites specifically methylated in RT-PCR-negative cell lines (but not in RT-PCR-positive cell lines) and then designed methylation-specific and unmethylation-specific primers to perform MSP for each gene (Fig. 1, A–C). Results of aberrant promoter methylation of LAMA3, LAMB3, and LAMC2 genes in NSCLC and SCLC cell lines are detailed in Table 1, and representative examples are illustrated in Fig. 2. The differences in methylation and MI between NSCLC and SCLC cell lines were statistically significant for LAMC2 (\( P = 0.0098 \)), LAMB3 (\( P = 0.0001 \)), and MI (\( P = 0.0004 \)). Both unmethylated and methylated bands were present in some cell lines at low frequencies (LAMA3, 2 of 40; LAMB3, 1 of 40, and LAMC2, 3 of 40). Aberrant methylation was present at very low frequency in peripheral blood lymphocytes and was completely absent in buccal swabs from healthy volunteers (Table 1). In NSCLC cell lines, 12 of 20 (60%) had at least one of the three chains methylated, whereas in SCLC cell lines, 19 of 20 (95%) had at least one of the three chains methylated (Fig. 3C). The above differences in the number of chains methylated between NSCLC cell lines and SCLC cell lines were statistically significant (\( P = 0.01 \)). Mean chain MI in SCLC cell lines (mean \( \pm \) SE, 2.25 \( \pm \) 0.204) was statistically significant (\( P = 0.0002 \)) from mean chain MI in NSCLC cell lines (mean \( \pm \) SE, 0.95 \( \pm \) 0.223). The concordance between expression and methylation in NSCLC and SCLC cell lines, respectively, for LAMA3 (100%, \( P < 0.0001 \)), LAMB3 (95%, \( P = 0.0002 \); 95%, \( P = 0.0004 \)), and LAMC2 (95%, \( P = 0.028 \); 85%, \( P = 0.035 \)) was statistically significant. The overall concordance between expression and methylation in lung cancer cell lines for LAMA3 (95%, \( P < 0.0001 \)), LAMB3 (95%, \( P < 0.0001 \)), and LAMC2 (90%, \( P = 0.0002 \)) was statistically significant.

**Aberrant Promoter Methylation of LAMA3, LAMB3, and LAMC2 Genes in Lung Tumors and Nonmalignant Tissue.**

We further studied the methylation patterns of LN5-encoding genes in NSCLC tumors, SCLC tumors, and carcinoids. The results are presented in Table 1, and representative
DISCUSSION

Metastases are the major cause of cancer deaths. To metastasize and grow, neoplastic cells must invade and migrate into surrounding tissues. The ability to block these processes offers a new approach to treating and perhaps preventing invasive cancers. The roles of the components of BM in tumorigenesis are complex (21). The reported role of LN5 in tumorigenesis shows apparent dichotomy, in part because immunostaining techniques do not examine all three gene products. In breast cancer, a down-regulation of LN5 mRNA (for all three genes) has been observed, and a role for LN5 in controlling tumor growth has therefore been proposed (9).

To understand the role of LN5-encoding genes in lung cancers, we examined the expression of LAMA3, LAMB3, and LAMC2. All of the three genes were expressed in bronchial cells, cultured airway epithelial cells, and lung tissues, whereas lung cancer cell lines had varying frequencies of losses of all
Table 1  Frequency of methylation of promoters of LN5-encoding genes in lung cancer cell lines, tumors, and control tissues

<table>
<thead>
<tr>
<th>Samples</th>
<th>Sample size (n)</th>
<th>LAMA3 (18q11.2)</th>
<th>LAMB3 (1q32)</th>
<th>LAMC2 (1q25–q31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSCLC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell lines</td>
<td>20</td>
<td>12 (60)</td>
<td>3 (15)</td>
<td>5 (25)</td>
</tr>
<tr>
<td>Primary tumors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>19</td>
<td>11 (58)</td>
<td>6 (32)</td>
<td>6 (32)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>15</td>
<td>4 (27)</td>
<td>3 (20)</td>
<td>2 (13)</td>
</tr>
<tr>
<td>Total tumors</td>
<td>36</td>
<td>15 (42)</td>
<td>9 (25)</td>
<td>8 (22)</td>
</tr>
<tr>
<td>Total NSCLC samples</td>
<td>56</td>
<td>27 (48)</td>
<td>12 (21)</td>
<td>13 (23)</td>
</tr>
<tr>
<td>SCLC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cell lines</td>
<td>20</td>
<td>16 (80)</td>
<td>17 (85)</td>
<td>12 (60)</td>
</tr>
<tr>
<td>Primary tumors</td>
<td>26</td>
<td>17 (65)</td>
<td>20 (77)</td>
<td>15 (58)</td>
</tr>
<tr>
<td>Total SCLC samples</td>
<td>46</td>
<td>33 (72)</td>
<td>37 (80)</td>
<td>27 (59)</td>
</tr>
<tr>
<td>Carcinoids</td>
<td>24</td>
<td>4 (17)</td>
<td>8 (33)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Total lung cancer samples</td>
<td>126</td>
<td>67 (53)</td>
<td>58 (46)</td>
<td>43 (34)</td>
</tr>
<tr>
<td>Nonmalignant tissues</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood lymphocytes</td>
<td>14</td>
<td>1 (7)</td>
<td>1 (7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Buccal swabs</td>
<td>12</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Nonmalignant peripheral lung</td>
<td>21</td>
<td>2 (9.5)</td>
<td>2 (9.5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Total nonmalignant samples</td>
<td>47</td>
<td>3 (6.4)</td>
<td>3 (6.4)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Note: The differences in methylation and MI between two groups that were significant and the concordances between expression and methylation in lung cancer cell lines are presented in text in “Results.”

Theoretically, loss of any of the five component chains of this laminin-integrin complex could disrupt the hemidesmosome and lead to invasion (27–30). LN5 can be down- or up-regulated, depending on specific microenvironmental features, whereas its absence could favor disassembly or reduction in the number of hemidesmosomes with a consequent failure of cell anchoring leading to an invasive and metastatic phenotype. Thus, whereas loss of any chain results in loss of the functional molecule, unopposed expression of one or more chains (especially C2) may aid invasion (3). The loss of LN5 may cause perturbations of the ECM and integrin signaling, affecting growth factors and cell cycle regulators and apoptosis. As postulated, these gene expression changes may have potential genetic instability in lung carcinoma due to loss of an ECM protein (31). Because LN5 is a component of BM, which is a major barrier for invasion of cancer, we presume that methylation of LN5 genes may help distinguish invasive from noninvasive cancers. We have found that methylation of LN5-encoding genes distinguishes invasive from noninvasive bladder cancers.3

Our data satisfy the criteria required for the demonstration of biological significance of methylation (24, 32); (a) aberrant methylation is frequent in the tumor type studied; (b) methylation is a rare event in nonmalignant and control tissues; (c) loss of expression is frequent in tumors; (d) aberrant methylation and expression are concordant; and (e) gene expression is restored after exposure to a demethylating agent. Our results strongly suggest that silencing of LAMA3, LAMB3, and LAMC2 genes by
methylolation plays an important role in pathogenesis of all types of lung cancers.

Higher frequencies of loss of LN5 chains have been observed in SCLC, as compared with NSCLC (11). Our results confirm and extend these findings and demonstrate the mechanism of loss of the LN5 chains. Whereas promoter methylation of LN5-encoding genes appears to be an important factor in the pathogenesis of invasive lung cancers, alternative mechanisms for disruption of the BM may exist. These include inactivation of LN5-encoding genes by mechanisms other than methylation, loss of LN5-encoding genes, or overexpression of matrix metalloproteinase 9 (33). Of interest, selective loss of integrin β4 has been reported in SCLC (34, 35). Our findings are of biological and potential clinical importance.

REFERENCES


Epigenetic Inactivation of Laminin-5-encoding Genes in Lung Cancers
