Frequent Inactivation of RAMP2, EFEMP1 and Dutt1 in Lung Cancer by Promoter Hypermethylation

Wen Yue,1 Sanja Dacic,2 Quanhong Sun,2 Rodney Landreneau,3 Mingzhou Guo,4 Wei Zhou,5 Jill M. Siegfried,1 Jian Yu,2 and Lin Zhang1

Abstract
Purpose: The goal of this study is to identify novel genes frequently silenced by promoter hypermethylation in lung cancer.

Experimental Designs: Bioinformatic analysis was done to identify candidate genes significantly down-regulated in lung cancer. The effects of DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine on the expression of the candidate genes were determined. Methylation-specific PCR was developed and used to analyze DNA methylation in cell lines and clinical specimen. Pathologic and functional analyses were done to study the role of one candidate gene, receptor activity-modifying protein 2 (RAMP2), in suppressing lung cancer cell growth.

Results: Among 54 candidate genes down-regulated in lung cancer, 31 were found to contain CpG islands in their promoters. Six of these 31 genes could be reactivated by 5-aza-2′-deoxycytidine in at least four of six lung cancer cell lines analyzed. Promoter hypermethylation of RAMP2, epidermal growth factor–containing fibulin-like extracellular matrix protein 1, and deleted in U Twenty Twenty cells was detected in 36% to 77% of 22 lung cancer cell lines and in 38% to 50% of 32 primary lung tumors, whereas hypermethylation of these genes was rarely found in the matched normal samples. The methylation frequencies of these genes in lung cancer were similar to those of commonly used methylation markers, such as RAS association domain family protein 1A, p16, and methylguanine-DNA methyltransferase. Immunohistochemistry showed that RAMP2 was down-regulated in a majority of lung tumors, and RAMP2 down-regulation was correlated with high tumor grade. Ectopic expression of RAMP2 inhibited lung cancer cell growth and caused apoptotic cell death. Knockdown of RAMP2 by RNA interference stimulated cell proliferation.

Conclusions: Studying the newly identified genes may provide new insight into lung tumorigenesis. These genes might be useful as molecular markers of lung cancer.

Lung cancer, which accounts for 12.3% of all cancers, is the most common form of cancer in the world (1). Lung cancer development is a multistage process involving activation of oncogenes and inactivation of tumor suppressor genes (2, 3). Epigenetic changes, especially hypermethylation of CpG islands in gene promoters, have recently emerged as an important mechanism for tumor suppressor gene silencing (4, 5). Promoter hypermethylation has been detected in lung cancer for a number of genes, such as p16, RAS association domain family protein 1A (RASSF1A), and methylguanine-DNA methyltransferase (MGMT; refs. 6–8). Further studies of the genes silenced by promoter hypermethylation in lung cancer would provide invaluable insight into the mechanisms underlying lung tumorigenesis.

Lung cancer is also the leading cause of cancer-related death in the United States (1). When detected, lung tumors are often in late stages and refractory to conventional anticancer therapies. The mortality of lung cancer could be greatly reduced through detection of the disease at the earliest stages. Promoter hypermethylation has been used as a molecular marker for...
Inactivation of RAMP2, EFEMP1 and Dutt1 in Lung Cancer

Table 1. Primers for MSP

<table>
<thead>
<tr>
<th>Genes</th>
<th>Priming site</th>
<th>Forward primer (5’ to 3’)</th>
<th>Reverse primer (5’ to 3’)</th>
<th>PCR product size (bp)</th>
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<tbody>
<tr>
<td>RAMP2</td>
<td>Genomic</td>
<td>AGGCCCCCTCCGAAGAAGCCCAGCCG</td>
<td>CGGGGCCGCGCGCCGCTCACCAGG</td>
<td>146</td>
</tr>
<tr>
<td></td>
<td>(M)</td>
<td>AAAGAGATGGATGGAGAAGGAGGAG</td>
<td>AAGGGCAGCGGCGCTCACCAGG</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(U)</td>
<td>AGGCTTCTCAGGCGGAGAGGAGAGG</td>
<td>CAAACCAACACACACACCTCACCCCA</td>
<td>148</td>
</tr>
<tr>
<td>EFEMP1</td>
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<td>AGGCTTCTCAGGCGGAGAGGAGAGG</td>
<td>TCCCCGACAGCTACCTCG</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>(M)</td>
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<td>TCCCCCAACACTACCTCA</td>
<td>162</td>
</tr>
<tr>
<td></td>
<td>(U)</td>
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<td>TCCCCCAACACTACCTCA</td>
<td>162</td>
</tr>
<tr>
<td>Dutt1</td>
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<td>GTAAATGTAGGAGGCTCGGGC</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>(M)</td>
<td>AAGCATTGCGGAAAGTGCAGCAG</td>
<td>ATAAAAATAAACGACTACG</td>
<td>196</td>
</tr>
<tr>
<td></td>
<td>(U)</td>
<td>AAGGTGTTGGAAAGGTTTATGAT</td>
<td>ATAAAAATAAACGACTACG</td>
<td>196</td>
</tr>
</tbody>
</table>

NOTE: M, methylated sequence; U, unmethylated sequence.

detection of lung cancer (9, 10). Hypermethylation of several genes, such as p16 and RASSF1A, can be found in tumors, smoking-damaged normal lung, sputum, and blood from lung cancer patients (11–15). However, additional methylation markers are necessary to achieve desirable sensitivity and specificity for lung cancer detection (9, 10).

In this study, we attempted to identify novel genes that are frequently inactivated in lung cancer through promoter hypermethylation. Genes significantly down-regulated in lung cancer were first identified through bioinformatic analysis. Those that contain CpG islands in their promoters and could be reactivated by the DNA methyltransferase inhibitor 5-aza-2’-deoxycytidine were further studied. Methylation-specific PCR (MSP) was developed and used to investigate their methylation status in lung cancer cell lines, primary lung tumors, and their matched normal tissues. As the result, several novel genes frequently silenced by promoter hypermethylation in lung cancer were identified, including receptor activity-modifying protein 2 (RAMP2), epidermal growth factor–containing fibulin-like extracellular matrix protein 1 (EFEMP1), and deleted in U Twenty Twenty cells (Dutt1). Pathologic and functional studies were done to determine the role of RAMP2 in suppressing lung cell growth. Further elucidation of the functions of these genes may lead to better understanding of lung tumorigenesis. These genes are potentially useful as molecular markers of lung cancer.

Materials and Methods

Bioinformatic analysis. SAGE databases7 at the National Center for Biotechnology Information were used to identify candidate genes that are down-regulated in lung cancer. A pool of three SAGE libraries from lung cancer (total, 159,128 tags) was compared with a pool of three libraries from normal lung tissues (total, 159,917 tags) using Digital Gene Expression Displayer program. A total of 347 genes down-regulated by at least 2-fold (P < 0.05) in lung cancer were further analyzed using the National Center for Biotechnology Information Expressed Sequence Tag databases (169,722 clones from 20 lung cancer libraries; 102,337 clones from 23 normal lung libraries). Three criteria were applied: (a) significantly down-regulated (P = 0) in lung cancer by SAGE, (b) not significantly up-regulated by Expressed Sequence Tag (P < 0.01), and (c) not significantly down-regulated in three other types of cancer by SAGE (P > 0.05). Therefore, genes with contradictory expression patterns in SAGE and Expressed Sequence Tag databases and those ubiquitously down-regulated in different types of cancer were excluded. In addition, seven genes reported to be down-regulated in lung cancer were included. The expression levels (copies per cell) of the candidate genes (Supplementary Table S1) were normalized based on the estimation that there are ~300,000 transcripts in one cell (16).

To identify CpG islands in the promoters of the candidate genes, 1 to 2 kb DNA sequence 5’ to the translation initiation site of each gene were analyzed using CpG Island Searcher8 and CpG Island Plot9 programs. The criteria for CpG island were (a) % GC > 55%, (b) observed CpG/expected CpG > 0.65, and (c) length > 500 bp.

Cell culture. The lung cancer cell lines used in the study were from American Type Culture Collection, except for 273T and 201T which were from the University of Pittsburgh Cancer Institute lung cancer program. Cells were maintained at 37°C and 5% CO2 in RPMI 1640 (Mediatech) supplemented with 10% defined fetal bovine serum (HyClone), 100 units/mL penicillin, and 100 μg/mL streptomycin (Invitrogen). For demethylation, cells were treated with 5 μmol/L 5-aza-2’-deoxycytidine (Sigma) for 6 days. Fresh medium was added after days 1, 2, and 3.

Western blotting. Cell lysates were collected, and Western blotting was done as previously described (17). The antibodies for Western blotting included rabbit antibodies against RAMP2 (Santa Cruz Biotechnology), caspase-3 (Stressgen Bioreagents), caspase-9 (Cell Signaling Technology), and monoclonal antibodies against V5 (Invitrogen) and o-tubulin (BD Biosciences).

Reverse transcriptase-PCR. Total RNA was isolated from cells using the RNAgent Total RNA Isolation System (Promega). First-strand cDNA was synthesized from 10 μg of total RNA using Superscript II reverse transcriptase (Invitrogen). Reverse transcriptase-PCR was done to amplify the candidate genes using the touchdown PCR conditions previously described (18). Primers for reverse transcriptase-PCR were listed in Supplementary Table S2.

Isolation of genomic DNA and bisulfite modification. Genomic DNA was isolated from lung cancer cell lines and tissues using QIAamp DNA Blood minikit and QIAamp DNA minikit (Qiagen), respectively. A mixture of 0.25 μg of genomic DNA along with 1.0 μg of carrier salmon sperm DNA (Promega) was used for bisulfite modification. DNA from lung cancer cell lines was modified using the previously described method (19). DNA from tissues was modified using the EZ DNA Methylation Gold kit (ZYMO Research) according to the manufacturer’s protocol. The modified DNA was ethanol precipitated and dissolved into 40 μL of distilled water.

Bisulfite sequencing and MSP. PCR was done in 20 μL of final volume using one-twentieth (2 μL) of bisulfite-modified DNA and 2 units of Platinum Taq DNA polymerase (Invitrogen). The cycle conditions included 35 to 40 cycles of 95°C for 20 s, 55°C for 30 s, and

6 http://cgap.nci.nih.gov/SAGE
7 http://cpgislands.usc.edu
8 http://www.ebi.ac.uk/emboss/cpgplot

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72°C for 90 s. For bisulfite sequencing, 400 to 800 bp fragments were amplified using the primers described in Supplementary Table S3. PCR products were purified and sequenced using the same primers for PCR as previously described (20). If a T peak was 50% or higher compared with a C peak at the same position, the base was considered to be partially methylated. For MSP, 50-bp to 200-bp fragments were amplified from bisulfite-modified DNA using the primers listed in Table 1 and the previously described primers for RASSF1A, p16, and MGMT (15, 19, 21). The PCR products were analyzed by electrophoresis on 2% agarose gels.

**Tissue samples.** The acquisition of the tissues was approved by the Institutional Review Board at the University of Pittsburgh. Frozen specimens, including 32 randomly selected nonsmall cell lung tumors and their matched histologically normal lung parenchyma adjacent to the tumors (within 1 cm of the discrete tumor margin) and the normal lung parenchyma distal to the tumors (at least 4 cm away from the tumors), were obtained from the University of Pittsburgh Cancer Institute lung cancer program. The clinicopathologic characteristics of the patients were summarized in Supplementary Table S4. Tissue microarray slides containing 95 cores of histologically confirmed

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**Fig. 1.** Identification of candidate genes. A, strategies for identifying novel genes silenced by promoter hypermethylation in lung cancer. B, Virtual Northern and Anatomic View analyses were used to compare the expression of the candidate genes in different tumor and normal tissues. As an example, RAMP2 was found to be specifically down-regulated in lung cancer. C, the expression of the candidate genes in six lung cancer cell lines with or without 5-aza-2'-deoxycytidine treatment was determined by reverse transcriptase-PCR. RASSF1A and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were used as controls. MW, molecular weight marker. D, RAMP2 protein expression was analyzed by Western blotting in the indicated cell lines with or without 5-aza-2'-deoxycytidine treatment.
nonsmall cell lung carcinomas and 49 cores of normal lung samples were purchased from US Biomax. These samples included 32 matched tumor/normal pairs and unmatched specimen (Supplementary Table S5).

**RAMP2 immunohistochemistry.** The sections were deparaffinized by xylene, rehydrated in decreasing concentrations of ethanol (100% twice followed by once each of 95% and 70%), and boiled twice with each for 5 min in 0.1 mol/L citrate buffer antigen retrieval solution (pH 6.0). The slides were stained by rabbit anti-RAMP2 antibodies (Santa Cruz Biotechnology) and then biotinylated goat anti-rabbit antibodies (Vector Laboratories). The signals were detected using Vectastain Elite avidin-biotin complex method kit following the manufacturer’s instructions (Vector Laboratories). Hematoxlin was used for counter-staining.

The staining distribution was scored based on the percentage of positive cells: 0, 0%; 1, 1-30%; 2, 31-60%; 3, 61-100%. The signal intensity was scored using this criteria: 0, no signal; 1, weak; 2, moderate; and 3, marked. The staining was considered to be positive if the sum of distribution and intensity scores was ≥2.

**Transfection and RNA interference.** The expression construct for RAMP2 was generated by cloning a PCR-amplified full-length human RAMP2 cDNA fragment into pCDNA3.1/V5-His vector (Invitrogen). The inserts were verified by restriction digestion and DNA sequencing. A549 and H1299 cells were transfected with RAMP2 or the control empty pCDNA3.1 vector using Lipofectamine 2000 (Invitrogen). For analysis of apoptosis, cells were stained with Hoechst 33258 (Invitrogen) after transfection and assessed through microscopic visualization of condensed chromatin and micronucleation as previously described (22). Treatment with pan-caspase inhibitor z-VAD-fmk (20 μmol/L; R&D Systems) was initiated 4 h before RAMP2 transfection. For colony formation assays, cells were plated into six-well plates at different dilutions and selected by G418 (400 μg/mL; Invitrogen) for transfected cells for 11 to 14 days. Colonies were visualized by crystal violet staining as previously described (23).

Fig. 2. CpG methylation of the candidate genes in lung cancer cell lines. A, CpG site distribution in the promoter regions of the candidate genes. Bisulfite sequencing regions and positions of MSP primers were indicated. B, summary of the bisulfite sequencing results for the indicated genes in eight lung cancer cell lines and normal lymphocytes (NL). C, bisulfite-modified genomic DNA with and without promoter hypermethylation of the indicated genes were mixed at different ratios and analyzed by MSP (25 ng of total input DNA per reaction). The ratio of 1:1000 was equivalent to 5 to 10 copies of methylated DNA mixed with 25 ng of unmethylated DNA. D, MSP was used to determine the methylation status of the indicated genes in 22 lung cancer cell lines. Right, numbers of MSP positives. M, PCR products amplified using primers specific for methylated DNA; U, PCR products amplified using primers specific for unmethylated DNA. Normal lymphocytes and in vitro methylated DNA were negative and positive controls, respectively. MW, molecular weight marker.
For RAMP2 knockdown, H1752 cells were transfected with the ON-TARGETplus small interfering RNA specific for RAMP2 (J-003701-05; Dharmacon) or the control scrambled small interfering RNA by Lipofectamine 2000. After 36 h, cells were incubated with 10 μmol/L bromodeoxyuridine (Sigma) for 2 h, then fixed and permeabilized with cold methanol for 10 min. Bromodeoxyuridine incorporation was visualized using monoclonal antihuman bromodeoxyuridine Alexa-Fluor 594 antibody (Invitrogen) according to the manufacturer’s protocol.

Statistical analysis. Statistical analysis was done using GraphPad Prism IV software. P values of <0.05 were considered to be statistically significant. The means (±SD) were displayed in the figures.

Results

Identification of candidate genes. To identify genes that are silenced in lung cancer, we compared the global gene expression profiles in lung cancer and normal lung tissues using National Center for Biotechnology Information SAGE databases (24, 25). Total 347 genes down-regulated in lung cancer by at least 2-fold compared with normal lung tissues (P < 0.05) were identified after analyzing 159,128 transcripts from three lung adenocarcinomas and 159,917 transcripts from three normal lung specimens (Fig. 1A). Using the criteria described in the Materials and Methods, we identified 47 genes specifically down-regulated in lung cancer (1-47, Supplementary Table S1; Fig. 1B). Additional seven genes previously reported to be significantly down-regulated in lung cancer were also included for further analysis (48-54, Supplementary Table S1; refs. 3, 26–29).

Gene expression changes in lung cancer cells treated with 5-aza-2'-deoxycytidine. To identify genes that are silenced by promoter hypermethylation, we analyzed promoter region for each of the 54 candidate genes as described in the Materials and Methods. CpG islands could be identified in the promoters of 31 genes (Supplementary Table S2). We then determined whether the expression of these 31 genes could be restored in lung cancer cells by 5-aza-2'-deoxycytidine. Total RNA was isolated from six lung cancer cell lines with or without 5-aza-2'-deoxycytidine treatment for 6 days. Reverse transcriptase-PCR indicated that the expression of 6 of these 31 genes was significantly enhanced in at least four of six lung cancer cell lines after the treatment, whereas the rest were either unchanged or altered in no more than three cell lines (Fig. 1C). In comparison, the expression of the positive control RASSF1A was reactivated by 5-aza-2'-deoxycytidine in five of six cell lines analyzed (Fig. 1C). We also used Western blotting to determine the protein expression of RAMP2, for which specific antibodies were available. RAMP2 protein level was indeed elevated in response to 5-aza-2'-deoxycytidine treatment in

Fig. 3. Promoter hypermethylation of the candidate genes in lung tumors and matched normal tissues. A, MSP was used to determine the methylation status of three candidate genes, along with RASSF1A, p16, and MGMT, in 32 matched sets of samples (Supplementary Table S4), including primary lung tumors (Tumor), histologically normal lung tissues adjacent to the tumors (Adjacent normal), and normal lung tissues distal to the tumors (Distal normal). Representative results from three sets of samples. M, PCR products amplified using primers specific for methylated DNA; U, PCR products amplified using primers specific for unmethylated DNA. B, the methylation status in 32 matched sets of samples. Right, numbers of MSP positives of 32 samples. C, RAMP2 expression was analyzed by immunohistochemistry for MSP-positive tumors 16, 19, and 32 and their matched normal samples. Magnification, × 200.
Calu1, H1299, and A549 cells but not in H1752 cells (Fig. 1D), consistent with reverse transcriptase-PCR results.

**Identifying CpG island hypermethylation.** To determine whether the six candidate genes are inactivated in lung cancer by promoter hypermethylation, we determined their CpG island sequences using bisulfite-modified genomic DNA from eight lung cancer cell lines. DNA from normal lymphocytes was used as a control. For RAMP2, EFEMP1, tissue inhibitor of metalloproteinase 3, and Dutt1, the majority (70-100%) of the CpG sites in their promoters were either completely or partially methylated in several cell lines (Fig. 2A and B; Supplementary Fig. S1). The methylation patterns matched their expression changes in response to 5-aza-2′-deoxycytidine treatment (Fig. 1C), suggesting that promoter methylation is responsible for their down-regulation in lung cancer. No consistent methylation was detected for SH3BGRL3 and HES2, suggesting that different CpG sites or other mechanisms are responsible for their down-regulation in lung cancer. Among the identified genes, only tissue inhibitor of metalloproteinase 3 has previously been shown to be frequently down-regulated by promoter hypermethylation in human lung cancer (15).

To further study their CpG island methylation, we developed MSP assays for RAMP2, EFEMP1, and Dutt1. In vitro methylated DNA and normal lymphocytes were used as controls. Promoter hypermethylation of all three genes could be reliably detected by MSP, with PCR results matching bisulfite sequencing results (data not shown). We also optimized PCR primers and conditions so that MSPs for these three genes were as sensitive as those for analyzing p16, MGMT, and RASSF1A (Fig. 2C).

**Promoter hypermethylation in lung cancer cell lines and tumors.** Next, we used MSP to determine whether these three genes are frequently targeted by promoter hypermethylation in lung cancer cell lines. The results indicated that all three genes were methylated in a large fraction of 22 lung cancer cell lines analyzed, with methylation detected in 14 (63.6%) for RAMP2, 8 (36.4%) for EFEMP1, and 17 (77.3%) for Dutt1, respectively (Fig. 2D). In comparison, promoter hypermethylation of RASSF1A, p16, and MGMT was detected in 20 (90.9%), 11 (50.0%), and 11 (50.0%) of these cell lines, respectively (Fig. 2D).

MSP was then used to analyze their methylation status in tumor and normal tissues. Matched samples from 32 patients, including their lung tumors, histologically normal lung tissues adjacent to the tumors, and histologically normal lung tissues distal to the tumors, were examined (Supplementary Table S4). The representative data were shown in Fig. 3A. The results indicated that RAMP2 was methylated in 14 tumors (43.8%), 4 adjacent normal (12.5%), and 1 distal normal...
(3.2%) samples (Fig. 3B). Similarly, EFEMP1 and Dutt1 were methylated in a large fraction of lung tumors but rarely methylated in the adjacent or distal normal tissues (Fig. 3B). In comparison, methylation of RASSF1A, p16, and MGMT was detected in 14 (43.8%), 15 (46.9%), and 12 (37.5%) lung tumors, respectively, and in one to three normal samples (Fig. 3B). Therefore, the methylation frequencies of the newly identified genes in lung cancer were similar to those of commonly used methylation markers. Analysis of six representative MSP-positive tumors by bisulfite sequencing verified that over 90% of the CpG sites in the RAMP2 promoter were methylated in each tumor (data not shown). In all cases, if a methylation event was detected for a distal normal sample, it was also found in the matched adjacent normal and tumor tissues (Fig. 3B). Together, these results suggested that RAMP2, EFEMP1, and Dutt1 are frequently inactivated by promoter hypermethylation in lung cancer.

**Expression of RAMP2 in lung tumors.** To determine whether promoter hypermethylation leads to loss of gene expression, immunohistochemistry was used to examine the expression of RAMP2, the most consistently methylated gene we identified, in three representative tumors showing RAMP2 hypermethylation and their matched normal samples. In each case, strong RAMP2 staining was detected in the normal samples but not in the corresponding tumor tissues (Fig. 3C).

An additional panel of tumor and normal samples on a tissue microarray, including 95 non-small cell lung tumor and 49 normal samples, among which were 32 matched pairs, were analyzed by immunohistochemistry for RAMP2 expression. The representative results were shown in Fig. 4A and B. Among these samples, 89.8% (44 of 49) of normal lung specimen were positive for RAMP2 staining, whereas only 22.1% (21 of 95) of the tumor samples were positive for RAMP2 expression (Supplementary Table S5). The difference between the tumor and normal samples was highly significant ($P < 0.001$, Fisher exact test; Fig. 4C). Among the 32 matched pairs, 22 tumors completely lose RAMP2 expression compared with their matched normal samples (Fig. 4A; Supplementary Table S5). Furthermore, loss of RAMP2 expression was found to be correlated with tumor grade, with RAMP2 immunostaining detected in 58.3% (7 of 12) of grade 1 tumors but in only 26.5% (9 of 34) grade 2 and 10.4% (5 of 49) of grade 3/grade 4 tumors.

**RAMP2 suppressed lung cancer cell proliferation.** To determine whether promoter hypermethylation leads to loss of gene expression, immunohistochemistry was used to examine the expression of RAMP2, the most consistently methylated gene we identified, in three representative tumors showing RAMP2 hypermethylation and their matched normal samples. In each case, strong RAMP2 staining was detected in the normal samples but not in the corresponding tumor tissues (Fig. 3C).

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RAMP2 is a member of the calcitonin receptor–like receptor family of receptors. It is localized in 3p12, a frequently used methylation marker. RAMP2, EFEMP1, and Dutt1 are frequently altered region in lung cancer (3). Targeted disruption of Dutt1 in mice caused abnormalities in lung development and predisposed mice to lung adenocarcinomas and lymphomas (39, 40). Epigenetic alterations of Dutt1 in rat lung tumors have been reported (41). The high frequency of human Dutt1 hypermethylation in lung tumors suggested that it also functions as a tumor suppressor.

Promoter hypermethylation has been proposed to be useful as molecular markers for cancer detection (9, 10). Because hypermethylation of a single gene only occurs in a subset of tumors and, depending on tumor staging, tumor DNA is circulated in only a fraction of patients (42), it is critical to develop a panel of methylation markers for improvement of sensitivity and specificity. For example, a recent study showed that a combination of eight specific methylation markers allowed detection of renal cancer with good sensitivity and reasonable specificity (43). Future studies will address whether the genes identified in this study can be combined with commonly used methylation markers, such as RASSF1A, p16, and MGMT, for lung cancer detection.

Together, our studies identified several novel genes that are frequently silenced by promoter hypermethylation in lung cancer. Understanding the functions of these genes may provide new insight into pathogenesis of lung cancer. These genes might be useful as molecular markers of lung cancer.

Acknowledgments

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References

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