Frequent Epigenetic Inactivation of RASSFIA in Human Bladder Carcinoma

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Abstract

Allelic deletion or transcriptional silencing of RASSF1, a putative tumor suppressor at 3p21.3, has been found in a considerable proportion of lung, breast, and ovarian cancers. In this study, we analyzed the expression and mutation status of three RASSF1 isoforms (A, B, and C) in 55 primary bladder carcinoma tissues and 10 bladder and prostate cancer cell lines. The RASSFIA transcript was not detected in 80% (4 of 5) and 100% (4 of 4) of bladder and prostate cell lines, respectively. Compared with normal bladder tissues, loss or significant reduction of RASSFIA was identified in 62% (34 of 55) of primary bladder carcinomas and 10 (83%) of 12 matched sets showed tumor-specific alteration of RASSFIA expression. Moreover, loss or abnormal down-regulation of RASSFIA was correlated with advanced tumor stage. RASSFIB was undetectable in 60% (3 of 5) of bladder cell lines and in 31% (17 of 55) of primary tumors, but none of these tumors showed altered expression exclusively in RASSFIB. RASSFIC transcript was detected in all cell lines and primary tumors we examined. Expression of RASSFIA and RASSFIC was reactivated in all nonexpressor cell lines by treatment with the demethylating agent 5-aza-2-deoxycytidine. Bisulfite DNA sequencing analyses revealed that aberrant hypermethylation at the CpG island in the RASSFIA promoter is strongly associated with the loss of RASSFIA expression in cell lines and uncultured primary tumors. Methylation-specific PCR and BsoUI digestion analyses also demonstrated that 97% (33 of 34) of RASSFIA-nonexpressing primary tumors are methylated. Although somatic mutations were not identified in RASSF1 transcripts expressed in unmethylated tumors, 24% (9 of 37) of methylated cell lines and primary tumors showed detectable reductions in genomic levels of RASSFIA suggesting that RASSFIA inactivation might be caused by both epigenetic and genetic mechanisms in a subset of bladder tumors. Together, our data suggest that RASSFIA inactivation may play a critical role in the malignant progression of human bladder carcinomas.

Introduction

Allelic loss at chromosome 3p21 is one of the most frequent genetic changes found in various types of human cancers, including lung, breast, and bladder cancers (1–3). Recently, RASSFIA identified at 3p21.3 was suggested as the major target tumor suppressor on the basis of its frequent epigenetic silencing and LOH in lung cancers (4). RASSF1 encodes several isoforms, including RASSFIA, RASSFIB, and RASSFIC, which are derived from alternative mRNA splicing and promoter usage (4). The three major isoforms have four common exons (exons 3–6), which encode a Ras association domain (5). RASSFIA has two 5’ exons (1α and 2αβ) and is predicted to encode a M, 39,000 peptide (4). RASSF1A contains an NH₂-terminal cystein-rich diacylglycerol/phorbol ester binding domain, and its COOH terminus shows high homology to Ras effector Nore1/Maxp1 (6). RASSFIB contains exons 1β and 2αβ and most likely encodes only the Ras association domain. Transcription of RASSFIC initiates in exon 2γ, and its protein product lacks similarity to Norel or any other known protein. In addition to containing a Ras association domain, RASSFIA and RASSFIC have PEST sequences, and a serine residue within this region was identified as a putative phosphorylation target in vitro for ataxia-telangiectasia-mutation (7).

It was reported previously that RASSFIA is epigenetically inactivated in 40–72% of primary lung tumors by de novo methylation at the CpG island in the promoter (4, 8, 9). Methylation-associated inactivation of RASSFIA was also observed in a considerable proportion of breast, ovarian, and nasopharyngeal cancer cell lines and primary tumors (8–12). In small cell lung cancers, allelic deletion at 3p21.3 is associated with RASSFIA methylation, suggesting that both genetic and epigenetic steps are crucial for RASSFIA inactivation in some tumor types. The tumor suppressor function of RASSFIA has been suggested by observations that exogenous expression of RASSFIA decreases in vitro-colony formation, suppresses anchorage-independent growth, and dramatically reduces tumorigenicity in vivo (4, 8). With these tumor suppression effects, the presence of a Ras association domain suggests that RASSF1 proteins may function as effector molecules in Ras or related growth inhibitory signaling pathways.

Carcinoma of the bladder is one of the most common malignancies occurring worldwide and the fourth most frequent cause of cancer death in men in the United States (13, 14). In Korea, bladder cancer accounts for ~2.5% of all cancers, with a male:female ratio of 4:1, and it is the most common genitourinary tumor (an estimated 2500 new cases and 550 deaths each year), with an annual rise in overall incidence of 2% (Korea Central Cancer Registry, 2000). An increased risk for bladder cancer has been associated with smoking and with occupational or environmental exposures to chemical carcinogens such as benzidine and β-naphthylamine (15). Cyto genetic and LOH studies detected deletion of chromosome 3p in ~30% of bladder cancers (3, 16–18). Frequent allelic losses at two discrete regions, 3p12–14 and 3p21–23, have been identified, and they showed a correlation with higher tumor grade and more advanced stage, suggesting the presence of multiple tumor suppressor genes in these regions and their implication in the pathogenesis of bladder cancers (3, 18). Recently, the tumor suppressor gene FHIT was cloned at 3p14.2, and its highly frequent alterations were identified in bladder cancers (19).

In the present study, we investigated the expression and mutation status of RASSF1 located at 3p21.3 in a series of primary bladder tumors and tumor cell lines to explore the candidacy of RASSF1 as a suppressor in bladder carcinogenesis. Our data demonstrate that RASSFIA expression is lost or down-regulated in a majority of bladder cancer cell lines and primary tumors by aberrant promoter hypermethylation, suggesting that epigenetic inactivation of RASSFIA may play a critical role in bladder carcinogenesis.
Materials and Methods

Tissue Specimens and Human Cell Lines. Fifty-five primary bladder carcinomas and 15 normal bladder tissues were obtained from 55 bladder cancer patients and 15 noncancer patients by surgical resection in the Kyung Hee University Medical Center (Seoul, Korea). Tissue specimens were snap-frozen in liquid N2 and stored at −70°C until used. Tumors and adjacent tissues were sectioned for histological examination, and 12 of the adjacent tissues were found not to contain cancer cells. Six human bladder cancer cell lines (J82, T24, HT1197, HT1376, 253J, and 253J-BV) and four human prostate cancer cell lines (LNCaP, DU145, PC3, and TSU-Pr1) were obtained from American Type Culture Collection (Rockville, MD) or Korea Cell Line Bank (Seoul National University, Seoul, Korea). Extraction of total cellular RNA and synthesis of cDNA were performed as previously described (20). Genomic DNA was extracted from the same cells of the tissues from the DNA phase after RNA was extracted.

Quantitative PCR Analysis. Our PCR-based strategies used for quantitative analysis of expression and genomic levels of RASSF1 were previously described (21). Briefly, 1:4 diluted cDNA (12.5 ng/50 μl PCR reaction) undergoing 24–36 cycles was observed to be within the logarithmic phase of amplification and yielded reproducible results with primers RSF-3 (sense; 5’-TCTGGGGCTCTGGTGGCAAA-3’) and RSF-4 (antisense; 5’-GAACCTTTAGTGAAGGCTTGTTG-3’) for RASSF1A; 1B (sense; 5’-CTGGTTTCCAGACGCCCAAGT-3’) and RSF-4 (antisense; see above) for RASSF1B; 1C (sense; 5’-GGAGGCCGCCCTTCTGGAAAGA-3’) and RSF-4 (antisense; see above) for RASSF1C; and an endogenous gene standard gene GAPDH (22). PCR was done for 34 cycles at 95°C (1 min), at 58–62°C (0.5 min), and at 72°C (1 min) in 1.5 mM MgCl2-containing reaction buffer (PCR buffer II; Perkin-Elmer). Ten μl of RT-PCR products were resolved on 2% agarose gels. Quantitation of RASSF1 expression levels was achieved by densitometric scanning of the ethidium bromide-stained gels. Absolute area integrations of the curves representing each specimen were then compared after adjustment for GAPDH expression. For genomic PCR analysis, 200 ng of genomic DNA was used for amplification of the exon 3 region of RASSF1 with intron-specific primers RSF3 (sense; 5’-ATGTTATATATGACATCGGGA-3’) and RSF3AS (antisense; 5’-CAGGCCATACGTGGTACC-3’). Integration and analysis were performed using the Molecular Analyst software program (Bio-Rad, Hercules, CA).

Methylation Analysis. One μg of genomic DNA in a volume of 50 μl was denatured by NaOH (final concentration, 0.3 M). Thirty μl of 10 mM hydroquinone and 520 μl of 3 M sodium bisulfite (pH 5.0) were added and incubated at 55°C for 16–20 h. DNA samples were purified using the Wizard DNA clean-up system (Promega Corp., Madison, WI), treated again with NaOH at 37°C for 15 min, precipitated with ethanol, and resuspended in distilled water. Fifty ng of bisulfite-modified DNA was subjected to PCR amplification of the CpG island in the RASSF1A promoter using primers PS (5’-CATCCCTTCCACCATTTTCCAT-3’) and PAS (5’-TCTATTACTCTCTTTTTATTT-3’). The PCR products were cloned into pcRII vectors (Invitrogen, Carlsbad, CA), and 10 clones of each specimen were sequenced by automated fluorescence-based DNA sequencing to determine the methylation status. For methylation-specific PCR analysis, PCR was performed with methylation-specific primers MS-1 (sense; 5’-TTTTTTTCTTAGCGGATC-3’) and MS-2 (antisense, 5’-CGTTTTTGGCTTTTCTCGG-3’) and unmethylation-specific primers UMS-1 (sense, 5’-TCACCATTTTTTTTTTTTTTTTTTTTTT-3’) and UMS-4 (antisense, 5’-CCCTTTTTTTTTTTTTTTTCTTTT-3’) using 200 ng of the bisulfite-modified genomic DNA as templates for 38 cycles at 95°C for 1 min, at 60–63°C for 1 min, and at 72°C for 1 min. For BstUI digestion assay, the CpG island region spanning two BstUI sites was amplified by PCR with primers PS (sense; see above) and PAS (antisense; see above) using 200 ng of the bisulfite-modified DNA as templates. Fifteen μl of the PCR products were digested with 20 units of BstUI and resolved on a 2% agarose gel.

Nonisotopic RT-PCR-SSCP Analysis. Nonisotopic RT-PCR-SSCP analysis was performed as described previously (20). The isoform-specific regions (1a, 1B, 2α, and 2γ) and exons 3–6 of RASSF1 transcripts were amplified with 10 sets of primers. Sequences of the primers used for our PCR-SSCP analysis will be obtained upon request. Twenty μl of the PCR products mixed with 5 μl of 0.5 M NaOH, 10 mM EDTA, 10 μl of denaturing loading buffer (95% formamide, 20 mM EDTA, 0.05% bromphenol blue, and 0.05% xylene cyanol), and 15 μl of dH2O. After heating at 95°C for 5 min, samples were loaded in wells precooled to 4°C. SSCP was performed using 8% nondenaturing acrylamide gels containing 10% glycerol at 4–8°C or 18–22°C.

5-Aza-2'-deoxycytidine Treatment. To assess reactivation of RASSF1 expression, six bladder and four prostate cancer cell lines were plated in six-well tissue plates 24 h before treatment. 5-Aza-2'-deoxycytidine (Sigma Chemical Co., St. Louis, MO) was added to the fresh medium at concentrations of 5 μM in duplicate, and cells were harvested after 4 days.

Results and Discussion

Loss of RASSF1A Expression in Bladder and Prostate Cancer Cell Lines. To explore the candidacy of RASSF1 as a suppressor in bladder carcinogenesis, we initially evaluated mRNA expression of three RASSF1 isoforms (−A, −B, and −C) in five bladder carcinoma cell lines (J82, T24, HT1197, HT1376, and 253J) by quantitative RT-PCR using isoform-specific primers. Two human cell lines, HL60 (lymphoma) and A549 (lung carcinoma), whose RASSF1 expression status was previously characterized, were included as expression and nonexpression controls, respectively (4). As shown in Fig. 1, all three RASSF1 isoforms were detected in HL60, whereas only RASSF1C transcript was observed in A549. RASSF1A expression was not found in four (T24, HT1197, HT1376, and 253J) of five bladder cell lines. The 253J-BV cell line, a metastatic subline of 253J, also showed no RASSF1A expression. RASSF1B expression was detectable only by nest-PCR approach in two cell lines (J82 and 253J-BV). RASSF1C transcripts were expressed in all cell lines, but two (HT1197 and HT1376) showed reduced levels compared with the other three cell lines. In addition, all of four human prostate carcinoma cell lines we analyzed also showed no expression of both RASSF1A and RASSF1B transcripts, and a marked reduction of RASSF1C expression was observed in the LNCaP prostate cell line (Fig. 1). Thus, expression of RASSF1A was lost in 80% (4 of 5) of bladder carcinoma cell lines, and three of these also showed no RASSF1B expression.

Frequent Alteration of RASSF1A Expression in Primary Bladder Carcinomas. Next we analyzed the expression status of RASSF1A mRNA in 55 primary bladder carcinomas. All of 15 normal
showed complete methylation at 15 CpG sites, whereas the RASSF1A promoter was. The promoter region (nucleotide sequences of the CpG island region amplified by PCR). The 15 CpG sites was amplified by PCR using sodium bisulfite-modified genomic DNA as templates, and 10 PCR clones of each specimen were sequenced (Fig. 3A). All RASSF1A-nonexpressing cell lines showed complete methylation at 15 CpG sites, whereas the RASSF1A promoter was found in 61.8% (34 of 55) of primary carcinomas (Table 1 and Fig. 1A). Tumor-specific loss or down-regulation of RASSF1A was identified in 10 (83.3%) of 12 matched sets (Fig. 1B). In addition, altered expression of RASSF1A was observed in 78.6% (22 of 28) of muscle-invasive tumors (T2–T4) but in 44.4% (12 of 27) of superficial tumors (T1). No correlation of abnormal RASSF1A expression with morphological patterns of tumors or age, sex, and smoking status of the patients was recognized (Table 1). RASSF1B mRNA was undetectable in 30.9% (17 of 55) of primary carcinomas, and tumor-specific reduction was observed in 5 (41.7%) of 12 matched sets. However, all these tumors also showed loss or abnormal reduction of RASSF1A, thus none of the primary tumors and cell lines carried abnormality exclusively in RASSF1B expression. RASSF1C transcript was easily detectable in all primary carcinomas. However, decreased expression of RASSF1C was reproducibly detected in 14.5% (9 of 55) of tumors. Collectively, these results demonstrate that loss or abnormal reduction of RASSF1A and RASSF1B is frequent in primary bladder tumors.

Hypermethylation of the CpG Island in the RASSF1A Promoter. To elicit the implication of promoter hypermethylation in abnormal expression of RASSF1, the cell lines were treated with the demethylating agent 5-aza-2-deoxycytidine. As shown in Fig. 2, expression of RASSF1A and RASSF1B was reactivated in all nonexpressing bladder and prostate cell lines. Compared with untreated control, RASSF1C level was significantly up-regulated in LNCaP after treatment, indicating that DNA methylation represses the transcription of all three RASSF1 isoforms in this cell line (data not shown).

To define the methylation status of the gene, we performed bisulfite DNA sequencing analysis of the CpG island in the RASSF1A promoter. The promoter region (nucleotide −139 to +3) spanning 15 CpG sites was amplified by PCR using sodium bisulfite-modified genomic DNA as templates, and 10 PCR clones of each specimen were sequenced (Fig. 3A). All RASSF1A-nonexpressing cell lines showed complete methylation at 15 CpG sites, whereas the RASSF1A-expressing J82 cells showed no methylation (Fig. 3B). Furthermore, complete or high rate of methylation was identified in RASSF1A-nonexpressing primary bladder tumors, whereas no methylation was found in the corresponding normal tissues.

To determine the overall frequency of RASSF1A methylation in bladder tumors, we performed methylation-specific PCR and BstUI digestion analyses for 55 primary tumors, including 12 matched sets. Methylation at CpG sites 1/2 and 10/11 protected two restriction sites for BstUI (CGCG) from bisulfite modification (Fig. 3A). Methylation-specific PCR analysis demonstrated that 56.4% (31 of 55) of primary carcinomas are methylated, whereas none of the 15 normal tissues are methylated (Fig. 4A). Tumor-specific methylation was found in 10

| Table 1 Expression of RASSF1 in human bladder cell lines and tissues |
|------------------|------------------|
| Bladder specimens | Abnormal expression* | Abnormal expressionb |
|                  | RASSF1A | RASSF1B |
| Cell lines       |        |        |
| Tissue           |        |        |
| Normal           |        |        |
| Carcinoma        |        |        |
| Stage            |        |        |
| T2–T1            |        |        |
| T2–T4            |        |        |
| Grade            |        |        |
| I                |        |        |
| II               |        |        |
| III              |        |        |
| Morphology       |        |        |
| Papillary        |        |        |
| Nonpapillary     |        |        |
| Age              |        |        |
| <40              |        |        |
| 41–60            |        |        |
| >61              |        |        |
| Sex              |        |        |
| Male             |        |        |
| Female           |        |        |
| Smoking          |        |        |
| None             |        |        |
| >10 years        |        |        |

* No expression or less than one-half of normal means was classified as abnormal expression.
A Numbers in parentheses are percentages.

Fig. 2. Reactivation of RASSF1 expression by 5-aza-2-deoxycytidine treatment. Six bladder and four prostate carcinoma cell lines were treated with the demethylating agent 5-aza-2-deoxycytidine (5 μM) for 4 days, and expressions of RASSF1A and RASSF1B isoforms were evaluated by quantitative RT-PCR. C, untreated control; T, treated.

Fig. 3. Methylation status of a CpG island in the RASSF1A promoter in bladder and prostate cell lines and primary bladder carcinoma tissues. The CpG island spanning the RASSF1A promoter was amplified by PCR with bisulfite-modified genomic DNA as templates. The PCR products were cloned, and 10 clones of each specimen were sequenced. A, nucleotide sequences of the CpG island region amplified by PCR. The 15 CpG sites (1–15) analyzed are shown in bold. B, schematic representation of methylation status at the 15 CpG sites in 10 cell lines and normal (N1–N5) and tumor (T1–T5) tissues obtained from five matched sets. □, complete methylation (70–100%); □, partial methylation (10–60%); □, unmethylation.

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assays, 33 (97.1%) of 34 primary bladder carcinomas with loss or abnormal reduction of 5'-deoxycytidine in 60% (33 of 55) of primary tumors (Fig. 4 methylated, whereas none of 21 normal tissues was methylated. Collectively, our data demonstrate that –aza-2'-deoxycytidine restored expression of RASSF1A.

Figure 4. Methylation analysis of the RASSF1A promoter in cell lines and primary carcinomas. A, methylation-specific PCR analysis. Genomic DNA was extracted from bladder and prostate cancer cell lines, primary bladder tumors (T1–T4), and adjacent normal (N1–N3) tissues. Fifty ng of bisulfite-modified DNA was subjected to PCR amplification of the RASSF1A promoter sequences using unmethylation-specific (U) and methylation-specific (M) primer sets. Twenty µl of the PCR products were resolved on a 2% agarose gel. B, Bisulfite digestion assay for the RASSF1A promoter. The CpG island region spanning two BstUI sites was amplified by PCR using bisulfite-modified DNA as templates. BstUI digestion of the 15 µl PCR products (192 bp) from methylated tumors generated two fragments (89 bp and 75 bp) in a 2% agarose gel.

(83.3%) of 12 matched sets. BstUI digestion assay also detected methylation in 60% (33 of 55) of primary tumors (Fig. 4B). In these assays, 33 (97.1%) of 34 primary bladder carcinomas with loss or abnormal reduction of RASSF1A expression were verified to be methylated, whereas none of 21 RASSF1A-expressing tumors and 15 normal tissues was methylated. Collectively, our data demonstrate that ~60% (33 of 55) of primary bladder carcinomas are aberrantly methylated at the CpG island in the RASSF1A promoter, and hypermethylation in this region is strongly associated with loss or abnormal down-regulation of RASSF1A expression.

Mutation Analysis of RASSF1. To investigate whether altered expression of RASSF1 is associated with allelic deletion of the gene, we evaluated RASSF1 gene levels by quantitative genomic PCR analysis. The RASSF1 gene was detected in all cell line and tissue specimens (Fig. 1). However, compared with normal or corresponding noncancerous bladder tissues, two bladder cell line (HT1197 and HT1376) and 7 (13%) of 55 primary tumors showed decreased genomic levels of RASSF1. Interestingly, all of these tumors showed RASSF1A CpG island hypermethylation and also revealed detectable reductions of RASSF1C mRNA expression. Although comprehensive LOH study was not performed, this observation suggests that RASSF1A inactivation might be caused by both epigenetic and genetic mechanisms in a subset of bladder tumors. For the screening of somatic mutations, RT-PCR-SSCP analysis was performed for the entire coding region of three isoform transcripts. However, we failed to detect any types of mutation leading to amino acid substitutions or frameshifts except for previously described polymorphisms at codons 53 (CGG to CGT) and 56 (CCC to CCT), whereas 35% (21 of 60) of the same set of tumors were found to carry p53 mutations, indicating that mutational alteration of RASSF1 is not a main genetic event in bladder carcinogenesis (11).

In the present study, we demonstrate that expression of RASSF1A is silenced in a considerable proportion of bladder cancer cell lines and primary tumors. Loss or abnormal reduction of RASSF1A expression was found in 80% (4 of 5) of bladder cancer cell lines and in 62% (34 of 55) of primary carcinomas. Exposure to the methylase inhibitor 5’-aza-2’-deoxycytidine restored expression of RASSF1A in all non-expressor cell lines. Methylation analyses revealed that 97% (37 of 38) of RASSF1A-nonexpressing cell lines and uncultured tumors acquire 5’ CpG island hypermethylation in the RASSF1A promoter, whereas none of RASSF1A-expressing cell line and tissue specimens are methylated. Furthermore, altered expression of RASSF1A showed an association with advanced tumor stage, suggesting that RASSF1A inactivation may contribute to the malignant progression of bladder tumors.

The 3p21 region, where the RASSF1 gene is located, undergoes frequent allelic losses in a variety of human malignancies, including bladder cancer (1–3). Although the LOH status of RASSF1 has not been evaluated in this work, two bladder cancer cell lines (HT1197 and HT1376) and 21% (7 of 33) of RASSF1A-methylated primary tumors showed low genomic levels of RASSF1, which, accompanied with decreased expression of RASSF1C, suggests that the unmethylated RASSF1 allele might be deleted in a subset of bladder tumors. In this context, the presence of only the methylated allele in RASSF1A-nonexpressing cell lines is consistent with either methylation of both alleles or loss of the unmethylated allele. Recently, RASSF1A inactivation by two hits (allelic loss at 3p21.3 and promoter methylation) was also demonstrated in small cell lung cancers (9).

In contrast with ubiquitous expression of RASSF1A and RASSF1C, RASSF1B expression has been detected in cells from the hematopoietic systems (4). In the present study, we observed that low levels of RASSF1B mRNA is expressed in normal and cancerous bladder tissues. Although we cannot exclude the possibility that the RASSF1B transcripts detected in tissue specimens are derived from the tumor-infiltrating lymphocytes, expression of RASSF1B in some bladder carcinoma cell lines and its tumor-specific loss support that inactivation of RASSF1B might be associated with bladder carcinogenesis. However, it should be noted that none of the RASSF1A-expressing tumors we examined carries exclusively RASSF1B alteration. In addition, the simultaneous reexpression of both isoforms’ transcripts by 5-aza-2’-deoxycytidine raises the possibility that hypermethylation in the RASSF1A promoter region might lead to the concomitant suppression of the RASSF1B promoter in a subset of tumors. In contrast with RASSF1A, RASSF1C expression has been observed in all cancer cell lines, and CpG sites in the presumed RASSF1C promoter region were not methylated in the cancer cell lines (4, 8–10). However, Vos et al. (12) found that six of nine transformed ovarian cell lines had lost the expression of RASSF1C. It was also demonstrated that RASSF1C binds Ras in a GTP-dependent manner both in vitro and in vivo and mediates the apoptotic effects of oncogenic Ras, suggesting that RASSF1C might be a tissue-specific, Ras-activated tumor suppressor. Interestingly, we observed that a RASSF1A-nonexpressing prostate cancer cell line LNCaP also carries a significant reduction of RASSF1C expression. Thus, additional molecular biological studies will be required to define whether there are the tissue-type- or isoform-specific roles of RASSF1 in human tumorigenesis.

It has been well documented that Ras proteins bind a diverse array of effector molecules and mediate tumor suppressive effects such as terminal differentiation and apoptosis as well as oncogenic effects (23, 24). In this context, it was suggested that RASSF1 might mediate the Ras-activated growth inhibition through its proapoptotic function and RASSF1A inactivation may be a tumorigenic mechanism that is distinct from the oncogenic activation of Ras signaling in tumors (8, 12). Loss of RASSF1 expression may shift the balance of Ras activities toward a growth-promoting effect without the necessity of Ras-activating mutations (10). In this study, we observed that some cancer cell lines harboring oncogenic mutations of Ha-Ras (T24 and TSU-Pr1) or N-Ras (HT1197) also show the epigenetic silencing of RASSF1A, suggesting that Ras activation and RASSF1 inactivation may not be
mutually exclusive in tumors (25). Thus, the role of RASSF1 in Ras-dependent growth control remains to be characterized.

In conclusion, the data presented here clearly demonstrate that RASSF1A (RASSF1B) undergoes epigenetic silencing in a majority of bladder cancer by methylation of the CpG island in the promoter region. Our observations also suggest that inactivation of RASSF1A might contribute to the malignant progression of bladder cancer. Additional studies will be required to elucidate the biological significance of RASSF1 inactivation in bladder tumorigenesis.

References
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