Frequent Epigenetic Silencing of RASSF1, a Newly Identified Putative Tumor Suppressor at 3p21, in Human Bladder Cancer

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Background RASSF1, a recently identified putative tumor suppressor at 3p21, encodes multiple isoforms with Ras effector domain. To explore the possible implication of RASSF1 alteration in bladder carcinogenesis, we characterized expression and mutation status of three major isoforms of RASSF1, A, B and C in 63 bladder specimens.

Methods Expression and mutation status of RASSF1 was characterized using quantitative PCR and PCR-SSCP analyses. Gene levels of RASSF1 were also measured by quantitative genomic PCR. To explore the possible involvement of aberrant promoter hypermethylation, 5 bladder carcinoma cell lines were treated with 5-aza-2’deoxycytidine and expression levels of RASSF1 mRNA were evaluated.

Results While expression of RASSF1 C isotype mRNA was observed at a similar level in all normal and tumor tissues, loss of expression of A and B isoforms was detected in 36%(20/55) and 22%(12/55) of carcinoma tissues, respectively. In addition, reduced or no expression of RASSF1 A isotype was identified in 23%(5/22) of Ta-T1 and 65%(15/23) of T2-T4, showing that abnormal expression of RASSF1 A isotype correlates with the malignant progression of bladder cancers. Furthermore, among 5 carcinoma cell lines examined, only 182 expressed RASSF1 A isoform whereas the other four cell lines expressed RASSF1 B but not A isoform, raising the possibility that the reciprocal regulatory loop might be exist between these two isoforms. However, no evidences for sequence alterations or allelic deletion of the gene were found by RT-PCR-SSCP and quantitative genomic PCR analyses, suggesting the epigenetic silencing of RASSF1 gene transcription in this type of cancer. To explore abnormal hypermethylation of the gene as a possible cause of gene silencing, three cell lines (T24, HT1197, HT1376, and 253J/BV) that express no RASSF1 were treated with a demethylating agent 5-aza-deoxycytidine. Interestingly, all of the cell lines showed reexpression of RASSF1 mRNA following treatment, indicating the abnormal methylation of RASSF1 may be a main epigenetic event leading to loss of RASSF1 expression in bladder cancer.

Conclusion Our study strongly suggests that inactivation of RASSF1, mainly due to epigenetic transcriptional silencing of the gene, might contribute to the malignant progression of human bladder cancers.