RAPID COMMUNICATION Jpn. J. Cancer Res. 86, 249–251, March 1995

Infrequent Somatic Mutation of the MTS1 Gene in Primary Bladder Carcinomas

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We examined a candidate tumor suppressor gene on chromosome 9p21, MTS1/CDK4I (multiple tumor suppressor 1/cyclin-dependent kinase 4 inhibitor), which has been found to be mutated frequently in cell lines derived from bladder carcinomas, for somatic mutations in 39 primary bladder cancers by means of SSCP (single-stranded conformational polymorphism) and DNA sequencing. Mutations were detected in two of these carcinomas; one was a 61-base deletion and the other a 1-base deletion. In both cases the homologous allele was missing, indicating that "two-hit" mutation of the MTS1 gene had taken place in these tumors. The results indicated that inactivation of the MTS1 gene is likely to be a contributing factor in some, but not the majority of, bladder cancers.

Key words: MTS1/CDK4I — Bladder cancer — Two-hit mutation

Loss of heterozygosity (LOH) of chromosome 9p21-22 has frequently been observed in carcinomas arising in a number of organs, including the urinary bladder,[1-4] implying that this chromosomal region contains a tumor suppressor gene which may play a crucial and common role in development and/or progression of a wide range of cancers.[5]

Recently, a gene encoding a 16-kD protein was isolated from 9p21-22.[6,7] This protein (p16) is a cyclin-dependent kinase inhibitor (CDK4I) which regulates the cell cycle in the G1/S phase.[8] Homozygous deletions of the p16 gene have been observed frequently in cancer-cell lines established from many tissues, including the bladder,[5] and germline mutations have been identified in patients belonging to families carrying a genetic predisposition to melanoma.[9,10] Furthermore, somatic mutations have been found in primary esophageal carcinomas, pancreatic cancers, and lung carcinomas.[11-13] As the gene encoding p16 appears to be involved in carcinogenesis of multiple tissues, it is now termed "multiple tumor suppressor gene 1 (MTS1)."

To clarify the role of MTS1 in primary bladder cancer, we investigated somatic mutation of this gene in 39 tumors obtained during surgery; 13 of them from the Department of Urology, Shinshu University and 26 from the Cancer Institute Hospital in Tokyo. Each specimen was immediately frozen in liquid nitrogen and stored at −80°C. DNA was extracted from frozen tissues as described elsewhere.[14] All cancers were histopathologically diagnosed as transitional cell carcinomas.

DNA sequences corresponding to exon 2 of MTS1, and intronic sequences flanking this exon were amplified by means of the polymerase chain reaction (PCR) with the following primers: MTS1-P1, 5′-GGCTCTACACAAGCTTCCT-3′; MTS1-P2, 5′-TGAGCTTTGGAAGCTCTCAG-3′. PCR was performed in 35 cycles of 30 s at 95°C, 30 s at 58°C, and 30 s at 72°C, as described elsewhere.[15] DNA sequences of the PCR products were determined through direct sequencing by the dideoxy chain termination method.[16] Primers for the DNA sequencing were 5′-ACACGCTGGTGGTGCT-3′, 5′-AGGTCCACGGGCAGAC-3′, and 5′-GTCATGATGATGGGC-3′. Mutation of exon 1 was screened by SSCP (single-stranded conformational polymorphism) analyses with the following primers: MTS1-EX1F, 5′-GGGAGCAGCATGGGAGCCG-3′; MTS1-EX1R, 5′-AGTCGCCCGCCATCCCCT-3′.[9]

No alteration was detected by SSCP analyses of exon 1. However, among the 39 primary bladder carcinomas examined by direct DNA sequencing of the PCR products from exon 2, we found somatic mutations in two cases. The size of the PCR product from one of these tumor DNA was small, and no band corresponding to the normal size was observed (Fig. 1A). DNA sequencing of this PCR product revealed a 61-bp deletion beginning in the intron, 9 bp upstream of the exon-intron boundary, and ending at the first nucleotide of codon 60 (Fig. 1B); this deletion was expected to cause aberrant splicing. In the other case, a one-base deletion resulting in a shift of the reading frame had occurred in codon 45

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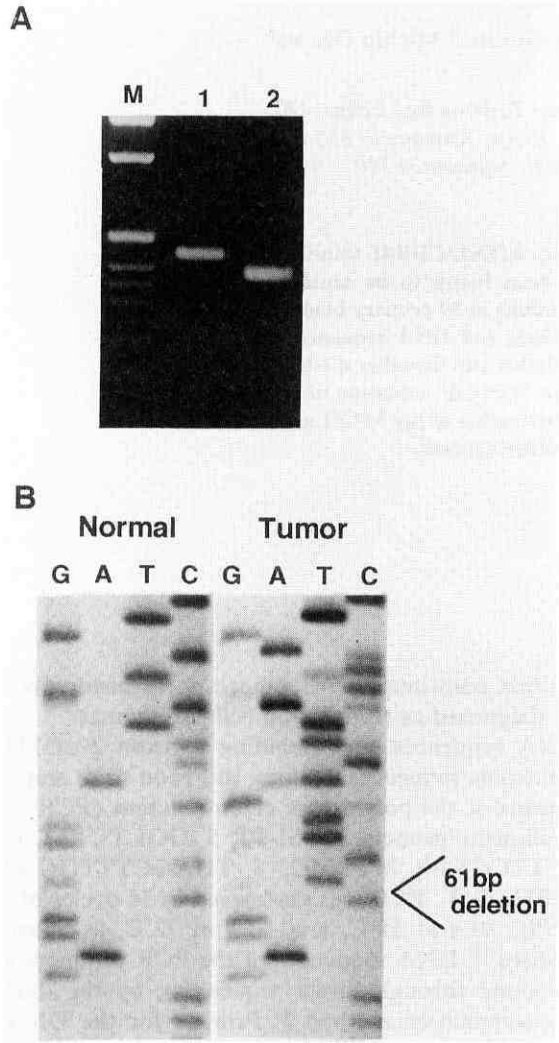

Fig. 1. A 61-bp deletion in the MTS1 gene in a bladder carcinoma (case 8). (A) Electrophoresis of PCR products of exon 2; M, size marker; lane 1, PCR product from normal DNA (429 bp); lane 2, PCR product from tumor DNA (368 bp). (B) DNA sequencing of PCR products. Left, normal DNA; Right, tumor DNA corresponding to the smaller band in lane 2 of Fig. 1A.

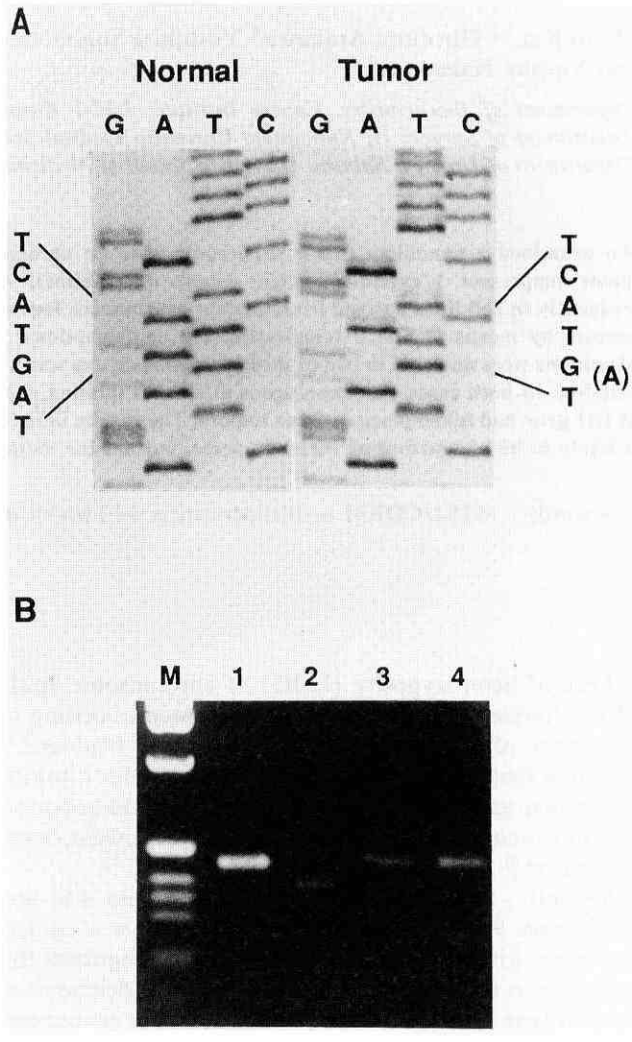

Fig. 2. A one-base deletion in the MTS1 gene in bladder carcinoma (case 11). (A) DNA sequencing of the PCR product reveals a 1-base deletion at the first letter of codon 45, which would result in a shift of the reading frame. (B) Electrophoresis of PCR product digested with restriction enzyme *Bsp*HI. M, size marker; lane 1, PCR product from normal DNA without enzyme digestion; lane 2, PCR product from normal DNA with enzyme digestion; lane 3, PCR product from tumor DNA without enzyme digestion; lane 4, PCR product from tumor DNA with enzyme digestion.

(Fig. 2A). This mutation was confirmed by digestion of the PCR product with *Bsp*HI, since the change from TCATGA to TCATGT destroyed a restriction site for this enzyme (Fig. 2B). Figs. 1 and 2 show that in both tumors the homozygous allele was absent, indicating a "two-hit" mutation inactivation of MTS1.

Although the carcinogenic pathway of bladder carcinoma remains unclear, a number of recent studies have revealed frequent genetic alterations in the short arm of chromosome 9 in bladder carcinomas.[1, 2, 5] MTS1/CDK4I became a candidate for the putative tumor suppressor associated with bladder cancer for several reasons: 1) many cell lines derived from bladder carcinomas carry homozygous deletion of this gene[6]; 2) germline mutations of MTS1 have been identified in patients belonging to families segregating an allele associated with hereditary melanoma[9, 10]; and 3) somatic mutations have been found in esophageal carcinomas, pancreatic cancers, and

lung carcinomas.[11–13] However, we and another group[17] have found a very low incidence of somatic mutations in primary bladder cancers even though our two "positive" tumors had clearly sustained two-hit mutations.

Complete loss of MTS1 function is very likely to cause deregulation of G1/S in the cell cycle. This change might favor cell growth, leading to a switch from slow to rapid growth or from rapid to more rapid growth. The finding that homozygous deletion at this gene locus was very frequently observed in cancer cell lines derived from various tissues but rarely in primary tumors may indicate that the gene dosage might affect cell growth; i.e., cells containing only one active copy of MTS1 might obtain a growth advantage, and tumor cells that have lost both copies might have an advantage for more rapid growth and/or for survival in culture media. This may be one of the reasons for infrequent somatic mutations in spite of the very frequent loss of heterozygosity at the MTS1 locus in tumors. However, it is also possible that another putative tumor suppressor gene exists in the p21-22 region near the MTS1 locus of chromosome 9.

We are grateful to Kiyoshi Noguchi for technical assistance. This work was supported by a Grant-in-Aid from the Ministry of Education, Science and Culture, Japan.

(Received October 26, 1994/Accepted January 13, 1995)

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