Frequent Association of p53 Gene Mutation in Invasive Bladder Cancer

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ABSTRACT

Structural alterations of the p53 gene were investigated to elucidate the molecular biological difference between superficial and invasive bladder cancer by polymerase chain reaction single-strand conformation polymorphism analysis. In 25 bladder cancers obtained from 23 patients, p53 gene mutations were investigated in exons regions 4 to 11. Twenty-four were transitional cell carcinomas, and the remaining one was a squamous cell carcinoma. Only one of 13 superficial bladder cancers, including pT1s, pTa, and pT1, was found to have p53 gene mutation. However, of 12 invasive bladder cancers with pT2, pT3, and pT4, six primary carcinomas, including a squamous cell carcinoma and one metastatic carcinoma, were found to have p53 gene mutations. The number of cancers examined in Grades 1, 2, and 3 was three, seven, and 15, respectively. p53 gene mutation was not found in any of the ten cancers with Grades 1 and 2, while eight of 15 bladder cancers with Grade 3 were found to have p53 gene mutation.

The results indicated that the incidence of p53 gene mutations appeared to be much higher in invasive-type and high-grade bladder cancers than in superficial and low-grade ones. Our results are compatible with the recently published results by Sidransky et al. (Science [Washington DC], 252: 706–709, 1991) showing that p53 gene mutations were frequently found in invasive bladder cancers by sequence analysis on polymerase chain reaction amplified products corresponding to exons 4 to 9. Our results are also compatible with previously reported results by Ohnuma et al. (Cancer Res., 50: 7081–7083, 1990) showing that the loss of chromosome 17p, revealed by analysis with restriction fragment length polymorphism, was frequent in high-grade bladder cancers. In this study, p53 gene mutations were often found in exon 4 as well as in other exons. Therefore, this region should also be examined for screening of mutations of this gene in bladder cancer. There appeared to be no consistent mutation sites in exons 4 to 11 of the p53 gene and no specific patterns of the mutation in bladder cancer.

INTRODUCTION

Bladder cancer is divided into two types: superficial bladder cancer and invasive bladder cancer. Superficial bladder cancers (pTis, pTa, and pT1) do not invade the muscle layer, whereas invasive bladder cancers (pT2, pT3, and pT4) involve the muscle layer. Superficial bladder cancers are usually low-grade (Grade 1 or Grade 2) tumors, and most of the invasive ones are high-grade (Grade 3) tumors. These two types of bladder cancer exhibit significantly different clinical behavior. Superficial bladder cancers usually occur and develop in multiple and low-grade forms with their specific papillary shape, and they frequently recur at the original site or occur at other new sites in the urinary bladder after transurethral resection. Most superficial bladder cancers have a good prognosis, but in 10 to 20% of the cases, cancer cells become more malignant showing an increase in the grade and/or infiltration into the muscle layer. On the other hand, invasive bladder cancers are commonly nodular shaped carcinomas with high-grade malignancy. Invasive bladder cancers are very aggressive, because they develop and progress rapidly and metastasize in an early stage.

Since point mutation of the H-raz gene was reported in the bladder cancer cell line (1), various human cancers have been studied for the presence of changes in oncogenes. There have been several reports on the alteration of the ras gene family (2) and on increased expression of EGF receptor (3, 4) in bladder cancers. Tumor suppressor genes, such as the retinoblastoma gene, were also implicated in a variety of cancers, and it is suggested that inactivation or loss of suppressor genes on a specific chromosome plays an important role in the development of cancer and tumor progression. Recent studies have shown that p53 gene may act as a tumor suppressor gene (5) and that its inactivation appears to be one of the most common genetic abnormalities in cancer. It is clear that losses of the specific chromosome are nonrandom and may be associated with the development of various cancers including kidney (6), lung (7), breast (8–10), and colorectal (11) cancers. The losses of heterozygosity of chromosomes 9q, 11p, and 17p were frequently observed at a high percentage in bladder cancers (12, 13), and loss of heterozygosity of chromosome 17p appeared in only high-grade tumors (14). It was reported by Sidransky et al. (15) that p53 gene mutations were detected in a high proportion of primary invasive bladder cancers by subcloning and sequencing PCR products of exons 5 to 9 of this gene. In other early studies, karyotype analysis revealed that monosomy 9 was frequently observed in superficial bladder cancers with near diploid modal chromosome numbers (16). It was also reported that monosomy 9 was not observed in the invasive type of bladder cancers (17). Deletion of 11p was more likely to be found in invasive bladder cancers than superficial bladder cancers (18).

In this study, we used PCR-SSCP analysis (19, 20), an efficient method to detect base changes, to determine whether the p53 gene alteration is involved in human bladder cancer.

MATERIALS AND METHODS

Samples and DNA Extraction. Twenty-five specimens of bladder cancers, including 24 transitional cell carcinomas and one squamous cell carcinoma, were obtained from 23 patients at cystectomy and transurethral resection performed at the National Cancer Center Hospital and Nara Medical University. These specimens were staged and graded according to the General Rule for Clinical and Pathological Studies on Bladder Cancer (21), which was adopted from the tumor-nodes-metastases (TNM) classification system of malignant tumors (International Union against Cancer, Geneva, 1978). pTis tumor is a flat tumor in the mucosa, what is called carcinoma in situ, and pTa tumor is a papillary tumor, which is also limited to the mucosa. pT1 tumor has invaded into the lamina propria but not into the muscle.
layer. pT2 tumor has penetrated less than half way through the muscle layer, whereas pT3a tumor has invaded the muscle layer to a depth greater than half way but still confined to the muscularis. pT3b tumor has involved the perivesical fatty tissue. pT4 tumor has extended into the prostate or other neighboring organs. These cancerous tissues, which were available in this analysis, were microscopically found to be occupied by cancer cells in a range of 20 to 70% of total cells in superficial tumors and that of 30 to 80% in invasive tumors. Patients had received neither chemotherapy nor radiation therapy prior to the operation. Twenty-four specimens were obtained from primary cancer tissue. In one patient with multiple cancers with extensive lymph node involvement, specimens were obtained from a primary nodular invasive carcinoma (11T), one primary carcinoma in situ lesion (11Tis), and one metastatic carcinoma of the internal iliac lymph node (11M). In this case, normal mucosa (11N) distant from the neoplastic lesions was also obtained as a control. These tissues were frozen in liquid nitrogen and stored at −80°C. Genomic DNA was extracted from tissues by proteinase K digestion and phenol/chloroform extraction according to the method of Sambrook et al. (22) with minor modifications. In PCR-SSCP and sequence analyses, human placenta DNA was used for control.

PCR. Oligonucleotides as primers for PCR were synthesized based on the published p53 gene sequence in each region from exons 4 to 11 (23). The designations and sequences for each primer are described as follows: PX4LT, GGAATTCGACGTTTTCCGCTTGC; PX4RT, GGAATTCATGCTATGCGTCTCT; PX5LT, GGAATTCCTTTTCTCAGTAC; PX5RT, GGAATTCGAGTCGACAGAGT; PX6LT, GGAATTCCTTTTCTCAGTAC; PX6RT, GGAATTCCTTTTCTCAGTAC; PX8LT, GGAATTCGACGTTTTCCGCTTGC; PX8RT, GGAATTCGACGTTTTCCGCTTGC; PX9LT, GGAATTCGACGTTTTCCGCTTGC; PX9RT, GGAATTCGACGTTTTCCGCTTGC; PX10LT, CTCTGTTGCTGCAGATC; PX10RT, GGAATTCAGTTGCAAACCAGACCTCAGG; PX4LT-2, CTCAGGGCAACTGACCGTGCA; PX4RT-2, CTCTGTTGCTGCAGATC. The designations and sequences for each primer are described in detail as follows: PX4LT, GGAATTCACCCATCTACAGTCC; PX4RT, GGAATTCAGTTGCAAACCAGACCTCAGG; PX5LT, GGAATTCGAGTCGACAGAGT; PX5RT, GGAATTCCTTTTCTCAGTAC; PX6LT, GGAATTCCTTTTCTCAGTAC; PX6RT, GGAATTCCTTTTCTCAGTAC; PX8LT, GGAATTCGACGTTTTCCGCTTGC; PX8RT, GGAATTCGACGTTTTCCGCTTGC; PX9LT, GGAATTCGACGTTTTCCGCTTGC; PX9RT, GGAATTCGACGTTTTCCGCTTGC; PX10LT, CTCTGTTGCTGCAGATC; PX10RT, GGAATTCAGTTGCAAACCAGACCTCAGG; PX4RT-2, CTCAGGGCAACTGACCGTGCA; PX4RT-2, CTCTGTTGCTGCAGATC. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region. Additional nucleotides for EcoRI sites were linked at their 5’ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 region.

SSCP Analysis. Two μl of PCR product were diluted 100-fold by a buffer consisting of 20 mM EDTA, 96% formamide, 0.05% bromophenol blue, and 0.05% xylene cyanoide and heated at 80°C for 2 min. Then 1 μl of this solution was applied to a 6% neutral polyacrylamide gel on an agarose gel. The gel was dried and exposed to X-ray film at −80°C for 2 to 4 h with an intensifying screen.

Cloning and Sequencing. PCR using PX4LT/PX4RT, PX5LT/PX6RT, PX8LT/PX8RT, or PX9LT/PX9RT as primer pair was performed as described above without [α-32p]dCTP. Amplified bands were purified by preparative gel electrophoresis and the GENECLEAN kit (BIO 101 Inc.; La Jolla, CA), followed by ligation to pUC18 vector. The recombinant plasmids were color selected by insertion mutagenesis of the β-galactosidase gene as described (22). Approximately 100 white recombinant colonies were picked up and pooled in super broth. After the mixed colonies were amplified, the double-stranded DNA was sequenced by the dideoxy chain termination method (24) using the Sequenase Version 2.0 enzyme (United States Biochemical, Cleveland, OH) and analyzed on a 5 and 8% polyacrylamide gel containing 7 M urea. Exon 4 was sequenced by the use of PX4LT and PX4RT primers. The oligonucleotides CACTGATTTGCTTTTAGT and CAGCACAAT-
Table 1  p53 gene mutation in human bladder cancer

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ND, not detected. 
* Carcinoma in situ (flat tumor). 
* This specimen consisted of squamous cell carcinoma alone without any transitional cell carcinoma component anywhere in the urinary bladder and other organs.

p53 GENE MUTATION IN HUMAN BLADDER CANCER

DISCUSSION

Understanding of human cancer at the molecular level provides us with new insights into the carcinogenic process and biological behavior of cancers. For example, c-erbB-2 or K-sawi amplification correlated well with biological malignancy in gastric cancer (25, 26) as well as amplification of c-erbB-2 (27) and hst-1 (28) in breast cancer, ras-related protein detected in the urine by immunoblot, using a sheep antibody against synthetic peptide for K-ras and H-ras p21, correlates with tumor stage and grade (29). Recently, several studies have reported the abnormality of a tumor suppressor gene associated with tumor stage and grade in bladder cancer.

Here we report the results of analyses on structural alterations of exons 4 to 11 of the p53 gene in 25 bladder cancer specimens using rapid and sensitive PCR-SSCP analysis. Our data show that the p53 gene mutation appears to be frequently associated with invasive and high-grade bladder cancers and rarely with superficial and low-grade bladder cancers. These results suggest that p53 gene mutation is a rather late event in tumor development and is involved in progression of bladder cancer. It is possible that p53 gene mutation is responsible for the conversion of superficial bladder cancer into invasive bladder cancer. Tsai et al. (12) and Olumi et al. (14) reported that allelic losses of chromosome 17p as well as 9q and 11p were most frequently observed in high-grade bladder cancers. Sidransky et al. (15) studied p53 gene mutation by sequence analysis on the PCR-amplified products corresponding to exons 5 to 9 (codon 328) in exon 9 was detected, and this deletion resulted in generation of a novel termination codon (TGA) at codon 344 (nucleotides 1244 to 1246) in exon 10.

separately to skip intron 7 by the primer pairs of each other, a mobility shift of the amplified exon 8 fragment could be clearly detected in 11M, 11Tis, and 8T (Fig. 1C), and a few bands of genetic polymorphism disappeared. In a previous study, polymorphic base substitutions were demonstrated to exist in intron 7. The intensity of normal allele bands is less than that of mutated bands in 11Tis and 11M. These results could not be explained by the presence of normal cells in cancerous tissue, and loss of the remaining allele was suggested in these specimens.

The region of exon 9 was analyzed by PCR-SSCP with the primers as described above. Mobility shifts were detected in exon 9 in 22T. (Fig. 1D).

Sequence Analysis. The exon 4 region was cloned from genomic DNA of the 12T specimen and sequenced. Comparison of the nucleotide sequences of this specimen and the published sequence of intact human p53 gene revealed the deletion of 21 base (CAGGGCAGCTACGGTTTCCGT or AGGGCAGCTACGGTTTCCGTC) pairs (codons 104 to 110 or 104 to 111) of exon 4. In 18T, sequence analysis of the mutated exon 4 showed a substitution from leucine (TTG) to serine (TCG) at codon 43. The exon 5–6 region was amplified and cloned from 7T and 20T. The sequence of these samples showed a substitution from alanine (GCC) to proline (CCC) at codon 159 in 7T (Fig. 2) and that from arginine (CGC) to histidine (CAC) at codon 158 in 20T. The exon 8 region was also cloned from 11M, 11Tis, and 8T. A single-point mutation was identified, resulting in substitution of valine (GTT) from phenylalanine (TTT) at codon 270 in both 11M and 11Tis, whereas a single base pair insertion at codon 280, transition from AGA (arginine) to ACG (threonine), resulted in a frameshift in 8T. In 22T, one base pair deletion of three consecutive thymines positioned between nucleotides 1195 (codon 327) and 1197 (codon 328) in exon 9 was detected, and this deletion resulted in generation of a novel termination codon (TGA) at codon 344 (nucleotides 1244 to 1246) in exon 10.
in bladder cancer and reported that p53 gene mutations were observed in 10 of 15 invasive bladder cancers, while one of 3 superficial bladder cancers was found to have this gene mutation. Our present results are compatible with these reports.

However, these data must be cautiously interpreted, because variable degrees of contaminating noncancerous cells are present in cancerous tissue and also because the cancer cells in tumorous tissue could be heterogeneous with respect to p53 gene mutation. We reported previously that the mutations of the p53 gene could be detected by the PCR-SSCP method used here, if the cells with mutated p53 gene were present in more than one eighth of the total cells (30). The presence of noncancerous cells in bladder cancer specimens used in the present study was at most 80% of the total cellular components. Accordingly, the difference in incidence of p53 gene mutations between superficial and invasive types of bladder cancers or between low-grade and high-grade bladder cancers is not likely due to the difference in the percentage of contaminating normal cells in the cancerous tissues.

The present study, however, does not exclude the possibility that the lower incidence of p53 gene mutations in superficial or low-grade bladder cancers, compared with that in invasive or high-grade ones, could be due to the possible presence of small proportions of cells with the mutation in superficial or low-grade bladder cancers. Heterogeneity of cancer cells with respect to p53 gene mutation was considered to be present in bladder cancers. Furthermore, the present analysis with the PCR-SSCP method did not detect the mutations in exons 1 to 3 of the p53 gene and also might not be able to detect some of the mutations even in exons 4 to 11 of the p53 gene. Thus, the incidence of p53 gene mutations reported here should be considered to be a minimal estimate.
amplified, cloned, and sequenced as described in the text. Using the PXSLT (sense) and PX6RT (antisense) primers, the point mutation was identified, resulting in a substitution from alanine (GCC) to proline (CCC) at codon 159.

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