[CANCER RESEARCH 52, 1393–1398, March 15, 1992]

Frequent Association of p53 Gene Mutation in Invasive Bladder Cancer[1]

Kiyohide Fujimoto,[2] Yukishige Yamada, Eigoro Okajima, Tadao Kakizoe, Hiroki Sasaki, Takashi Sugimura, and Masaaki Terada[3]

Genetics Division [K. F., Y. Y., H. S., T. S., M. T.], National Cancer Center Research Institute, and Department of Urology [T. K.], National Cancer Center Hospital, 1-1, Tsukiji 5-chome, Chuo-ku, Tokyo 104, and Department of Urology, Nara Medical University, 840 Shijo-cho, Kashihara, Nara 634 [K. F., E.O.], Japan

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 exon 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 pTis, 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 5 to 9. Our results are also compatible with previously reported results by Olumi *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-*ras* 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[4] 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

Received 9/9/91; accepted 12/27/91.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This study was supported in part by Grants-in-Aid from the Ministry of Health and Welfare for a Comprehensive 10-Year Strategy for Cancer Control, Japan.

[2] Recipient of a Research Resident Fellowship from the Foundation for Promotion of Cancer Research.

[3] To whom requests for reprints should be addressed.

[4] The abbreviations used are: EGF, epidermal growth factor; PCR, polymerase chain reaction; SSCP, single-strand conformation polymorphism.

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 cancer tissue 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, GGAATTCACCCATCTACAGTCC; PX4RT, GGAATTCAGGGCAACTGACCGTGCA; PX4RT-2, CTCAGGGCAACTGACCGTG; PX5LT, GGAATTCCTCTTCCTGCAGTAC; PX6RT, GGAATTCAGTTGCAAACCAGACCTCAGG; PX7LT, GGAATTCTCCTAGGTTGGCTCTGAC; PX7RT, GGAATTCAAGTGGCTCCTGACCTGGA; PX8LT, GGAATTCCTATCCTGAGTAGTGGTAA; PX8RT, GGAATTCCTGCTTGCTTACCTCG; PX9LT, GGAATTCTTGCCTCTTTCCTAGCA; PX9RT, GGAATTCCCAAGACTTAGTACCTG; PX10LT, CTCTGTTGCTGCAGATC; PX10RT, GCTGAGGTCACTCACCT; PX11LT, GGAATTCTGTCTCCTACAGCCAC; and PX11RT, GGAATTCTGACGCACACCTATTGC.

The number in each designation indicates the region of exon of the p53 gene subjected to examination by PCR-SSCP analysis. "LT" and "RT" indicate primers upstream and downstream, respectively, in each region. Additional nucleotides for *Eco*RI sites were linked to all primers at their 5′ end except PX4RT-2, PX10LT, and PX10RT. PX4RT-2 was used as a downstream primer for PCR-SSCP analysis, whereas PX4LT and PX4RT were used as PCR primers for cloning and sequencing of exon 4. One hundred ng of genomic DNA were amplified in a total volume of 25 μl in a buffer recommended by Perkin Elmer/Cetus, Norwalk, CT, containing 1 mM to 1.5 mM $MgCl_2$, [α-^{32}P]dCTP (3000 Ci/mmol, 10 Ci/ml), and 0.5 unit of Taq polymerase. Thirty cycles of reaction at 94, 55, and 72°C for 30, 30, and 60 s, respectively, were run in a DNA Thermal Cycler (Perkin/Elmer/Cetus).

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 cyanol and heated at 80°C for 2 min. Then 1 μl of this solution was applied to a 6% neutral polyacrylamide gel on each lane. Ten % glycerin was added in the gel for analysis of exon 11. Electrophoresis was performed at 30 W for 1 to 6 h, depending on the length of the amplified nucleotide. The gel was dried and exposed to X-ray film at −80°C for 2 to 24 h with an intensifing 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-^{32}P]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 CACTGATTGCTCTTAGGT and CAGCACATGACGGAGGTT in addition to PX5LT and PX6RT primers were used as sequence primers for the exon 5–6 region, whereas exon 8 was sequenced by the PX8LT and PX8RT primers. Exon 9 was sequenced by use of the PX9LT and PX9RT primers.

RESULTS

PCR-SSCP Analysis. The results of PCR-SSCP analysis on exons 4 to 11 of the p53 gene are summarized in Table 1. All the specimens were diagnosed histopathologically and analyzed by PCR-SSCP. Twenty-four primary bladder cancers and one metastatic carcinoma of the internal iliac lymph node obtained from a total of 23 patients were examined. All 24 primary bladder cancers were transitional cell carcinomas, except for the one squamous cell carcinoma (12T) that was pT3 and Grade 3 without any evident primary lesions in other organs. Of 24 specimens of primary carcinoma, 13 specimens were superficial carcinomas including one pTis, 7 pTa, and 5 pT1, and 11 specimens were invasive ones, including one pT2, 9 pT3, and one pT4. Three specimens, 11T, 11Tis, and 11M, were obtained from the same patient.

Six carcinomas of 11 invasive transitional cell carcinomas contained p53 gene mutations. In addition, one squamous cell carcinoma had p53 gene mutation. In contrast, none of the 13 superficial carcinomas was found to have p53 gene mutations except one specimen of carcinoma *in situ*, 11Tis (Table 1).

Four of 9 specimens with pT3, both of one pT2 and one pT4 specimen, and one carcinoma *in situ*, pTis tumor, showed p53 gene mutation, while none of 7 specimens with pTa and 5 specimens with pT1 showed p53 gene mutation.

All specimens were graded on a scale of 1 to 3 (Table 1). Nine superficial bladder cancer specimens were classified as low grade (Grade 1 or 2), and 4 specimens, as high grade (Grade 3). Twelve invasive bladder cancer specimens except one Grade 2 carcinoma were classified as high grade. Eight of 15 specimens with high-grade carcinoma showed p53 gene mutations, while none of 10 low-grade cancers showed the mutation.

In one patient, four different specimens were obtained from primary main nodular invasive carcinoma (11T), carcinoma *in situ* (11Tis), lymph node metastatic carcinoma (11M), and normal mucosa (11N). We could not detect p53 gene mutation in the primary invasive carcinoma (11T) as in normal mucosa (11N). However, we detected the p53 gene mutations in 11Tis and 11M specimens.

The p53 gene mutation was detected by PCR-SSCP analysis in the region of exons 4 (2 cases), 5 (2 cases), 8 (2 cases), and 9 (1 case). Polymorphic base substitution from CCC (proline) to CGC (arginine) at codon 72 generates four bands, two pairs of single-stranded conformation, on the PCR-SSCP analysis of exon 4. 12T showed apparent mobility shift and faintness of normal bands (Fig. 1*A*), suggesting loss of the remaining allele. 18T also showed mobility shift of the mutated band.

PCR-SSCP analysis of an amplified 419-base pair fragment by the PCR primers PX5LT and PX6RT showed mobility shift of two complementary strands of a DNA fragment and disappearance of normal allelic bands in 7T (Fig. 1*B*). This suggested that loss of the remaining allele or the same mutation of both alleles was present. In 20T, a mutated band was clearly detected with normal bands.

The 658-base pair fragment containing exon 7, intron 7, and exon 8 was amplified by PX7LT and PX8RT. This analysis revealed no mobility shift and the presence of polymorphism in this region. However, when exons 7 and 8 were amplified

Table 1 *p53 gene mutation in human bladder cancer*

Bladder cancer specimens	Histopathology		p53 gene mutation		
	Stage	Grade[a]	Exon	Codon	Amino acid change
Superficial carcinoma					
3T	pTaNoMo	G_2		ND[b]	
4T	pTaN0M0	G_2		ND	
6T	pTaN0M0	G_2		ND	
9T	pT1N0M0	G_2		ND	
10T	pT1N0M0	G_3		ND	
11Tis	pTis[c] in pT3bN4M0	G_3	8	270	Phe(TTT) → Val(GTT)
13T	pTaN0M0	G_1		ND	
14T	pT1N0M0	G_3		ND	
15T	pTaN0M0	G_2		ND	
16T	pTaN0M0	G_1		ND	
17T	pT1N0M0	G_2		ND	
21T	pT1N0M0	G_3		ND	
23T	pTaN0M0	G_1		ND	
Invasive carcinoma					
1T	pT3N0M0	G_2		ND	
2T	pT3bN0M0	G_3		ND	
5T	pT3bN1M0	G_3		ND	
7T	pT3N0M0	G_3	5	159	Ala(GCC) → Pro(CCC)
8T	pT2N0M0	G_3	8	280	Frameshift caused by 1-base pair insertion Arg(AGA) → Thr(ACG)
11T	pT3bN4M0	G_3		ND	
11M	pT3bN4M0	G_3	8	270	Phe(TTT) → Val(GTT)
12T[d]	pT3bN0M0	G_3	4	104–110 or –111	21-base pair deletion
18T	pT4N4M0	G_3	4	43	Leu(TTG) → Ser(TCG)
19T	pT3N0M0	G_3		ND	
20T	pT3bN1M0	G_3	5	158	Arg(CGC) → His(CAC)
22T	pT3N1M1	G_3	9	327or 328	Stop codon at codon 344 caused by 1-base pair deletion (TAT TTC → TAT TCA)

[a] Classified under the provision of the General Rule for Clinical and Pathological Studies on Bladder Cancer as described in the text.
[b] ND, not detected.
[c] Carcinoma *in situ* (flat tumor).
[d] This specimen consisted of squamous cell carcinoma alone without any transitional cell carcinoma component anywhere in the urinary bladder and other organs.

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. 1*C*), 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 allelic 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. 1*D*).

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.

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-*erb*B-2 or K-*sam* amplification correlated well with biological malignancy in gastric cancer (25, 26) as well as amplification of c-*erb*B-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

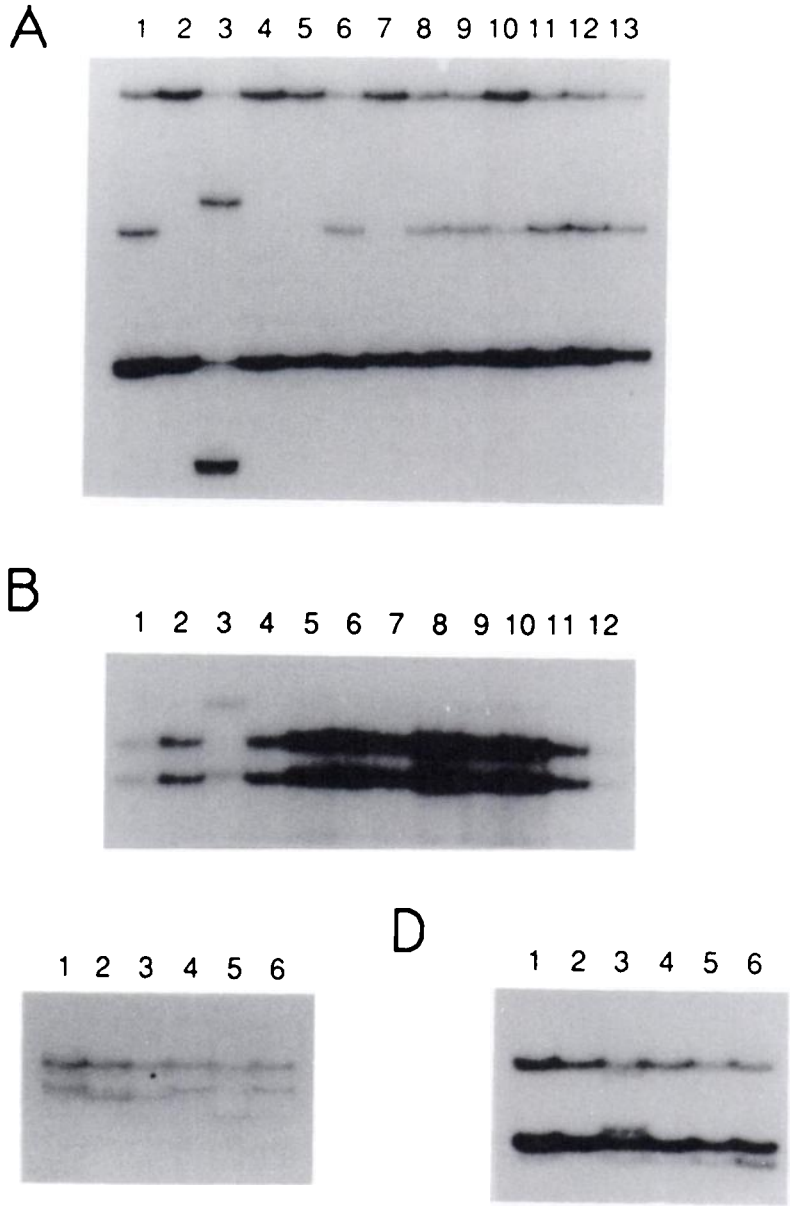

Fig. 1. PCR-SSCP analysis of human bladder cancers. PCR-SSCP analyses of DNAs extracted from human bladder cancers were performed as described in the text. *A*, mobility shift of 12T (*Lane 3*) and polymorphic base substitution (*Lanes 1, 6, 8, 9, 10, 11, 12, 13*) in exon 4. *Lanes* in *A*: *1*, 8T; *2*, 10T; *3*, 12T; *4*, 13T; *5*, 14T; *6*, 16T; *7*, 17T; *8*, 11T; *9*, 11Tis; *10*, 11M; *11*, 11N (normal mucosa as a control); *12*, 15T; and *13*, human placenta. *B*, mobility shift of 7T in exon 5–6 (*lane 3*). *Lanes* in *B*: *1*, 5T; *2*, 6T; *3*, 7T; *4*, 8T; *5*, 9T; *6*, 10T; *7*, 12T; *8*, 13T; *9*, 14T; *10*, 16T; *11*, 17T; and *12*, human placenta. *C*, two kinds of mobility shifts in the region of exon 8. 11M (*Lane 2*) and 11Tis (*Lane 3*) exhibit the same shift pattern. *Lanes* in *C*: *1*, 11T; *2*, 11Tis; *3*, 11M; *4*, 11N; *5*, 8T; and *6*, human placenta. *D*, mobility shift of 22T in exon 9. *Lanes* in *D*: *1*, 19T; *2*, 21T; *3*, 22T; *4*, 23T; *5*, 12T; and *6*, human placenta. In all analyses, human placenta DNA was used for control.

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 for 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.

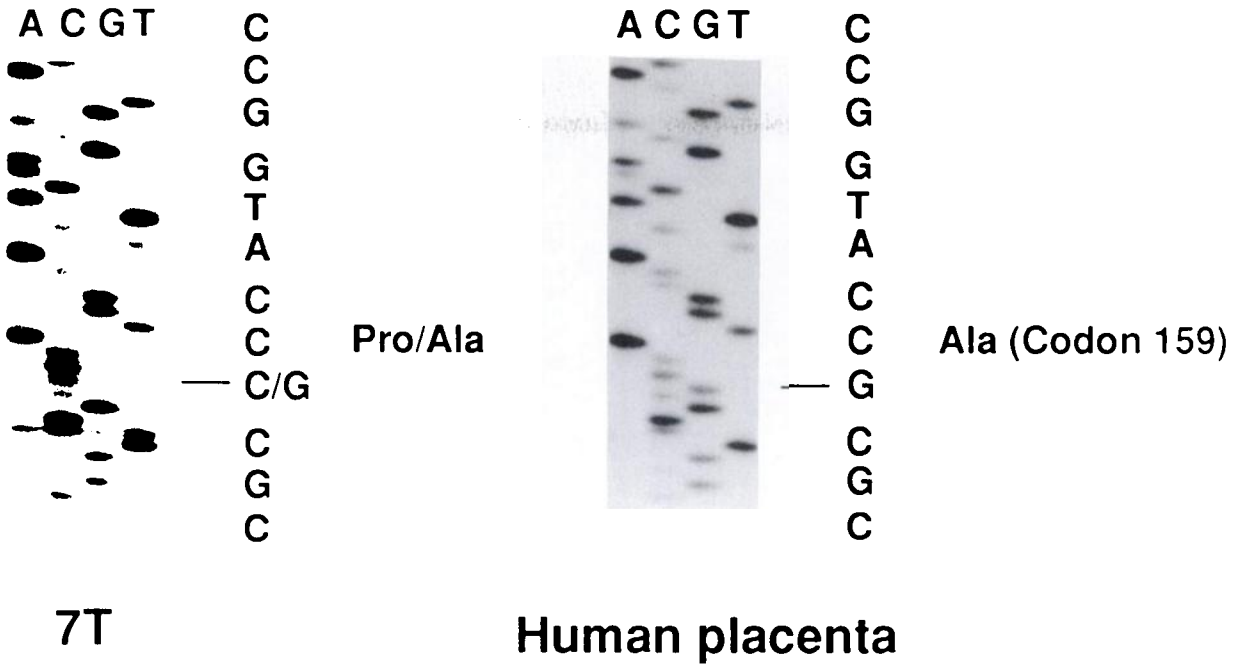

Fig. 2. Identification of point mutation in the p53 gene of invasive bladder cancers. The exon 5 region of the p53 gene from 7T or from human placenta was amplified, cloned, and sequenced as described in the text. Using the PX5LT (sense) and PX6RT (antisense) primers, the point mutation was identified, resulting in a substitution from alanine (GCC) to proline (CCC) at codon 159.

The present results as well as the already published data (15) of sequence analysis of the mutated p53 gene show that, unlike hepatocellular carcinomas (31), there were no consistent patterns of p53 gene mutation in bladder cancer. Nonetheless, an immunohistochemical method to detect mutant p53 with a long half life (32) might also be useful clinically. Further studies are required to determine whether p53 gene mutation can serve as an important predictor for the aggressiveness of the tumor or can be a useful marker for selection of a more suitable treatment and to elucidate whether there are consistent patterns of mutation in bladder cancer.

REFERENCES

1. Reddy, E. P., Reynolds, R. K., Santos, E., and Barbacid, M. A point mutation is responsible for the acquisition of transforming properties by the T24 human bladder carcinoma oncogene. Nature (Lond.), *300:* 149–152, 1982.
2. Fujita, J., Srivastava, S. K., Kraus, M. H., Rhim, J. S., Tronick, S. R., and Aaronson, S. A. Frequency of molecular alterations affecting *ras* protooncogenes in human urinary tract tumors. Proc. Natl. Acad. Sci. USA, *82:* 3849–3853, 1985.
3. Neal, D. E., Marsh, C., Bennett, M. K., Abel, P. D., Hall, R. R., Sainsbury, J. R. C., and Harris, A. L. Epidermal-growth-factor receptors in human bladder cancer: comparison of invasive and superficial tumours. Lancet, *1:* 366–368, 1985.
4. Berger, M. S., Greenfield, C., Gullick, W. J., Haley, J., Downward, J., Neal, D. E., Harris, A. L., and Waterfield, M. D. Evaluation of epidermal growth factor receptors in bladder tumours. Br. J. Cancer, *56:* 533–537, 1987.
5. Finlay, C. A., Hinds, P. W., and Levine, A. J. The p53 proto-oncogene can act as a suppressor of transformation. Cell, *57:* 1083–1093, 1989.
6. Zbar, B., Brauch, H., Talmadge, C., and Linehan, M. Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma. Nature (Lond.), *327:* 721–724, 1987.
7. Yokota, J., Wada, M., Shimosato, Y., Terada, M., and Sugimura, T. Loss of heterozygosity on chromosome 3, 13, and 17 in small-cell carcinoma and on chromosome 3 in adenocarcinoma of the lung. Proc. Natl. Acad. Sci. USA, *84:* 9252–9256, 1987.
8. Ali, I. U., Lidereau, R., Theillet, C., and Callahan, R. Reduction to homozygosity of genes on chromosome 11 in human breast neoplasia. Science (Washington DC), *238:* 185–188, 1987.
9. Lundberg, C., Skoog, L., Cavenee, W. K., and Nordenskjöld, M. Loss of heterozygosity in human ductal breast tumors indicates a recessive mutation on chromosome 13. Proc. Natl. Acad. Sci. USA, *84:* 2372–2376, 1987.
10. Mackay, J., Steel, C. M., Elder, P. A., Forrest, A. P. M., and Evans, H. J. Allele loss on short arm of chromosome 17 in breast cancers. Lancet, *2:* 1384–1385, 1988.
11. Vogelstein, B., Fearon, E. R., Hamilton, S. R., Kern, S. E., Preisinger, A. C., Leppert, M., Nakamura, Y., White, R., Smits, A. M. M., and Bos, J. L. Genetic alterations during colorectal-tumor development. N. Engl. J. Med., *319:* 525–532, 1988.
12. Tsai, Y. C., Nichols, P. W., Hiti, A. L., Williams, Z., Skinner, D. G., and Jones, P. A. Allelic losses of chromosomes 9, 11, and 17 in human bladder cancer. Cancer Res., *50:* 44–47, 1990.
13. Fearon, E. R., Feinberg, A. P., Hamilton, S. H., and Vogelstein, B. Loss of genes on the short arm of chromosome 11 in bladder cancer. Nature (Lond.), *318:* 377–380, 1985.
14. Olumi, A. F., Tsai, Y. C., Nichols, P. W., Skinner, D. G., Cain, D. R., Bender, L. I., and Jones, P. A. Allelic loss of chromosome 17p distinguishes high grade from low grade transitional cell carcinomas of the bladder. Cancer Res., *50:* 7081–7083, 1990.
15. Sidransky, D., Eschenbach, A. V., Tsai, Y. C., Jones, P., Summerhayes, I., Marshall, F., Paul, M., Green, P., Hamilton, S. R., Frost, P., and Vogelstein, B. Identification of p53 gene mutations in bladder cancers and urine samples. Science (Washington DC), *252:* 706–709, 1991.
16. Smeets, W., Pauwels, R., Laarakkers, L., Debruyne, F., and Geraedts, J. Chromosomal analysis of bladder cancer. III. Nonrandom alterations. Cancer Genet. Cytogenet., *29:* 29–41, 1987.
17. Gibas, Z., Prout, G. R., Pontes, J. E., Connolly, J. G., and Sandberg, A. A. A possible specific chromosome change in transitional cell carcinoma of the bladder. Cancer Genet. Cytogenet., *19:* 229–238, 1986.
18. Babu, V. R., Lutz, M. D., Miles, B. J., Farah, R. N., Weiss, L., and Van Dyke, D. L. Tumor behavior in transitional cell carcinoma of the bladder in relation to chromosomal markers and histopathology. Cancer Res., *47:* 6800–6805, 1987.
19. Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K., and Sekiya, T. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. Proc. Natl. Acad. Sci. USA, *86:* 2766–2770, 1989.
20. Orita, M., Suzuki, Y., Sekiya, T., and Hayashi, K. Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. Genomics, *5:* 874–879, 1989.
21. Japanese Urological Association and the Japanese Pathological Society. General Rule for Clinical and Pathological Studies on Bladder Cancer, Ed. 1. (In Japanese). Tokyo, Japan: Kanehara Press, 1980.
22. Sambrook, J., Fritsch, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual, Ed. 2. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.
23. Buchman, V. L., Chumakov, P. M., Ninkina, N. N., Samarina, O. P., and

Georgiev, G. P. A variation in the structure of the protein-coding region of the human *p53* gene. Gene, *70:* 245–252, 1988.

24. Sanger, F., Nicklen, S., and Coulson, A. R. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA, *74:* 5463–5467, 1977.
25. Yokota, J., Yamamoto, T., Toyoshima, K., Terada, M., Sugimura, T., Battifora, H., and Cline, M. J. Amplification of c-*erb*B-2 oncogene in human adenocarcinomas *in vivo*. Lancet, *1:* 765–767, 1986.
26. Hattori, Y., Odagiri, H., Nakatani, H., Miyagawa, K., Naito, K., Sakamoto, H., Katoh, O., Yoshida, T., Sugimura, T., and Terada, M. K-*sam*, an amplified gene in stomach cancer, is a member of the heparin-binding growth factor receptor genes. Proc. Natl. Acad. Sci. USA, *87:* 5983–5987, 1990.
27. Tsuda, H., Hirohasi, S., Shimosato, Y., Hirota, T., Tsugane, S., Watanabe, S., Terada, M., and Yamamoto, H. Correlation between histologic grade of malignancy and copy number of c-*erb*B-2 gene in breast carcinoma. A retrospective analysis of 176 cases. Cancer (Phila.), *65:* 1794–1800, 1990.
28. Tsuda, H., Hirohashi, S., Shimosato, Y., Hirota, T., Tsugane, S., Yamamoto, H., Miyajima, N., Toyoshima, K., Yamamoto, T., Yokota, J., Yoshida, T., Sakamoto, H., Terada, M., and Sugimura, T. Correlation between long-term survival in breast cancer patients and amplification of two putative oncogene-coamplification units: *hst*-1/*int*-2 and c-*erb*B-2/*ear*-1. Cancer Res., *49:* 3104–3108, 1989.
29. Stock, L. M., Brosman, S. A., Fahey, J. L., and Liu, B. C.-S. *Ras* related oncogene protein as a tumor marker in transitional cell carcinoma of the bladder. J. Urol., *137:* 789–792, 1987.
30. Yamada, Y., Yoshida, T., Hayashi, K., Sekiya, T., Yokota, J., Hirohashi, S., Nakatani, K., Nakano, H., Sugimura, T., and Terada, M. p53 gene mutations in gastric cancer metastases and in gastric cancer cell lines derived from metastases. Cancer Res., *51:* 5800–5805, 1991.
31. Hsu, I. C., Metcalf, R. A., Sun, T., Welsh, J. A., Wang, N. J., and Harris, C. C. Mutational hotspot in the p53 gene in human hepatocellular carcinomas. Nature (Lond.), *350:* 427–428, 1991.
32. Hinds, P. W., Finlay, C. A., Quartin, R. S., Baker, S. J., Fearon, E. R., Vogelstein, B., and Levine, A. J. Mutant p53 DNA clones from human colon carcinomas cooperate with *ras* in transforming primary rat cells: a comparison of the "hot spot" mutant phenotypes. Cell Growth & Differ., *1:* 571–580, 1990.