Aberration of chromosomes 8 and 11 in bladder cancer as detected by fluorescence in situ hybridization

Abstract Although a bladder cancer-specific abnormality in chromosomes or genes has not been reported, chromosomal regions that tend to become abnormal have been recognized. In this study, we investigated abnormalities in chromosomes 8 and 11. There were 27 patients with bladder cancer, 16 males and 11 females, who participated in this study. Abnormalities in chromosomes 8 and 11 were investigated by the fluorescence in situ hybridization (FISH) method. Probes used in this study were chromosome 8α-satellite and chromosome 11α-satellite (Oncor Co.). Of 27 cases, 15 cases were positive for chromosome 8 (55.6%) and ten cases were positive for chromosome 11 (37.0%). Since the FISH method detects chromosomal abnormality by the number of signals generated in cancer cells, this method is objective and simple and thus may be applicable in clinical practice.

Key words Bladder cancer · FISH method · Chromosomal abnormality

Introduction

Cytodiagnosis has been used for screening and diagnosis of malignant tumors, monitoring the course of cancer therapy and early discovery of recurrence as an established diagnostic technique. In bladder cancer, cytodiagnosis can use decidual cells in spontaneous urine as a sample, and thus is less stressful to patients. Therefore, cytodiagnosis is frequently incorporated as a routine test applied to outpatients consulting the urologic department. In cytodiagnosis, Papanicolaou-stained preparations are macroscopically observed for the morphology of cell mass and individual cells and cancer cells are diagnosed using the morphological characteristics of cancer cells based on experience [24, 27]. Of these cases 30–40% were found to be positive; in cases showing a low degree of atypism, around 10% were positive [7, 8, 17, 23, 30]. Since these diagnostic indices are based on the degree of atypism such as size and nonuniformity of nuclei and cells, nuclear-cytoplasmic ratio (N/C) and agglutination and distribution of nuclear chromatin, it is impossible to avoid subjective factors such as the experience and knowledge of the examiners. Furthermore some cancer cells exhibit intermediate features between benign and malignant tumors. False positives resulting from cytodiagnosis are actually obtained in routine clinical examinations that often cause problems in later diagnoses and difficulty in dealing with patients. Positive results for CA19–9 and CEA are as low as 10–20% [23, 28], and thus, there are no tumor markers that can be called specific to tumors in urinary tract epithelium.

In recent years, cancers have been recognized as diseases that are induced by gene abnormality or accumulation of chromosomal abnormalities and molecular biological examinations such as detection of gene mutations by the microsatellite method [22, 30], detection of mutant CD44 expression by the RT-PCR method [19] and telomerase activity measurement [4, 18, 32] have been actively performed. The fluorescence in situ hybridization (FISH) method is a differential staining method for chromosomes developed by Pinkel et al. in 1986 [26]. In this method, chromosomal regions that are complementary to a specific DNA are labeled with fluorescence by directly hybridizing DNA probes to chromosomal preparations on glass slides. Using the FISH method, Ichikawa et al. [3] reported that in mammary cancer, chromosome 1 polysomy was detected in 85%, chromosome 11 polysomy was detected in 55% and chromosome 17 polysomy was detected in 35% of the chromosomal preparations. Jibiki et al. [15] reported
that the chromosome 17 monosomy was detected in a significant number of stomach cancer cases, and Herman et al. [11] reported Y chromosomal abnormality. Regarding bladder cancer, deletion of chromosome 9 was reported [14]. Using the FISH method, we investigated the incidence of oncogene c-myc in chromosome 8 and oncogene H-ras in chromosome 11.

Materials and methods

Materials

There were 27 patients (16 males and 11 females) ranging in age from 39 to 87 years (67.0 ± 10.6 years old) with incipient bladder cancer detected between January 1997 and March 1998. These were diagnosed following transurethral resection of the tumors or punch biopsy. Three cases showed false positives for a tumor on urinary cytodiagnosis but on histological examination were diagnosed as cystitis. In seven cases tumors were suspected on cystoscopy but both urinary cytodiagnosis and histological examination did not show malignancy. These cases were used as comparative controls. Normal human peripheral lymphocytes were used as normal controls.

Methods

Sample preparation

The bladder was lavaged twice with 50 ml of physiological saline and 100 ml of lavage fluid was collected. The lavage fluid containing cells was centrifuged at 1200 rpm for 5 min and the supernatant was discarded. To the cell pellet, 5 ml of 0.075 M KCl was added and pipetted, then the solution was incubated at 37 °C for 10 min. Two milliliters of Carnoy’s fixative (acetic acid:methanol = 1:3) was added and the cells were fixed at 4 °C for 15 min. After centrifuging at 1200 rpm for 5 min, Carnoy’s fixative was exchanged. The cell pellet was mixed with 50 μl of Carnoy’s fixative and 10 μl of resuspended cells were dropped on a glass slide and air-dried. This was then incubated at 65 °C for 4 h.

Denaturation of preparations

The preparations were soaked in 70% formaldehyde at 75 °C for 5 min, 70% ethanol at −20 °C for 5 min, then 100% ethanol at room temperature for 5 min.

Preparation of probes

To 8.5 μl formaldehyde, 1.5 μl probes, chromosome 8α-satellite (D8Z2) or chromosome 11α-satellite (D11Z1) (Oncor Co.), were added. After heat treatment on a heatblock at 75 °C, the probes were cooled immediately in ice. To the denatured probes, 10 μl hybridization solution (mixture of 15 μl bovine serum albumin (BSA) 20 mg/ml, 30 μl 10 × standard saline citrate (SSC) and 30 μl 50% dextran sulfate) were added. The adjusted probes were mounted on slide preparations and uniformly distributed by covering with parafilm. Then, the slides were incubated in a tightly closed box filled with 2 × SSC at 37 °C for 45 min. The parafilm was removed and the slides were sequentially soaked with shaking (1 200 rpm) on a rotary shaker in the dark for 5 min in 4 × SSC, 0.1% Triton-X-100/× SSC, then 4 × SSC.

After the slides were soaked in 2 × SSC and left standing for 5 min, 30 μl counter staining solution (25 μl 0.25 μg/μl propidium iodide, 5 μl paraphenylenediamine) was dropped onto the slides and mounted with a cover glass. Excess solution was aspirated with a paper towel and the slides were sealed with nail polish lacquer and left standing for 15 min. Under a fluorescence microscope, 100 nuclei were counted and when 15% or more nuclei showed a single signal, the specimen was regarded as monosomic. When 20% or more nuclei showed three or more signals, the specimen was regarded as polysomic. These definitions were based on the criteria proposed by Ichikawa et al. [3]. For statistical analysis, the relationships between grades and FISH and between stages and FISH were analyzed using the Mann-Whitney U test, and comparison between urinary cytodiagnosis and the FISH method was analyzed using the sign test.

Results

General patient data and results obtained from urinary cytodiagnosis, the FISH method and histopathological examination are shown in Table 1. Urinary cytodiagnosis was performed at least three times and the highest value obtained was used as the result. The results of examining chromosomes 8 and 11 in normal control lymphocytes by the FISH method are shown in Fig. 1. Since a pair of chromosomes is present in human nuclei, two signals are detected in a normal cell nucleus. Figure 2 shows typical chromosomal abnormalities detected by the FISH method. Three signals from chromosome 8 were detected in Case 24 (histology: CIS) and four signals from chromosome 11 were detected in Case 22 (histology: TCC, G3, pT1b). There were no cases showing monosomy.

Of 27 patients histopathologically diagnosed as having malignant bladder tumor (including one case of small cell carcinoma), abnormality in chromosome 8 was detected in 15 patients (55.6%) and 10 of these patients (37.0%) also showed abnormality in chromosome 11. Both chromosomes were normal in 12 patients (44.4%).

Of the three cases that were diagnosed as dysplasia on histological examination but false positive for tumor on urinary cytodiagnosis, one case was negative for both chromosomes 8 and 11 on FISH, one case was positive for chromosome 8 and negative for chromosome 11, and one case was positive for both chromosomes. In three cases that were diagnosed as cystitis by histological examination but false positive for tumors by urinary cytodiagnosis, all cases were negative for both chromosomes 8 and 11 on FISH. Seven cases where the histology did not show malignancy were also negative for both chromosomes 8 and 11 (Table 2). Two dysplasia cases that were positive for one of the chromosomes by the FISH method underwent intravesical