RESEARCH COMMUNICATION

Molecular Cytogenetic Analysis of Urothelial Carcinomas using Urine Samples

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Abstract

Urinary cells obtained from voided urine specimens of 46 patients with urothelial carcinomas (UCs) and 10 normal individuals were analyzed with 3 different centromeric fluorescence *in situ* hybridization (FISH) probes. The overall sensitivity of cytology was 48.9% compared to 95.7%with the FISH technique. The minimum values were found for stage Ta and grade 1 (90.5 and 89.4) and sensitivity of FISH in other stages and grades was 100%. Chromosome 3 demonstrated the most frequent chromosomal abnormality in all samples (43%), followed by chromosome 17 (32%) and chromosome 7 (25%). There was a statistically significant association between the number of cell abnormalities in chromosome 17 and the tumour stage (p value=0.02). No relationship was found between the type of chromosomal abnormality and grade. Thus feasibility and reliability of a FISH based approach was confirmed for detection of UC in urine samples.

Key Words: Urothelial carcinoma - detection - urine samples - chromosome abnormalities - FISH

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Introduction

Urothelial carcinoma (UC) is the most common malignancy of the bladder and upper urinary tract. Cystoscopy and cytology have long been the primary techniques used to detect and monitor urothelial carcinoma (Koss et al 1985; Farrow et al., 1990). However, while cytology has a high specificity for bladder cancer detection, it has a relatively poor sensitivity (Fitzgerald et al., 1995; Schamhart et al., 1998). This pitfall together with the extensive training required to interpret urine cytology have led to the development of new tests for the detection of urothelial carcinoma in urine. These developments mainly involve the detection of antigens or the levels of a product in the urine of patients with bladder cancer (Schmetter et al.,1997; Ramakumar et al.,1999;Sarosdy et al., 1995; Soloway et al., 1996). Most of these techniques have a significantly higher sensitivity but lower specificity than cytology for urothelial carcinoma detection (Sarosdy et al., 1997; Schmetter et al., 1997; Landman et al., 1998; Ramakumar et al., 1999).

Recent studies suggest that the bladder tumor progression is accompanied by increased chromosomal instability and aneuploidy (Sasaki et al., 1992; Zhao et al., 1999). Frequent alterations of a variety of chromosomes, including chromosomes 9, 17, 7, 3, 4, 11, 1 and others have been reported and fluorescence in situ hybridization (FISH) can be used to detect cells with these chromosomal alterations (Sandberg et al., 1994, Zhang et al., 1997; Junker et al 1999). Previous investigators have demonstrated that FISH can be used to detect urothelial carcinoma in voided urine or bladder washing specimens and also assessed the sensitivity and specificity of FISH for the detection of urothelial carcinoma in urine specimens (Cajulis et al 1995; Zhang et al., 1997).

Our purpose was first, to evaluate the feasibility of performing fluorescence in situ hybridization (FISH) on routine urine samples and to compare the relative sensitivities of urine cytology and FISH for detecting urothelial carcinoma and second, to examine any relationship between stage and grade of bladder tumors with type of chromosomal abnormality (according to FISH results).

Materials and Methods

Patients and sample collection

Urine specimens from 46 patients were collected in a 4-month period. All of these patients had transitional cell carcinoma and treated at the urooncology ward by TUR or radical cystectomy. Two voided urine samples were obtained from each patient immediately before surgery for FISH and cytology analysis. Voided urine specimens

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had a median volume of 25 ml. All 46 urine samples were analyzed using both cytology and FISH.

Cytology

The cytology specimens were processed the same day by cytopathology laboratory in our center. Slides were stained using a modified Papanicolaou technique. The main criteria used to render a diagnosis of malignancy included increased nuclear-to-cytoplasmic ratio, hyperchromasia, nuclear membrane irregularities that can be subtle, macro nucleoli, and chromatin clumping with irregular chromatin distribution. The results of cytology test were classified as positive, negative or suspicious.

Urine preparation for FISH

Urine specimens for FISH were processed the same day, usually within 2 to 6 hours of reception. The volume of urine used for FISH ranged from 15 to 50 ml (median 25). Urinary cells were sedimented at 1200g for 10 minutes. The cell pellet was resuspended in 15 ml hypotonic solution (0.075 M KCl) for 10 minutes. The cells were then sedimented again at 1200g for 10 minutes and resuspended in 10 ml 3:1 methanol: glacial acetic acid. This procedure was repeated at least 2 times and the final cell pellet was generally resuspended in 50 to 200 µl (depending on size) of residual 3:1 methanol: acetic acid.

Slide preparation

Three µl of the cell suspension were spread on at least 4 slides for each patient after several dilution and concentration of the cell suspension to ensure the optimal cell density for the analysis.

FISH

The 3 probes were directly labeled probes to the peri-centromeric regions of chromosomes 3, 7 and 17 (Aquarius probes, Cytocell, UK). The Chr.3, Chr.7 and Chr.17 probes were labeled with green, red and green fluorophores, respectively. Slides were incubated in 2X saline/sodium citrate (SSC) at 37˚C for 2 minutes and placed in 70%, 85% and 100% ethanol for 2 minutes each and then FISH probe mix (1µl probe mix, 9µl hybridization mix) was placed on each spot containing specimen. The slide was then cover slipped, sealed with rubber cement, and underwent denaturation at 73˚C for 3 minutes on a flat bed PCR machine. The slide was then incubated at 37˚ C overnight in a humidified chamber and washed in 0.4x SSC at 73˚C for 2 minutes and rinsed in 2x SSC/0.05% Tween 20 at room temperature. Then 10 µl of DAPI counter-stain were placed on each spot and the slide was cover slipped (Ghaffari et al., 1998a:1998b).

Scoring

All cases were evaluated without knowledge of the cystoscopy, cytology or pathology results. All slides were scanned and the numbers of Chr.3, Chr.7, and Chr.17 signals in the cells were determined. The Positive test was assessed using the criteria determined by (Halling et al., 2000). In these cases, 100 cells (50 cells for mixed probes of Chr.7, and Chr.17 and 50 cells for Chr.3) were evaluated for chromosomal aberrations. A positive FISH result was defined as five or more urinary cells with gains or losses of chromosomes.

Table 1. Comparison between Sensitivity of Cytology and FISH Results by Stage of Tumor

No. Cases	Stage UC	FISH*	Cytology*
22	Ta	20 (90.5)	7 (31.8)
8	T1	8 (100)	4 (50.0)
14	T2	14 (100)	9 (64.2)
2	T3	2 (100)	2 (100)

Data are Number and Percentage Values

Table 2. Comparison between Sensitivity of Cytology and FISH Results by Grade of Tumor

No. Cases	Grade UC	FISH	Cytology
19	G1	17 (89.4)	5 (26.3)
10	G2	10 (100)	3 (30.0)
17	G3	17 (100)	14 (82.0)

Results

Overall, 46 patients with transitional cell carcinoma of the bladder and 10 samples from normal individuals (mean age 50) were enrolled in this study. All of the samples were confirmed by pathology. The mean age of patients was 65 (SD=15.8). Three patients were females (6%) and 44 patients were males (94%). The sensitivity of cytology was 48.93 according to the urine samples results. This sensitivity was increased by stage and grade of tumors. The minimum sensitivity was 31.8 (stage Ta) and maximum sensitivity was 100 (stage T3). The minimum and maximum sensitivity of cytology were seen in grades I and III respectively (26.3 and 82.3).

There was a significant relationship between the cytology results and grade of tumors (specially grade 2) (p=0.03). The overall sensitivity of FISH was 95.7. The minimum sensitivity were seen in stage Ta and grade I

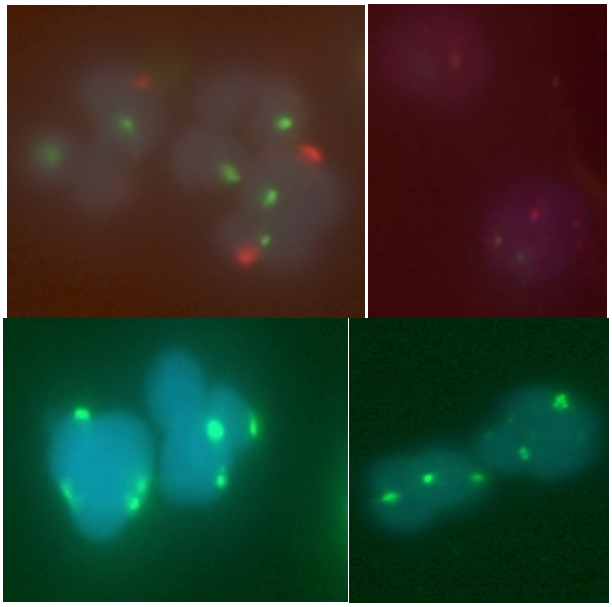

Figure 1. FISH Findings. Upper left: right side, cell with normal chr.7 & tetrasomy of chr.17; Upper right : tetrasomoy of chr.7 & normal chr.17; Lower left: two cells with trisomy (Right) & tetrasomy of chr.3 (Left); Lower right: two cells with normal (Right) & trisomy of chr.3 (Left)

Table 3. Mean Number (SD) and Percentage of Cells with Chromosomal Aberrations by Grade of Tumor

Grade	Chr.3		Chr.7		Chr.17	
G1	13.7 (6.9)	41	10.8 (9.3)	32	8.9 (4.4)	27
G2	15.1 (6.1)	43	8.5 (3.7)	24	11.8 (5.8)	33
G3	16.5 (5.3)	47	7.5 (3.9)	21	11.3 (5.3)	32

Table 4. Mean Number (SD) and Percentage of Cells with Chromosomal Aberrations by Stage of Tumor

Grade	Chr.3		Chr.7		Chr.17	
Ta	14.4 (6.6)	40	10.4 (8.2)	29	11.1 (4.6)	31
T1	8.0 (5.8)	29	5 (5.4)	18	14.0 (2.7)	53
T2	14.8 (4.3)	44	8.2 (2.6)	25	10.5 (5.6)	31
T3	26.5 (7.8)	55	9 (4.2)	19	12.5 (6.4)	26

(90.9 and 89.4) respectively. The sensitivity of FISH in other stages and grades was 100. Other data regarding the sensitivity of cytology and FISH techniques according to the stage and grade of tumors are summarized in Tables 1 and 2. In each sample 100 cells were analyzed by FISH technique (see Figure 1)(50 cells for mixed probes of Chr.7, and Chr.17 and 50 cells for Chr.3). The mean number (SD) & percentage of cells with chromosomal abnormality in each sample according to the stage and grade of tumors are summarized in Tables 3 and 4.

Chromosome 3 was the most frequent chromosomal abnormality in all samples (43%) followed by chromosome 17 (32%) and chromosome 7 (25%). There was a statistically significant association between the number of cell abnormality in chromosome 17 with stage of tumors (p value=0.02). However, the p values for other chromosomal abnormalities (Chr 3, Chr 7) were not significant. No relationship was found between the type of chromosomal abnormality and grade of tumor.

Discussion

Urothelial carcinomas are relatively common tumors. Early and low-grade urothelial carcinomas are more difficult to diagnose. Patients may present with vague symptoms such as intermittent microscopic or gross hematuria and mild abdominal pain. Cystoscopy and other radiographic tests may be non diagnostic. Clinicians often depend on urine cytology to make a diagnosis of urothelial malignancy and to follow up patients with previous diagnosis after surgery or other therapy.

In this study we used 3 centromeric probes for chromosomes 3, 7 and 17 to detect urothelial carcinoma in voided urine specimens. Our results demonstrate that a FISH assay using this combination of probes has high Sensitivity for the detection of urothelial carcinoma. The overall sensitivity of FISH was statistically significantly greater than the overall sensitivity of cytology (95.7 %vs. 48.93%) for the detection superficial and invasive tumor. The sensitivity of cytology increases by stage of tumors. Cytology had the minimum sensitivity in low stages of disease whereas the FISH technique had much higher sensitivity for detecting low stage tumors. Also FISH demonstrated a statistically significant better sensitivity than cytology for any pathological grade. We reviewed the literature to determine if the sensitivities of cytology by grade and stage found in our study were different than previously reported. Among these studies the sensitivity of cytology ranged widely from 0% to 86%, 3% to 88% and 38% to 100% for grade 1, 2 and 3 tumors, respectively (Rife et al.,1979; Koss et al 1985; Fitzgerald et al., 1995; Ellis et al.,1997; Schamhart et al.,1998; Thomas et al.,1999; Miyake et al., 1998). The overall sensitivities of cytology for grade 1, 2 and 3 tumors for all of the studies were 21%, 53% and 78%, respectively.

According to our results the number of cells with chromosome 17 aberrations had a statistically significant relationship with stage of disease. This finding may help clinicians to determine the stage by using FISH results.

In conclusion, FISH is a powerful alternative to cytology for monitoring patients with superficial urothelial carcinoma for tumor recurrence. Increased sensitivity of FISH for patients with carcinoma in situ and invasive urothelial carcinoma could significantly reduce urothelial carcinoma mortality. Further studies are needed to examine the sensitivity & specificity of FISH and role of this test for diagnosis, follow up and predicting prognosis (progression) in transitional cell carcinoma patients.

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