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Evaluation of upper urinary tract tumors by FISH in Chinese patients

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Abstract Upper urinary tract tumor (UUTT) usually presents a high grade and stage, and recurs frequently. The aim of this study was to evaluate the utility of a fluorescence in situ hybridization (FISH) assay on chromosomes 3, 7, 9, and 17 as a reliable and noninvasive method for the diagnosis of Chinese patients with UUTT. Urine specimens from 50 patients with UUTT and 25 donors without evidence of urothelial tumors were analyzed by cytology and FISH. Voided urine samples from 20 normal individuals were used to establish the cut-off values for FISH assay. The McNemar test was applied for sensitivity and specificity. The overall sensitivity of FISH was statistically significantly greater than that of cytology (84.0 vs. 40.0%, P=0.000). The overall specificities of FISH and urine cytology were all 96.0% (P=1.000). Polysomy in chromosomes 3, 7, and 17 were 38, 42, and 30%, respectively. Heterozygous and homozygous loss of the p16 locus was found in 36 and 32%, respectively. FISH analysis performed on cells collected from voided urine is feasible, and FISH could prove to be a reliable and less invasive ancillary test and improve the sensitivity of urine cytology in the diagnosis of UUTT. © 2010 Elsevier Inc. All rights reserved.

1. Introduction

Upper urinary tract tumor (UUTT) is not a frequent urologic cancer, representing only around 5% of all urothelial tumors [1]. But UUTT usually presents a high grade and stage [2], which emphasizes the need for an early diagnosis and an effective treatment. Moreover, as with bladder cancer, UUTT recurs frequently in patients with low-grade urothelial carcinoma managed by periodic endoscopic surveillance and resection. Recurrence rates ranged from 19.5 to 88.5% [3–5]. The natural history of UUT tumor is characterized by a high risk (40–75%) of having recurrent bladder tumors [6]. Although the incidence of UUTT after primary bladder cancer is low (0.7–4%) [7], it presents clinical significance and some management difficulties. Therefore, accurate diagnosis of the tumors and long-term surveillance are mandatory.

Diagnosis of UUT tumors is mainly based on urine imaging techniques, cytology, and ureteroscopy. The imaging techniques mostly used are those that detect a filling defect, such as intravenous pyelography (IVP), retrograde pyelography (RGP), and computed tomography (CT). IVP, RGP, and CT should not be used as the sole diagnostic tools because of their low sensitivity in the detection of small tumors and other causes [8]. Cytologic examination of exfoliated cells in voided urine is an excellent tool for detecting high-grade urothelial tumors, with sensitivity as high as 95% and specificity higher than 90%. Its role in the diagnosis of UT is controversial, however, because its sensitivity can be below 50% [1,9], especially for low-grade tumors [10,11]. It is possible to find transitional cells with equivocal morphology in urine samples, reflecting the limited value of this technique in distinguishing low-grade tumors from reactive urothelial changes [12]. Moreover, the results of cytology are operator dependent and unreliable in case of infection [13,14], and instrumentation can lead to false-positive results [10,11]. Ureteroscopy, although a highly sensitive, is considered an invasive procedure that can be associated with severe complications [13], especially in the examination of more proximal locations of the urinary tract. It may also fail to detect microscopic disease that may have significant clinical importance, such as carcinoma in situ [15]. A number of studies have focused on the evaluation of urinary

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markers that hold promise as noninvasive adjuncts for the detection of bladder cancer (e.g., BTA Stat, NMP 22, and Immunocyt), but their sensitivity and specificity are still unsatisfactory [16,17]. Therefore, a reliable, noninvasive method of detecting UUTT and for monitoring patients, who are at greater risk of developing UUTT, is urgently needed.

The fluorescence in situ hybridization (FISH) assay is a multitarget molecular assay that has detected up to four chromosomal changes that have been associated with transitional-cell carcinoma, including the centromeres of chromosomes 3, 7, and 17, as well as for the p16 locus at 9p21, and was shown to have high sensitivity and specificity for the detection of bladder cancer in a noninvasive way [16−20].

Since abnormal karyotypes in UUTT do not differ from those found in UC of the bladder [21], the aim of the present study was to evaluate the utility of FISH on chromosomes 3, 7, 9, and 17 as a noninvasive method for the diagnosis of Chinese patients with UUTT.

2. Materials and methods

2.1. Patients and samples

Sixty patients from Nanfang Hospital and Armed Police Hospital, who were suspected to have UUTT according to imaging or other clinical evidence, were enrolled in this study between January 2008 and March 2010. Ten of the patients were subsequently excluded for different reasons (four cases due to a lack of cells on the slide processed for FISH, two cases had simultaneous bladder cancer, and four cases were diagnosed as calculus). Finally, voided urine samples from 50 patients with pathologically confirmed UUTT and provided sufficient data for analysis of the FISH and cytology performances, and were enrolled in the experimental group for the sensitivity study.

A separate specificity study of FISH and cytology was conducted in voided urine from 25 donors without evidence of urothelial tumors, who were used as controls. In addition, voided urine samples from 20 normal individuals were used to establish the cut-off values for FISH assay ahead of the experiment.

Patients and volunteers were enrolled in the study after giving the institutional review board approved, signed, and written consent.

The laboratory personnel and study cytopathologist were blinded to ureteroscopy results. The urologists, review histopathologists, and patients were all unaware of the urine testing results. No clinical study results were used to make management decisions.

2.2. FISH analysis

First-time voided urine specimens were collected for FISH and cytology in the morning the day before treatment and processed on the same day, usually within 2 hours of receipt. Urinary cells were sedimented at 1500 rpm for 8 minutes. The cell pellet was resuspended in 15 mL hypotonic solution (0.075 mol/L potassium chloride) for 10 minutes. The cells were then sedimented again at 1,500 rpm for 10 minutes and resuspended in 10 mL 3:1 methanol/glacial acetic acid. This procedure was repeated two more times and the final cell pellet was generally resuspended in 1/50−1/100 of urine volume. Two slides, each one for two probes, made from the voided urine samples were prepared for FISH.

The probe mix used consisted of centromeric enumeration probes of chromosomes 3 (CEP3), 7 (CEP7), and 17 (CEP17), and locus-specific identifier probes to the 9p21 locus location of the p16 tumor suppressor gene (LSI 9p21 or p16), all provided by GP Medical Technologies, Ltd. (Beijing, China). Two DNA probes were mixed together as a set double-target FISH and paired as follows: chromosome 3 (rhodamine) and chromosome 7 (FITC), chromosome 17 (FITC) and p16 (rhodamine).

Slides were incubated in RNase A (100 μg/mL) at 37°C for 15 minutes, pepsin (pH 1.0) at 37°C for 10 minutes, and 2× saline/sodium citrate (SSC; pH 7.0) at room temperature twice (5 minutes once). The slides were then placed in 70, 85, and 100% ethanol for 2 minutes each and denatured in 2× SSC/70% formamide at 76°C for 5 minutes. The FISH probe mix (2 μL probe mix, 7μL hybridization mix, and 1 μL water) was denatured at 76°C for 5 minutes. Denatured slides were rehydrated in 70, 85, and 100% ethanol at −20°C for 3 minutes each. The slides were then dried and 10 μL of the denatured probe were placed on slides. The slide was then coverslipped, sealed with rubber cement, and incubated at 37°C overnight in a humidified chamber. The slides were washed in 2× SSC/50% formamide at 46°C three times (5 minutes each), 2× SSC at 46°C for 10 minutes, 2× SSC/0.1%NP-40 at 46°C for 5 minutes, and 70% ethanol at room temperature for 3 minutes. Finally, 10 mL of 4',6-diamidino-2-phenylindole-2 HCl (DAPI) counterstain was placed on each well, and the slides were coverslipped and air dried. Visualization of the signals was done using a computer applied imaging system.

The evaluation of the samples was carried out by two different observers blinded to the group of patients analyzed. Scanning of the slides was performed basically by considering cytologically atypical nuclei suggestive of malignancy (big nuclear size, irregular nuclear shape, patchy, and often lighter nuclear DAPI staining). For each probe, 100 nuclei were evaluated. The criteria for FISH abnormality were determined by evaluating urine specimens from 20 normal individuals. Means and three times SDs of the percentages of nuclei with abnormal signal patterns were calculated as the cut-off values (Table 1).

The probe was considered abnormal when the percentage of abnormal cells was greater than the cut-off

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