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The UroVysion fluorescence in situ hybridization assay is an effective tool for monitoring recurrence of bladder cancer

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Abstract

The newly developed UroVysion fluorescence in situ hybridization (FISH) probe was applied to urine specimens from 19 patients being monitored for recurrence of bladder cancer. The results for the multi-target DNA FISH assay were compared with independent analyses of urine cytology and flexible cystoscopy. Patients with tumors identified through the cystoscopy exam were biopsied and/or underwent surgery. In 12 patients with normal cytoscopy, cytology and FISH were also normal. Therefore, the specificity of these two tests was 100%. In 7 patients, a tumor was diagnosed by cystoscopy, and 3 of them had abnormal urine cytology while 6 of them had an abnormal result in the FISH assay. Accordingly, the sensitivity was 43% for the cytology and 87% for the FISH test. Interestingly, a pT1G3 tumor in a bladder diverticulum was not detected by cytology or the FISH test. These results agreed with a large series previously published using similar FISH probes and support the proposal for a multicenter trial to confirm the usefulness of the UroVysion probe as a screening tool to select patients for cystoscopy. © 2004 Elsevier Inc. All rights reserved.

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1. Introduction

Cystoscopy and urine cytology are standard modalities to monitor patients with transitional cell carcinoma of bladder. However, cystoscopy is an invasive and expensive procedure and cytology has a relatively poor sensitivity for the detection of bladder cancer, particularly in histologically well or moderately differentiated tumors [1]. Efficient laboratory tests are needed for effective patient follow-up and monitoring of tumor recurrence, and cystoscopy could be reserved for cases where recurrence is strongly suspected. Voided urine and saline bladder washings can be easily obtained and are ideal sources of cells for screening or follow up of bladder carcinomas. In recent years, several adjunctive tests using urine for detection of bladder cancer have been proposed and investigated, including the bladder tumor antigen test (BTA, BTA stat, and BTA Trak), the nuclear matrix proteins 22 (NMP22) and BLCA-4, the fibrin-fibrinogen degradation products (FDP), and the telomeric repeat amplification protocol assay [1–3]. Although the majority of these tests have been shown to have a higher sensitivity than the cytology evaluation for bladder cancer detection, their specificity is unacceptably low.

The enumeration of copies of chromosomes in interphase cells by fluorescence in situ hybridization (FISH) assays has been successfully used as a screening tool in genetic and cancer studies [4]. However, a precise selection of targets to be used as FISH probes for monitoring particular disease is necessary. The targets must be highly specific and frequently included among the abnormalities characterizing the disease. In some conditions, especially solid tumors, such highly specific and sensitive targets are not available,

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a fact that has hampered the applicability of the FISH technology as a screening tool for these conditions. Recent progresses in the development of DNA labeling strategies using new fluorophores have partially circumvented this problem. The new methodological developments allowed the design of multicolor FISH probes to simultaneously target four different chromosome regions in a single cell. This multicolor strategy is particular useful for analysis of solid tumors characterized by extensive polyploidy or even polysomy for multiple chromosomes such as bladder tumors [5,6]. These tumors may benefit from a multicolor FISH probe even if the target chromosomes were not involved in specific changes [7].

In the current study, we prospectively assessed the sensitivity and specificity of a panel of DNA FISH probes (UroVysion FISH assay) in the follow-up of patients with bladder cancer, and compared with the urine cytology.

2. Materials and methods

2.1. Patients

Between February and June 2001, 19 patients with a history of bladder cancer who provided informed consent were included in this prospective study. All patients were under surveillance after resection of bladder tumors. Fresh voided urine samples were collected from each patient before routine flexible cystoscopy in the outpatient clinic, part of the voided urine was sent to the pathology laboratory for routine cytological examination and part to the cytogenetics laboratory for FISH analysis. Cystoscopy, cytology and FISH examinations were performed independently and physicians and investigators involved in one test were unaware of the results of the other tests. Cytology was considered positive if tumor or highly suspicious cells were found. Patients who were found with suspected lesions or a tumor during the flexible cystoscopy were scheduled for biopsy or a transurethral bladder tumor resection. The size, location, and number of tumors found on cystoscopy were recorded. Tumors were graded according to the World Health Organization grading system and staged according to TNM classification [8,9].

2.2. Fluorescence in situ hybridization assay

From 20 to 50 ml of voided urine were received from each patient mixed 2:1 with 2% Carbowax and stored at 4°C until processing within 24 h. Exfoliated urothelial cells were sedimented by centrifugation at 600 × *g* for 10 min and fixed in multiple changes of 3:1 v:v methanol:glacial acetic acid. The cell pellet was suspended in fixative and 10 ul of the suspension were applied onto a microscope slide. The slide was checked under phase contrast microscopy for proper cell density (~100–200 cells visible in the field of the 20× objective lens) and adjustments to the cell density were made if necessary. The FISH assay was performed with the UroVysion Bladder Cancer DNA probe kit (Abbott Laboratories, Abbott Park, IL). The probe set consisted of three repetitive sequences recognizing the centromeric regions of chromosomes 3, 7, and 17, and a unique locus sequence that hybridizes to 9p21. These DNA sequences were directly labeled with the fluorophores SpectrumRed, SpectrumGreen, SpectrumAqua, and SpectrumGold, respectively. The panel of probes was selected and tested in previous studies [7,10]. The FISH assay was performed on 1- to 3-day-old slides according to a codenaturation protocol. Briefly, slides were incubated in 2×SSC for 2 min at 73°C, digested in 0.008% pepsin in 0.01M HCl for 10 min at 37°C, washed in PBS, fixed in 1% formaldehyde for 5 min at room temperature and dehydrated. The UroVysion probe solution was applied to the selected area on the slide, the area was covered and sealed with rubber cement. Codenaturation of probe and chromosomal DNAs was achieved incubating slides at 80°C in a dry oven for 8 min and hybridization was allowed to occur in a moist chamber at 37°C for 20 h. Posthybridization washes were performed with 0.4×SSC/0.3% NP-40 at 73°C for 2 min, followed by 2×SSC/0.1% NP-40 at room temperature for 1 min. After air-drying, DAPI II was applied for chromatin counterstaining. The analysis was performed according to the kit instructions. The hybridization area was scanned with a 40× objective for cytologically atypical nuclei, represented by features such as nuclear enlargement, irregular contour, and patchy and often lighter-stained chromatin, because these cells were demonstrated as more likely to be polysomic. Approximately 50 cytologically atypical nuclei were scored for the number of fluorescent red, green, aqua and gold signals. An abnormal nucleus was defined as carrying gain in copy numbers for at least two of the DNA targets or homozygous loss for 9p21 signals. An abnormal specimen was defined as carrying more than 16% of cells with gain for multiple chromosomes or 48% of cells with 9p21 homozygous loss.

3. Results

Of the 19 patients enrolled in the study, 16 were male and 3 were female and their mean age was 68 years (range 58–80). The results for urine cytology evaluation, FISH assay, cystoscopy procedure and surgical pathology are combined for each patient in Table 1. In summary, bladder tumors were found in 7 patients. Of these, the urine cytology evaluation was positive for detection of tumor cells in 3 patients (sensitivity = 43%), and the FISH assay identified the specimen as abnormal in 6 patients (sensitivity = 86%). Both urine cytology and FISH results were normal in the 12 patients who had normal cystoscopy findings (specificity = 100%). Interestingly, in patient #6, a grade 3 pT1 tumor was located in the bladder diverticulum, and his cytology and FISH results were normal.

Table 1
Results of FISH assay, cytology, cystoscopy and surgical pathology in the 19 patients

Patient ID#	FISH	Cytology	Cystoscopy	Surgical pathology	
				Stage	Grade
1	Abnormal	Positive	Positive	pT2	G3
2	Normal	Normal	Normal		
3	Normal	Normal	Normal		
4	Abnormal	Positive	Positive	Ta	G2
5	Normal	Normal	Normal		
6	Normal	Normal	Positive	T1	G3
7	Abnormal	Normal	Positive	T1	G2
8	Normal	Normal	Normal		
9	Normal	Normal	Normal		
10	Normal	Normal	Normal		
11	Abnormal	Normal	Positive	Ta	G1
12	Normal	Normal	Normal		
13	Normal	Normal	Normal		
14	Normal	Normal	Normal		
15	Abnormal	Normal	Positive	Ta	G1
16	Normal	Normal	Normal		
17	Abnormal	Positive	Positive	T2	G2
18	Normal	Normal	Normal		
19	Normal	Normal	Normal		

Comparing the two tests using sediments of urinary cells, FISH was more effective than cytology. Included among the tumors identified by the FISH assay with the UroVysion probe were one tumor classified as Grade 3 (G3), three tumors Grade 2 (G2), and two tumors Grade 1 (G1). The cytology evaluation of urine sediments detected only one G3 tumor, and two G2 tumors; none of the G1 tumors was detected by cytology.

4. Discussion

The early detection of recurrence in patients with a history of bladder cancer may significantly impact the effectiveness of local therapies. The standard follow-up procedure for early detection of recurrent bladder cancer includes cystoscopy and cytological analysis of exfoliated cells found in urine at regular intervals. Cystoscopy is the method of choice, however, is an invasive and expensive procedure. Conversely, cytology of urinary cells is well known for its low sensitivity in well or moderately differentiated tumors [1–3]. The sensitivity of the cytology evaluation for grade 1, 2, and 3 tumors is approximately 20%, 50%, and 80%, respectively. Therefore, urine cytology evaluation is not a reliable alternative to reduce the number of cystoscopy procedures. Several other methods for noninvasive detection of bladder cancer in urine have yielded impressive results but they have yet to be transferred to the clinic. Overall sensitivity of these tests was 67 to 87% for BTA stat, 70 to 82% for NMP22, 81% for FDP, and 80 to 85% for telomerase [2,3,11]. Conversely, specificity of cytology is around 96 to 100% whereas specificities of BTA stat, NMP22, FDP, and telomerase are 69 to 90%, 79 to 86%, 75 to 91%, and 66 to 80%, respectively.

Microsatellite techniques have been used as a tool for detection of loss of heterozygosity (LOH) and genomic instability in neoplasia. The LOH test appears to be a robust procedure for screening patients with bladder cancer with 100% specificity and 96% sensitivity [12]. Nevertheless, the validity of the established cutoff values must be confirmed in a large number of patients.

Chromosomal aneuploidy has been associated with solid tumors in general, and bladder tumors specifically [5,6,13]. Most frequently, the chromosomal changes are gain in the copy numbers per cells, although many examples of loss of regions harboring tumor suppressor genes have been identified. Some chromosomal changes are specific for particular tumor types and are considered critical to the initiation and progression of these tumors. However, it has also been identified that a variety of solid tumors are characterized from their early stages by an extensive chromosome duplication followed by probably random chromosome gains and losses, a phenomenon named chromosomal instability. The chromosomal aneuploidy in interphase cells was initially investigated by flow cytometry but this test showed multiple limitations in its clinical application. The interphase FISH assays have been demonstrated as effective tools for detection of chromosome aneuploidy and may be processed and analyzed in automated devises [5,14]. However, it was the more recent development of multicolor DNA probe sets that have significantly increased the ability of testing single interphase cells for numerical chromosomal abnormalities.

In this study, the overall sensitivity of the UroVysion FISH assay was greater than the overall sensitivity of cytology (86% vs. 43%). Urine specimens from two patients with G1 tumors and one patient with a G2 tumor, all negative by cytology, could be identified as abnormal by the FISH test. Although it is difficult to generalize this finding because of small size of the study patient population, our results are concordant with a large series of bladder carcinomas [10], in which the sensitivity of FISH was significantly greater than that of cytology (81% vs. 58%, $p = 0.001$). These authors also reported a significant better sensitivity for FISH than cytology for the detection of pTis ($p = 0.046$), pT1-pT4 ($p = 0.025$), and G3 ($p = 0.003$) tumors. The difference in the sensitivity of FISH and cytology for pTa ($p = 0.058$) and G2 ($p = 0.059$) showed a trend toward statistical significance, but no difference for G1 tumors was found. Therefore, our results in patients with low-grade tumors need to be supported by a larger series. Intriguingly, patient #6 had bladder tumor but abnormal cells were not found in both cytology and FISH tests. His tumor was stage pT1 G3, therefore low tumor burden likely was not a cause for the lack of abnormal cells in urine. The tumor, however, was located in a bladder diverticulum, which probably has prevented the evacuation of urine with tumor cells.

5. Conclusion

This pilot prospective study found a superior sensitivity of the UroVysion FISH assay compared to urine cytology for the detection of recurrence of bladder cancer, whereas the specificity of these tests was similar. Therefore, the UroVysion FISH assay is supported as a screening tool for selection of patients with a history of bladder cancer that should be submitted to cystoscopy. Prospective studies in a large series are strongly encouraged to test the predictive power of this panel and to validate the possible extension of the intervals between cytoscopic controls.

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