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MONITORING INTRAVESICAL THERAPY FOR SUPERFICIAL BLADDER CANCER USING FLUORESCENCE IN SITU HYBRIDIZATION

BENJAMIN R. KIPP, R. JEFFREY KARNES,* SHANNON M. BRANKLEY, AARON R. HARWOOD, V. SHANE PANKRATZ, THOMAS J. SEBO, MICHAEL M. BLUTE, MICHAEL M. LIEBER, HORST ZINCKE† AND KEVIN C. HALLING‡,§

From the Departments of Laboratory Medicine and Pathology (BRK, SMB, ARH, TJS, KCH), Urology (RJK, MMB, MML, HZ) and Biostatistics (VSP) Mayo Clinic and Foundation, Rochester, Minnesota

ABSTRACT

Purpose: We evaluated fluorescence in situ hybridization (FISH) for assessing the response to therapy in patients with superficial bladder cancer receiving bacillus Calmette-Guerin or other intravesical therapies.

Materials and Methods: A total of 37 patients receiving intravesical therapy for superficial bladder cancer were enrolled in this study. Urine specimens were collected for FISH analysis just prior to the first intravesical therapy in 31 cases and just prior to or within 2 months following the last intravesical therapy in 37. FISH was done using the UroVysion probe set (Abbott Laboratories, Abbott Park, Illinois) with results considered positive if 5 or more cells demonstrated polysomy. Biopsy, cystoscopy and/or cytology results were then compared to FISH results to evaluate the usefulness of the test for monitoring intravesical therapy.

Results: Of the patients 25 had a negative and 12 had a positive post-therapy FISH result. All 12 patients with a positive post-therapy FISH result had tumor recurrence, while tumor recurrence was observed in 13 of the 25 with a negative post-therapy FISH result (HR 4.6, 95% CI 1.9 to 11.1, $p < 0.001$). Of the patients with tumor recurrence 7 of 12 with a positive post-therapy FISH result had muscle invasive tumor and 2 of 25 with a negative post-therapy FISH result had muscle invasive tumor (HR 9.4, 95% CI 1.9 to 45.3, $p = 0.001$).

Conclusions: FISH appears to be useful for monitoring patients with superficial bladder cancer for the response to intravesical therapy. Patients with a positive FISH result at the end of treatment are at high risk for progression to muscle invasive bladder cancer.

KEY WORDS: bladder; bladder neoplasms; mycobacterium bovis; in situ hybridization, fluorescence; outcome and process assessment

There are approximately 55,000 new cases of bladder cancer each year in the United States,[1] of which 70% to 75% are classified as superficial bladder cancer (stage Ta, T1 or TIS).[2,3] When followed with time, 60% to 70% of these tumors recur and 20% to 30% of these recurrent tumors progress to a higher stage or grade.[4–6] Intravesical therapies, such as bacillus Calmette-Guerin (BCG), mitomycin C and thiotepa, are frequently used in addition to transurethral resection to eradicate tumor that cannot be controlled endoscopically, prevent tumor recurrence and/or prevent tumor progression.[4] Although intravesical therapies seem to decrease tumor recurrence and progression, a high proportion of Tis and T1 tumors progress to muscle invasive disease.[2,4,7] Currently there is no way to accurately predict which patients have responded favorably to BCG or other intravesical therapies.

Fluorescence in situ hybridization (FISH) is a technique that uses fluorescently labeled DNA probes to assess cells for chromosomal alterations. FISH can be used to identify cells in urine that have chromosomal abnormalities consistent with a diagnosis of urothelial carcinoma (UC).[8] UroVysion is a Food and Drug Administration approved, multicolored FISH probe set developed to detect recurrent bladder cancer.[9] Studies show that UroVysion has a significantly higher sensitivity than cytology for detecting UC, while maintaining the high specificity of cytology.[9–15] In addition, UroVysion has been shown to detect recurrent bladder cancer before it is clinically evident by cystoscopy.[9,11,13,15] In this study we assessed the ability of FISH using UroVysion to determine the response of patients with superficial bladder cancer to BCG and other intravesical therapies.

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* Financial interest and/or other relationship with American Medical Systems and Pfizer.
† Financial interest and/or other relationship with Oncura.
‡ Requests for reprints: Hilton 920B, Mayo Clinic, 200 First St. Southwest, Rochester, Minnesota 55905 (telephone: 507-284-7616; FAX: 507-284-0670; e-mail: halling.kevin@mayo.edu).
§ Financial interest and/or other relationship with Vysis.

MATERIALS AND METHODS

Patient population. One female and 36 males receiving intravesical therapy for superficial bladder cancer were enrolled in this study between March 2001 and August 2002. Patient age was 50.2 to 86.4 years (median 75.3, mean 72.2). Bladder cancer stage preceding intravesical therapy was pTa in 17 patients, Tis in 15 and T1 in 5. Of the 17 patients with a pTa tumor 4, 5 and 6 were diagnosed with grades 1 to 3 disease, respectively, while 2 had no tumor grade recorded. In the Tis tumor cohort all 15 cases were grade 3. In the T1 tumor cohort 3 cases were diagnosed as grade 2 and the remaining 2 were diagnosed as grade 3. Of the patients in this study 33 were treated with BCG (Tice strain), 2 received 20 mg mitomycin C/20 ml distilled H_2O and 2 received 30 mg thiotepa/30 ml distilled H_2O. Treatment most often consisted of a 6 course regimen once weekly for 6 weeks. When available, urine specimens were collected for FISH analysis just prior to the first intravesical therapy (31 cases) and just prior to or within 2 months following the last installation of intravesical therapy (37). Patient clinical followup was 6 to 29

months (median 16.0, mean 18.2). At followup patients with positive biopsy, cytology or cystoscopy results were identified as having tumor recurrence. This study was approved by the Mayo Clinic institutional review board.

In this study we did not directly compare the usefulness of FISH and cytology because there was not enough sample to perform the 2 tests in most patients. However, previous studies have consistently demonstrated that FISH is more sensitive than cytology[9–15] and, thus, we believed that it was reasonable to focus on the usefulness of FISH for detecting recurrent tumor in this patient population.

Slide preparation. Cells were isolated from urine for FISH analysis, as previously described.[8,15] Depending on estimated cellularity 5 to 30 μl cell suspension were added to a 0.6 cm slide well and 10 to 40 μl were added to an adjacent 0.6 cm slide well. The specimen was allowed to dry and further assessed for cellularity with a phase contrast microscope. If necessary, additional cell suspension was added to 1 well until the desired cell density was achieved. Cell density was considered optimal for FISH analysis when cells were numerous but not overlapping.

FISH. The probe mix used consisted of directly labeled DNA probes to the pericentromeric regions of chromosomes 3 (CEP3), 7 (CEP7) and 17 (CEP17), and to the 9p21 locus (LSI 9p21). The CEP3, CEP7, CEP17 and LSI 9p21 probes are labeled with Spectrum Red, Spectrum Green, Spectrum Aqua and Spectrum Gold fluorophores (Vysis, Downers Grove, Illinois), respectively. FISH hybridization was performed as previously described.[15]

Interpretation of FISH slides. Slides were evaluated by technologists blinded to patient clinical history and pathological findings. Slides were assessed by scanning for cytologically atypical cells and determining the number of CEP3, CEP7, CEP21 and 9p21 signals in these cells. Atypical cytological features included patchy and lighter nuclear DAPI (4,6-diamidino-2-phenylindole) staining, nuclear enlargement and irregular nuclear contour. As previously described,[15] slides were diagnosed as positive for malignancy if 5 or more cells showed polysomy, 10 or more demonstrated trisomy or greater than 20% demonstrated 9p21 homozygous deletion. Polysomy is defined as the gain of 2 or more chromosomes in a cell. If a positive diagnosis was rendered, 100 consecutive urothelial cells (noninflammatory or nonsquamous cells were considered urothelial) were analyzed to determine the percent of urothelial cells in urine that demonstrated an abnormal FISH pattern. All patients with a positive FISH result in this study were found to have polysomy. There were no patient samples that showed trisomy or homozygous 9p21 deletion.

Statistics. Patients were classified into groups according to FISH results, including pre-therapy positive or negative, post-therapy positive or negative, and combinations of pre-therapy and post-therapy results. Total followup in days was determined in the various groups for 2 end points, namely tumor recurrence and the onset of muscle invasive disease. Followup in subjects who did not have recurrence or progression to muscle invasive disease was recorded as the number of months under observation and each month was counted as 30 days of followup. In those experiencing recurrence or progression the date of the event was recorded and used to calculate the days of followup. The log rank test was used to compare median time to recurrence in the different groups of patients, as defined by the presence or absence of a positive FISH result. When possible, proportional hazards regression models were fit to the data. These models were used to estimate the HR and 95% CI to summarize the risk of disease in those with positive vs negative FISH results. Kaplan-Meier survival curves were also constructed to illustrate the differences in time to event, that is tumor recurrence or the incidence of muscle invasive tumor, in the groups of interest.

RESULTS

A total of 68 urine specimens were collected for FISH analysis from 37 patients undergoing intravesical treatment for UC. Specimens were obtained just prior to the first installation of intravesical therapy in 31 patients (pre-therapy diagnosis), and just prior to the last installation of intravesical therapy in 31 and within 2 months of the last intravesical therapy in 6 (post-therapy diagnosis).

Of the 31 patients with a pre-therapy and post-therapy FISH result 13 had negative pre-therapy and post-therapy results, 1 had a negative pre-therapy but positive post-therapy result, 9 had a positive pre-therapy but negative post-therapy result and 8 had positive pre-therapy and post-therapy results (table 1). Figure 1 shows the percent of abnormal cells observed in the 18 patients who had at least 1 positive FISH result (ie a positive pre-therapy or post-therapy FISH result). Of the patients 15 (83.3%) had a decrease in the percent of abnormal cells following therapy, 2 (11.1%) had an increase and 1 (5.6%) had the same percent of abnormal cells.

Table 1 shows patient followup for tumor recurrence and statistical comparisons among the groups, as defined by pre-therapy and post-therapy FISH results. Patients with a positive pre-therapy FISH result were at a risk for tumor recurrence that was 3.3 times as high as the risk in those with a negative pre-therapy result ($p = 0.009$). The risk of recurrence was even greater in those with a positive post-therapy FISH result. These patients were at a risk for tumor recurrence that was 4.6 times as high as the risk in those with a negative post-therapy result ($p < 0.001$, table 1, fig. 2). A comparison of time to recurrence among the 4 groups defined by pre-therapy and post-therapy FISH results indicated that there were differences in risk among the 4 groups thus defined ($p = 0.021$). Those with positive findings on the pre-therapy and post-therapy FISH evaluations were at 5.3 (95% CI 1.7 to 16.4) times the risk of tumor recurrence as those with negative findings on the 2 evaluations.

The associations between FISH results and muscle inva-

TABLE 1. *Time to recurrence by FISH result*

FISH Result	No. Pts	Followup (person-days)	No. Recurrences (%)	Cox Proportional HR (Wald-type 95% CI)	p Value* (log rank test)
Before therapy:					0.009
Neg	14	5,833	7 (50.0)	1.0 (referent)	
Pos	17	2,534	14 (82.4)	3.3 (1.3–8.5)	
After therapy:					<0.001
Neg	25	8,712	13 (52.0)	1.0 (referent)	
Pos	12	1,163	12 (100.0)	4.6 (1.9–11.1)	
Before/after therapy:					0.021
Neg/neg	13	5,698	6 (46.2)	1.0 (referent)	
Neg/pos	1	135	1 (100.0)	4.3 (0.48–38.1)	
Pos/neg	9	1,596	6 (66.7)	2.8 (0.9–9.1)	
Pos/pos	8	938	8 (100.0)	5.3 (1.7–16.4)	

* Comparison across all groups.