Fluorescence in situ Hybridization (FISH) for Recurrent Bladder Cancer: Detection of Genetic Alterations in Bladder Cancer Cells

Background Information

Bladder cancer is the fifth most common type of cancer in the United States. Ninety percent of bladder cancer cases are transitional cell carcinomas (TCC). At presentation, about 75% of tumors are superficial, of which 50 to 80% will have one or multiple recurrences, and 15 to 25% will progress to muscle-invasive tumors.

Follow-up cystoscopy and urine cytology have been used to detect recurrence and tumor progression in patients with superficial TCC. However, low-grade tumors tend to have false negative cytology results.

Several genetic alterations have been identified to occur at high frequency in bladder cancer. These include the loss of a portion of chromosome 9 (presumably carrying a tumor suppressor gene), as well as numerical change in chromosomes 3, 7 and 17.[1] Using a multicolor set of fluorescent DNA probes (UroVysion, Abbott Molecular, Vysis, Des Plaines, IL), several studies have demonstrated that detection of chromosomal abnormalities by fluorescence in situ hybridization (FISH) has higher sensitivity in detection of TCC recurrence than does cytology, while maintaining high specificity.[2-4] This technique has been validated for use as an adjunct to urinary cytology utilizing thin layer liquid-based preparations.[5]

Clinical Indications

Cleveland Clinic Laboratories now offers analysis of chromosomal abnormalities in urine cytology specimens using multicolor, multitarget UroVysion FISH probes (chromosomes 3, 7, 17 and 9p21 locus). FISH can be utilized as an ancillary test increasing the sensitivity of urinary cytology in the detection of TCC.

Methodology

FISH can be performed on previously prepared thin layer, liquid-based cytology slides (ThinPrep, Cytyc, Boxborough, MA) or, alternatively, on fresh voided/instrumented urine specimens shipped in 70% ethanol.

ThinPrep slides from voided or instrumented urine are prepared and stained with Papanicolaou stain.[6] The transitional cells are identified and marked on the slides. Using a pretreatment kit, the slides are protease digested, fixed in formaldehyde, washed and dehydrated. Hybridization is performed using a four-color multitarget interphase FISH probe kit. The kit includes directly labeled probes to the peri-centromeric regions of chromosomes 3 (CEP3-red), 7 (CEP7-green) and 17 (CEP17-aqua), and to the locus 9p21 (LSI 9p21-gold). The cells are analyzed using fluorescence microscopy.

Interpretation

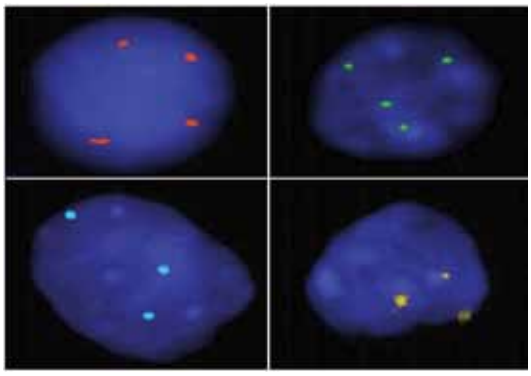
Optimal samples have a minimum of 25 transitional cells for analysis.

Positive test:

Four or more cells showing gain of 2 or more chromosomes (3, 7 and 17) in the same cell, OR loss of 9p21 locus in 12 or more cells, OR isolated gain of one of the chromosomes 3, 7, or 17 in >10% of the analyzed cells.The loss of 9p21 must be homozygous to be classified as positive.

Negative test:

Relevant chromosomal changes are not found after screening of the entire slide. The cellularity is limited for cases having fewer than 25 urothelial cells to count.



Multicolor fluorescence in situ hybridization performed on a slide prepared from a voided urine specimen from a patient with bladder cancer. The cells display gain of extra copies of chromosome 3 (red), 7 (green), and 17 (aqua). Three 9p21 signals (gold) are present in this case.

Limitations of the Assay

False negative results might occur if less than 25 transitional cells are available for analysis.

References

1. Sandberg AA, Berger CS. Review of Chromosome studies in urological tumors: Cytogenetics and molecular genetics of bladder cancer. *J Urol.* 1994;151:545-560.
2. Halling K, King W, Sokolova I *et al*. A comparison of cytology and fluorescence in situ hybridization for the detection of bladder carcinoma. *J Urol.* 2000;165:1768-1175.
3. Bubendorf L, Grilli B, Sauter G *et al*. Multiprobe FISH for enhanced detection of bladder cancer in voided urine specimens and bladder washings. *Am J Clin Pathol.* 2001;116:79-85.
4. Halling K, King W, Sokolova I *et al.* A comparison of BTA stat, hemoglobin dipstick, telomerase and Vysis UroVysion assays for the detection of urothelial carcinoma in urine. *J Urol.* 2002;167(5):2001-6.
5. Skacel M, Tsiftsakis E, Pettay J *et al.* Validation of a multicolor fluorescence in situ hybridization assay for detection of transitional cell carcinoma on fresh and archival thin-layer, liquid-based cytology slides. *Anal Quant Cytol Histol*. 2001;23(6):381-387.
6. Cytyc Corporation. Operator's manual: ThinPrep processor 1992.

Test Overview

Test Name	FISH for bladder cancer without urinary cytology	FISH for bladder cancer with urinary cytology
Patient Preparation	None	None
Reference Range	Absence of relevant chromosomal changes	
Specimen Requirements	40 ml fresh urine with equal amount of 70% ethanol added to sample in clean 100 ml urine container. Send specimen refrigerated. ThinPrep slides are also acceptable. The slide will be retained at Cleveland Clinic Laboratories per regulatory guidelines.	
Billing Code	82149	82151
CPT Code	88120(x1)	88112(x1); 88120(x1)

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