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Immunohistochemistry

Using fluorescent in situ hybridization (UroVysion FISH) for monitoring of recurrence of urothelial carcinoma.

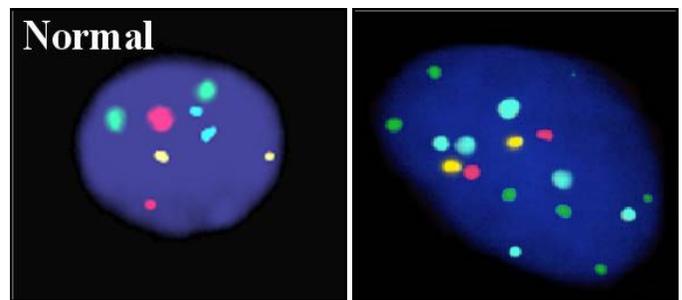
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Monitoring of patients with a previous diagnosis of urothelial carcinoma can be a very challenging task. This month, we highlight the utility of fluorescent in situ hybridization (FISH) techniques to assist in the care and management of these patients.

As with many other neoplasms, urothelial carcinomas occurs in both non-invasive and invasive varieties. Urothelial carcinoma in situ (CIS), a non-papillary flat lesion in which the surface epithelium is lined by epithelial cells showing high-grade atypia, has a significant risk of progression to invasive carcinoma, and is often seen in association with invasive carcinoma. Low-grade non-invasive papillary neoplasms commonly recur (48-71% of patients) but death from these neoplasms occurs in less than 5% of patients. However, high-grade non-invasive papillary neoplasms have a higher incidence of recurrence and higher risk of progression to invasive carcinoma.

Periodic urine cytologic examination is a common method of monitoring these patients, although these specimens can be notoriously difficult to interpret, and overall sensitivity is not impressive. For this reason, investigators have searched for other methods for monitoring these patients for recurrence. Genetic studies have shown that there appears to be 2 subtypes of urothelial neoplasia, that show a good correlation with morphologic categories. Low-grade non-invasive papillary tumors are genetically stable. In these tumors, partial or complete losses of chromosome 9 are common, present in roughly 50% of



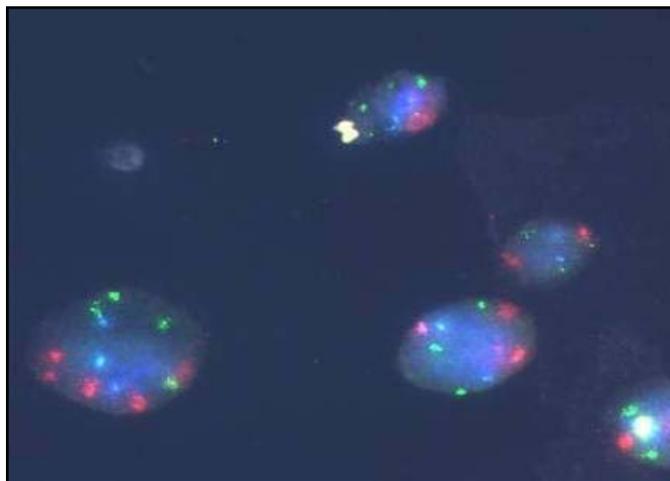
Computer-assisted images show normal urothelial cell with 2 copies of chromosomes 3 (red), 7 (green), and 17 (aqua), and 2 signals from the 9p21 locus (gold). In contrast, the abnormal cell on the right shows 6 copies of both chromosomes 7 (green) and 17 (aqua), indicating polysomy. (Photos courtesy of Mr. Scott Donovan of Vysis)

cases. Interestingly, loss of this chromosome has also been described in urothelial hyperplasia and in morphologically normal urothelium. Loss of the Y chromosome is the next most frequent abnormality, although again the significance of this finding is unclear, since it has also been identified in morphologically unremarkable urothelium from patients with no history of bladder cancer. In contrast, invasive carcinomas and high-grade CIS are genetically unstable, and show a much greater variety of chromosomal aberrations. Fortunately, neoplastic urothelium readily exfoliates in the urine, so abnormal cells from these tumors are frequently present in voided urine samples. Based on these findings, investigators have developed a panel of FISH probes that is useful in early diagnosis and in identifying recurrence of high-grade urothelial neoplasia, including high-grade CIS. This panel of probes, now commercially available from Vysis as the UroVysion kit, is FDA-approved for monitoring patients for recurrence of urothelial carcinoma.

UroVysion consists of a mixture of 4 different DNA probes, each with a different fluorophore. Three centromere probes for chromosomes 3, 7, and 17 are employed (marked with red, green, and aqua fluorophores), as well as a probe for the 9p21 locus, linked to a gold fluorophore. These probes allow for the detection of aneuploidy of chromosomes 3, 7, and 17, detectable as extra (i.e., >2) signals in the nucleus for each of the markers. Gains involving 2 or more of these 3 chromosomes are reportedly present in at least 95% of cases of high grade urothelial carcinoma, a condition referred to as polysomy. Low grade urothelial tumors typically lack polysomy. The criteria used for a "positive" UroVysion test is the detection of 4 or more cells that have gains in 2 or more of chromosomes 3, 7, and 17 in the same cell, or the identification of 12 or more cells that show deletion of the 9p21 locus. A negative UroVysion test does not rule out urothelial carcinoma, since low grade urothelial carcinomas usually do not have abnormalities detectable by UroVysion. Also, a positive UroVysion test is not completely specific for urothelial carcinoma, as occasional patients with prostate cancer or metastatic cancer involving the urinary tract may also have a positive UroVysion test.

A number of studies have reported very promising results for UroVysion as compared to other methods of monitoring patients for bladder cancer recurrence. One study (reported in the May 2002 edition of the Journal of Urology) included 280 voided urine specimens from 265 patients, 75 with biopsy-proven urothelial carcinoma. UroVysion was 81% sensitive and 96% specific for detection of urothelial cancer, which surpassed the performance of other methods for detecting cancer, including the BTA stat assay (78% sensitive and 74% specific), hemoglobin dipstick (74% sensitive and 51% specific), and the telomerase assay (46% sensitive and 91% specific). Reported figures for urine cytology are 58% sensitive and 96% specific. Other studies have reported similar and sometimes higher figures for UroVysion.

As one might expect, UroVysion can also be of value in helping to resolve "borderline" cytology cases. Additionally, it has also provided "anticipatory positive" results. In patients with negative cystoscopy



Photomicrographs (non-computer generated) of UroVysion FISH on abnormal urothelial cells. Note extra copies of several chromosomes. (Photo courtesy of Mr. Scott Donovan of Vysis).

and negative urine cytology, a high percentage of patients with a positive UroVysion test will develop recurrent tumor within 6-12 months, allowing these patients to be more closely monitored.

UroVysion FISH testing on urine is now available at ProPath.

References:

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