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Fluorescence in situ hybridization in diagnostic cytology☆

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Summary Fluorescence in situ hybridization (FISH) is a technique that uses fluorescently labeled DNA probes to detect chromosomal alterations in cells. FISH can detect various types of cytogenetic alterations including aneusomy (ie, abnormalities of chromosome copy number), duplication, amplification, deletion, and translocation. Because tumor cells generally contain chromosomal alterations, FISH is able to detect cells that have chromosomal abnormalities consistent with neoplasia in exfoliative and aspiration cytology specimens. This review will discuss the utility of FISH for the detection of bladder, lung, pancreatobiliary, and esophageal carcinoma in cytologic specimens.

1. Introduction

For more than a half century, the Papanicolaou stain and other cytochemical stains have been the primary modality used to identify tumor cells in cytologic specimens. However, in recent years, ancillary techniques such as DNA ploidy analysis by flow cytometry/image cytometry, immunocytology, and fluorescence in situ hybridization (FISH) have been increasingly used to facilitate the identification of neoplastic cells in cytologic specimens.

It is now widely recognized that tumors result from genetic and epigenetic alterations that activate oncogenes and inactivate tumor suppressor genes. Mutations within tumors occur at both the chromosomal and molecular level. Types of chromosomal alterations that occur in tumors include aneuploidy, deletion, amplification, and translocation.

FISH is a technique that uses fluorescently labeled DNA probes to detect chromosomal alterations in cells. FISH can detect various types of cytogenetic alterations including aneusomy (ie, abnormalities of chromosome copy number), duplication, amplification, deletion, and translocation. There are 2 types of FISH probes: chromosome enumeration probes (CEPs) and locus-specific indicator (LSI) probes. CEPs are used to detect aneusomy, whereas LSI probes are generally used to detect deletion, duplication, or amplification of specific genes.

Because tumor cells generally contain chromosomal alterations, FISH should be able to detect cells that have chromosomal abnormalities consistent with neoplasia in exfoliative and aspiration cytology specimens. This has proven to be the case, and FISH is now used to detect bladder and biliary tract cancers in urine and biliary tract brushings [1,2], respectively, and will likely soon be used to identify tumor cells in a variety of other cytologic specimens. The remainder of this review will discuss how FISH has been used to identify tumor cells in various types of cytologic specimens.

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2. FISH for bladder cancer detection

In the mid- to late 1990s, data began to appear in the literature suggesting that FISH might be a useful test for detecting bladder cancer in voided urine and bladder washing specimens [3-7]. In 2000, Sokolova et al [8] introduced what would become the first commercially available FISH probe set for bladder cancer detection in voided urine. This 4-target, multicolor FISH probe set, UroVysion (Abbott Molecular Inc, Des Plaines, IL), contains CEP probes for chromosomes 3, 7, and 17, and an LSI probe to the 9p21 band that are labeled with red, green, aqua, and gold fluorophores, respectively.

Since the development of the UroVysion probe set, numerous studies have compared the sensitivity and specificity of FISH with urine cytology. A recent review of the published literature [9] comparing the performance of FISH with conventional cytology reveals that FISH is more sensitive than cytology for all grades and stages of urothelial carcinoma (Table 1) [1,10-20]. The lower sensitivity of FISH for low-grade tumors is explained by the fact that some of these tumors are diploid or near-diploid tumors with relatively few chromosomal abnormalities [21,22]. However, failing to detect some low-grade tumors is less of a concern than missing high-grade tumors because low-grade tumors behave less aggressively.

In these same studies, the overall specificity of FISH was slightly lower than the specificity of urine cytology (85% versus 93%). The FISH assay is quite sensitive, and it is not uncommon for the assay to be positive for a patient in whom tumor cannot be identified. Several studies have now demonstrated that FISH can detect recurrent urothelial carcinoma before it is clinically evident by cystoscopy [10,11,19,23,24]. In the US Food and Drug Administration (FDA) trial that led to approval of UroVysion, Sarosody et al [10] reported that there were 36 patients with a negative cystoscopic examination but a positive FISH result. With continued longitudinal follow-up, 15 (42%) of these cases were found to have biopsy-proven tumor recurrence, with time to tumor diagnosis of 3 to 16 months (mean, 6.0 months). Conversely, among 68 patients who had a negative cystoscopy and a negative FISH result, only 13 (19%) had a biopsy-proven recurrence at 3 to 19 months (mean, 11.2 months; χ^2, P = .014). The patients with a positive FISH result but negative cystoscopy were referred to as "anticipatory positive" cases. A Kaplan-Meier curve showed that the time to tumor recurrence was significantly less (P = .014) for patients with anticipatory positive FISH results compared with those with negative FISH results. A recent report by Yoder et al [24] found that approximately 27% of patients with a negative or atypical cytology result had a positive FISH result but no evidence of tumor. However, approximately 65% of these patients were found to have tumor recurrence within 29 months, which further suggests that positive FISH results cannot be ignored despite an absence of clinically detectable tumor.

Table 1 Published sensitivities and specificities of cytology and FISH for the detection of bladder cancer [a]

Stage/grade	Weighted means	
	Cytology	FISH
Overall sensitivity	42	75
Ta	28	67
Tis	73	97
T1	67	90
T2-4	74	92
Grade 1	18	50
Grade 2	45	76
Grade 3	69	90
Overall Specificity	93	85

[a] Data compiled from 12 different studies [1,10-20] exploring the utility of cytology and FISH for the detection of urothelial carcinoma.

The UroVysion probe set received FDA approval for monitoring patients with bladder cancer for tumor recurrence in 2001 and for assessing patients with hematuria (gross and microscopic) for bladder cancer in 2005 [1,10]. Relatively few studies have compared FISH with other bladder tumor markers. However, two studies have compared FISH with BTA stat (a point-of-care bladder cancer detection immunoassay) for the detection of recurrent tumor in patients with a history of bladder cancer, and both have found that FISH is as sensitive as or more sensitive than BTA stat and significantly more specific than this assay [10,25].

FISH, with UroVysion, can be performed on any type of urine specimen including voided urine, urine obtained by catheterization, bladder and ureteral washings, and stomal urine specimens. However, voided urine is the specimen type that is approved by the FDA. An explanation of how to perform the UroVysion FISH assay is provided with the UroVysion test packet insert. However, modifications to this methodology have been reported, which allow laboratories to perform this assay specific to their practice [23,26,27].

When analyzing urinary cells, nonneoplastic cells generally show 2 copies for each of the 4 probes with the UroVysion probe set. Occasionally, normal cells will show only 1 copy of 1 or more of the 4 probes because of signal overlap or incomplete hybridization. Urothelial carcinoma cells, on the other hand, will show 1 of several types of chromosomal abnormality, which include polysomy, tetrasomy/near-tetrasomy, trisomy, and 9p21 loss. Representative examples of each of these types of chromosomal abnormality are shown in (Fig. 1A-D). In the authors' laboratory, the frequency of the different types of chromosomal abnormalities among our positive cases is as follows: polysomy, 90%; tetrasomy, 7%; trisomy 7 or 3, 2%; and homozygous 9p21 loss, 1%. The finding of polysomy generally correlates with the presence of a higher-grade tumor, whereas homozygous 9p21 loss alone almost invariably correlates with the presence of a low-grade papillary tumor. Relatively little data are available regarding the clinical correlates of tetrasomy and trisomy diagnoses.