Clinical FISH Testing for the Diagnosis of Solid Tumors

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Background

Cancer genomes contain numerical and structural somatic alterations, including single base substitutions, structural rearrangements, small insertions, small deletions, and copy number variation [1, 2]. Theodor Boveri, a German zoologist, is credited for first describing the association between numerical chromosome anomalies and cancer in his 1914 monograph *Concerning the Origin of Malignant Tumours* [3]. Nearly a century has passed since this sentinel paper and cancer biologists are still trying to better understand aneuploidy and its role in carcinogenesis. Recent large-scale DNA copy number analyses of tumors highlight how common aneuploidy is in malignant tumors. Beroukhim and colleagues [1] evaluated 3,131 tumors comprising 26 different tumor types and identified, on average, 24 gains (median = 12) and 18 losses (median = 12) for each tumor. In addition, approximately 17 % of the genome was amplified and 16 % was deleted in a typical cancer specimen.

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Fluorescence in situ hybridization (FISH) is a technique that uses fluorescently labeled DNA probes to detect chromosomal abnormalities in peripheral blood, paraffin-embedded tissue, or cytology specimens. Since tumor cells generally contain chromosomal alterations, FISH is able to detect cells that have chromosomal abnormalities consistent with neoplasia. Different types of DNA probes can be designed to target different chromosomal alterations including aneuploidy, deletions, amplifications, and translocations. Centromere enumeration probes (CEP) are designed to target highly repetitive human α-satellite DNA sequences (171 base pair repeats) located near the centromeres of individual chromosomes. These probes are used to enumerate the number of copies of a given chromosome in an individual cell. Locus-specific identifier (LSI) probes are designed to hybridize to specific chromosomal regions and can detect chromosomal loss (e.g., *CDKN2A*, *TP53*), gains/amplifications (e.g., *EGFR*, *HER2*), or translocations (e.g., *EML4-ALK*, *BCR-ABL*). LSI probes are becoming more popular because in addition to diagnosing cancer, alterations in specific genes (e.g., HER2 amplification) help predict whether a patient will respond to a targeted therapy (e.g., Herceptin). This chapter will focus on the diagnosis of solid tumors by FISH and discuss how this technique has changed clinical practice over the past decade.

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Clinical Applications

FISH for Bladder Cancer Detection

Bladder cancer is estimated to account for 73,510 new cases and 14,880 deaths in 2012 [4]. Most patients with bladder cancer present with painless and intermittent hematuria. However, only 10–20 % of patients with gross hematuria and 2–5 % with microscopic hematuria will actually have bladder cancer [5]. The diagnostic work-up for patients with bladder cancer includes initial cystoscopy followed by biopsy or resection of suspicious lesions, urine cytology, and upper urinary tract evaluation. Urine cytology complements cystoscopy in that it can detect tumors that are not visible by cystoscopy. However, urine cytology has relatively poor sensitivity for detecting bladder cancer, especially low-grade tumors. The reported combined sensitivity of cytology for grade 1, 2, and 3 tumors has been shown to be 21, 53, and 78 %, respectively [6]. Because urine cytology suffers from low sensitivity, numerous tumors markers have been investigated to help increase the diagnostic sensitivity [5].

FISH testing has become an excellent tool for overcoming some of the limitations of cytology. Much of the success of FISH testing for the diagnosis of solid tumors can be attributed to the FISH UroVysion assay™ (Abbott Molecular, Abbott Park, IL), which was the first commercially available FISH probe set for the detection of bladder cancer. In 2000, Sokolova et al. published the first manuscript describing UroVysion, a four-target, multicolor FISH probe set containing CEP probes targeting chromosomes 3, 7, and 17 and an LSI probe targeting 9p21 [7]. Halling et al. published a study later that year, which was the first to demonstrate the clinical utility of this probe set. This study evaluated urine from 265 patients being evaluated for bladder cancer and found that the overall sensitivity of FISH for detecting bladder cancer (81 %) was significantly higher than urine cytology (59 %) [6]. Two FDA trials followed, the first published by Sarosdy et al. in 2002, which led to the FDA approval of UroVysion for the detection of recurrent bladder cancer [8]. The second trial was published in 2006 and led to the FDA approval of UroVysion for the detection of bladder cancer in patients with gross or microscopic hematuria and no history of bladder cancer [9]. Numerous follow-up studies have compared the sensitivity and specificity of FISH to urine cytology and have consistently shown that FISH is more sensitive than cytology for all grades and stages of urothelial carcinoma. A meta-analysis evaluating 14 different studies found that the sensitivity of FISH and cytology for detecting bladder cancer was 72 and 42 %, while the specificity of FISH and cytology was 83 and 96 %, respectively [10].

There are several additional clinical indications for UroVysion testing [11]. Multiple reports have suggested that FISH can help identify which patients with equivocal cytology diagnoses (atypical or suspicious) are most likely to have bladder cancer [12–16]. The clinical management of patients with equivocal cytology diagnoses is challenging because fewer than half of these patients will have bladder cancer on clinical follow-up. This can lead to unnecessary and expensive clinical investigations [17]. Multiple studies now suggest that patients with an equivocal cytology and positive FISH result are at very high risk for having bladder cancer and should be followed more aggressively [12–16]. FISH is also useful for assessing patients undergoing bacillus Calmette–Guerin (BCG) treatment for noninvasive bladder cancer. A 2005 study by Kipp et al. found that patients with a positive FISH result following intravesical therapy were 4.6 times more likely to have recurrent bladder cancer and 9.4 times more likely to have follow-up muscle-invasive bladder cancer than patients with a negative FISH result [18]. Similar results were obtained by Mengual et al. [19] and Savic et al. [20], who found that patients with a positive post-BCG FISH result had 3.0 and 3.8 times higher risk of tumor recurrence, respectively. Although other tumor markers are currently available for diagnosing bladder cancer (e.g., BTA-STAT, NMP22), due to its high sensitivity and specificity, FISH with the UroVysion probe set continues to be one of the most commonly used molecular markers for detecting this type of cancer in urine cytology specimens.

FISH for the Detection of Pancreatobiliary Tract Malignancy

Hepatobiliary and pancreatic cancers represent over 82,000 newly diagnosed cancers a year in the USA, with pancreatic cancers and liver cancers representing the majority of these cases [4]. Although carcinomas of the pancreatobiliary tract ducts (pancreatic adenocarcinoma or cholangiocarcinoma) comprise only a small fraction of these malignancies, they are often lethal and can occur anywhere along the hepatic and common bile duct system [21]. An earlier diagnosis of pancreatobiliary tract malignancy is critical because it may allow for surgical resection of a tumor. It may also allow patients to become candidates for neoadjuvant chemoradiotherapy followed by a liver transplant, which has shown to decrease mortality in patients with cholangiocarcinoma [22]. However, differentiating pancreatobiliary tract malignancies from nonmalignant etiologies such as primary sclerosing cholangitis, choledocholithiasis, chronic pancreatitis, and surgical trauma can be difficult because pancreatobiliary tumors often grow longitudinally along the bile duct and do not generally present as large masses [23, 24]. Due to the difficulty of obtaining adequate biopsies within the pancreatobiliary ducts, routine cytology brushings or washings are often collected for diagnosing malignancy. Unfortunately, routine cytology has relatively poor sensitivity for detecting malignancy in biliary tract specimens with reports ranging from 6 to 80 %, and many reported sensitivities below 50 % [24–27].

In 2004, our group evaluated the value of FISH and the UroVysion probe set for detecting malignancy using pancreatobiliary tract brushing and bile specimens collected during endoscopic retrograde cholangiopancreatography [28]. Although the UroVysion probe set was not specifically tailored for the detection of pancreatobiliary tract tumors, we found that FISH improved the detection of these tumors when compared to routine cytology. Based on these data, we implemented FISH and the UroVysion probe set as a clinical assay in late 2004. Subsequent reports from our group and others confirmed that FISH is a valuable ancillary test for the evaluation of cytologic specimens obtained from pancreatobiliary tract strictures [23, 29–31]. Fritcher et al. [23] published the most comprehensive study evaluating the role of FISH in the detection of pancreatobiliary tract malignancy. This study included 500 patients undergoing clinical evaluation for suspicious pancreatobiliary tract strictures with clinical cytology and FISH results, as well as extensive clinicopathologic follow-up. The results of this study showed that the sensitivity of FISH was significantly higher than cytology (43 % vs. 20 %, $P<0.001$) for detecting malignancy. This study also found that a patient with a polysomy FISH result was over 77 times more likely to have malignancy than a patient with a negative FISH result. Based on these data, it has become routine practice at our institution to perform both routine cytology and FISH on all pancreatibiliary tract brushing specimens from indeterminate pancreatobiliary strictures when there is a suspicion for carcinoma.

FISH for the Detection of Lung Cancer

Cytology (brushings and washings) and biopsy specimens collected during flexible bronchoscopy are used for the diagnosis of suspected lung cancer in patients with indeterminate pulmonary nodules and endobronchial lesions. The overall diagnostic sensitivity of routine cytology bronchial brushing and washing specimens ranges from 44 to 94 % (mean, 72 %) and 27 to 90 % (mean, 68 %), respectively [32]. Peripheral lung tumors are more difficult to diagnose than centrally located tumors by cytology, with sensitivities ranging from 6 to 83 % (mean, 45 %) in brushings and 4 to 43 % (mean, 28 %) in washings [32]. In addition, the size of the tumor is also important with smaller tumors being more difficult to detect [33]. Data suggest that there remains a clinical need for a molecular marker such as FISH to increase detection rates in small (<2 cm in diameter) peripherally located tumors, where the combined sensitivity of bronchoscopic techniques has been reported to be 34 %, compared to 63 % for larger (>2 cm) tumors [33].

Sokolova et al. have been credited for publishing the first DNA-based FISH probe set to be used clinically to improve the detection of lung cancer on cytology specimens [34]. This FISH probe set (originally called LAVysion, Abbott Molecular, Inc.) consisted of locus specific probes to 5p15, 7p12 (*EGFR*), 8q24 (*C-MYC*), and a CEP to chromosome 1. However, it was suggested in this manuscript that it would be advantageous to substitute CEP6 for CEP1 due to staining quality. Several subsequent studies have evaluated the clinical utility of this FISH probe set (with CEP 6) and have shown that FISH increases the sensitivity of lung cancer detection when combined with routine cytology, as compared to routine cytology alone, while maintaining high specificity [35–40]. Two of the larger studies [35, 36] evaluating this FISH probe revealed similar sensitivity (61–65 %) and specificity (92–95 %) using RC and FISH on brushing specimens. In addition, these studies suggest that FISH may be helpful in detecting early stage and peripheral lung cancers that are difficult to detect using conventional cytology. Subsequent studies by Savic et al. and Schramm et al. have also shown that FISH may be particularly useful in patients with equivocal (atypical or suspicious) cytology results [37, 38].

A recent study by our group [40] found that FISH significantly increased the detection of lung cancer using a reflex algorithm in routine clinical practice. In this algorithm, routine cytology, which is relatively inexpensive and has high specificity, is initially performed. Specimens not found to be positive for cancer (i.e., negative, atypical and suspicious) by routine cytology are then sent on for FISH analysis. Based on data from nearly 300 specimens using this algorithm, FISH detected 32 % more peripheral lung cancers than routine cytology alone. The FISH test was most beneficial for detecting small (<2 cm), peripheral cancers where FISH detected 15 cancers (44 %) that were not detected by cytology.

Other FISH probe sets have also been evaluated with similar success. A group from MD Anderson designed and evaluated a probe set comprising chromosomal loci 3p22.1 (containing the *GC20*, *RPL 14*, *CD39A*, and *PMGB*) and 10q22.3 (surfactant protein A gene, *SP-A*) and showed that loss of either of these chromosomal loci by FISH, when combined with cytologic atypia, is more sensitive than cytology alone for diagnosing lung cancer in sputum specimens [41, 42]. Liu et al. [43] recently developed a FISH assay with probes targeting chromosomes 3, 7, and 8. In their study, the overall sensitivity of FISH on brushing specimens when combined with routine cytology was significantly higher (95 % vs. 76 %; $P<0.001$) than routine cytology alone and detected more squamous cell carcinomas and late stage tumors. However, as with any diagnostic molecular assay, all these new FISH probe sets will need to be validated using specimens from the intended patient population to determine their true clinical utility. In addition, these FISH lung assays should be evaluated as part of a testing algorithm that includes bronchoscopic biopsy, bronchial brushing routine cytology, and other bronchoscopic methods currently used in clinical practice.

FISH Barrett's Esophagus Associated Neoplasia

Barrett's esophagus (BE) is a pre-neoplastic condition in which the squamous epithelium of the distal esophagus undergoes transformation to intestinal metaplasia. Patients with BE have up to a 60-fold increased risk of developing esophageal carcinoma when compared to the general population [44, 45]. Current American College of Gastroenterologists guidelines suggest that patients with BE undergo routine surveillance for the detection of dysplasia or malignancy which includes the collection of four-quadrant biopsy specimens every 1–2 cm of affected esophagus [44]. Limitations to this procedure are that it is time consuming and results in a large number of biopsies. Brush cytology has been suggested as an alternative to endoscopic biopsy since cytology can collect cells from a larger surface area in less time than it takes to collect the numerous biopsies suggested by practice guidelines [46]. Unfortunately, cytology has limited sensitivity for detecting dysplasia or malignancy

because it can be difficult to differentiate reactive epithelium from dysplasia by routine cytology and because the cytologic features of early dysplastic cells are not significantly different from non-neoplastic Barrett's cells [47].

As with the other body sites discussed in this chapter, FISH has been shown to increase the sensitivity of detecting esophageal dysplasia and cancer over routine cytology alone [44, 46, 47–51]. Recent studies at our institution suggest that a probe set consisting of probes to 8q24, 9p21, 17q11.2, and 20q13.2 has high sensitivity and specificity for the detection of BE-associated dysplasia and esophageal adenocarcinoma [44]. Fritcher et al. evaluated this four-probe set and found that FISH was significantly more sensitive than routine cytology for detecting low-grade dysplasia, high-grade dysplasia, and adenocarcinoma in patients with BE [49]. The specificity of cytology, digital image analysis, and FISH among patients ($n=14$) with tissue showing only benign squamous mucosa was 93, 86, and 100 % ($P=0.22$), respectively. In addition, all 33 patients with a polysomic FISH result had HGD and/or EA within 6 months of the FISH test. Similar results were published by Rygiel et al. who concluded that this probe set (with the exception that the *P16* probe was removed) was useful for predicting BE patients at risk for developing high-grade dysplasia and adenocarcinoma [50].

Methodology

FISH can be performed on many different types of specimens including peripheral blood, paraffin-embedded tissue, and cytology specimens that are fresh or processed with standard (i.e., formalin, ethanol, etc.) or commercially available fixatives [52]. A number of different FISH protocols have been used for clinical testing with varied success. The steps involved in FISH analysis of tissue specimens can be divided into specimen collection, cell harvest, slide preparation, prehybridization, hybridization, washing, and microscopic analysis. Detailed protocols for preparing specimens for clinical testing can be found in previous publications [28, 34, 39] or package inserts from FISH probe kits. In general, the process begins by placing the collected cells or tissue on a glass slide for prehybridization. Prehybridization is a series of chemical treatments of the specimen that enables the FISH probes to efficiently hybridize to their cellular targets without disrupting cell morphology. This includes treatment with a protease such as pepsin to increase the accessibility of the probe to DNA. This is an important step in the process because overdigestion can lead to a decrease in FISH signal intensity and underdigestion can result in inefficient hybridization which makes it difficult to determine the true number of FISH signals in a cell. Following prehybridization, cellular DNA and FISH probe DNA are denatured and the FISH probes are allowed to hybridized to their cellular DNA targets. FISH hybridization is generally performed by placing approximately 1–10 μL of FISH probe on the cells being interrogated, placing a coverslip on top of this area, sealing the edges of the coverslip with rubber cement to prevent dehydration of the probe, and placing the slide in either a humidified chamber or a programmable temperature controlled slide processing system such as the ThermoBrite® Denaturation/Hybridization System (Abbott Molecular, Inc., Des Plaines, IL) [52].

Depending on the specimen being hybridized, co-denaturation of probe and target DNA is performed at approximately 70–75 °C for 3–5 min. The slides are then allowed to incubate at 37 °C overnight. After a minimum of 4 h hybridization time (preferably 8–16 h), slides are washed in a detergent such as 0.4× SSC/0.3 % NP-40 to remove nonspecifically bound probe. A fluorescent nuclear counterstain, such as 4′6′-diamidino-2-phenylindole (DAPI; Abbott Molecular, Inc., Des Plaines, IL) is then placed on the slide and the slide is coverslipped for analysis. Slides are microscopically assessed ("scanned") using a fluorescence microscope equipped with the appropriate filters that allow for visualization of the different colored fluorescent probes. FISH scanning and interpretation will be further discussed later in this chapter.

Regulatory Issues

Two Food and Drug Administration (FDA) trials have been conducted for UroVysion that evaluated different clinical claims for detecting bladder cancer [8, 9]. Minor deviations from the test packet have also been published with good results [11]. FDA trials have not been conducted for FISH probe use in diagnosing malignancies of the pancreatobiliary tract, lung, or Barrett's-associated neoplasia. Laboratories that offer laboratory developed FISH assays, i.e., assays that use non-FDA-approved specimens or methodologies that deviate from the FDA-approved methodology, need to comply with CLIA requirements, and should perform appropriate validation studies before using these tests clinically. This would include establishing test accuracy, precision, analytical sensitivity, analytical specificity, reference range, and reportable range. General recommendations on what is required for the validation of laboratory developed tests have recently been published [53]. In addition, a paper that specifically discusses how to go about validating a lab-developed FISH assay has been published [54]. However, this publication described the validation of a FISH assay that is used to detect MLL rearrangements for leukemia in paraffin-embedded specimens and consequently their may be some differences in the specifics of how to go about validating a FISH assay that is used for the detection of malignant cells in cytologic specimens. The CAP Molecular Pathology checklist also has a useful section that describes many of the regulatory requirments for FISH assays. For instance, the CAP checklist indicates that at least one photograph of a representative normal cell (with FISH signals captured) be kept as part of the

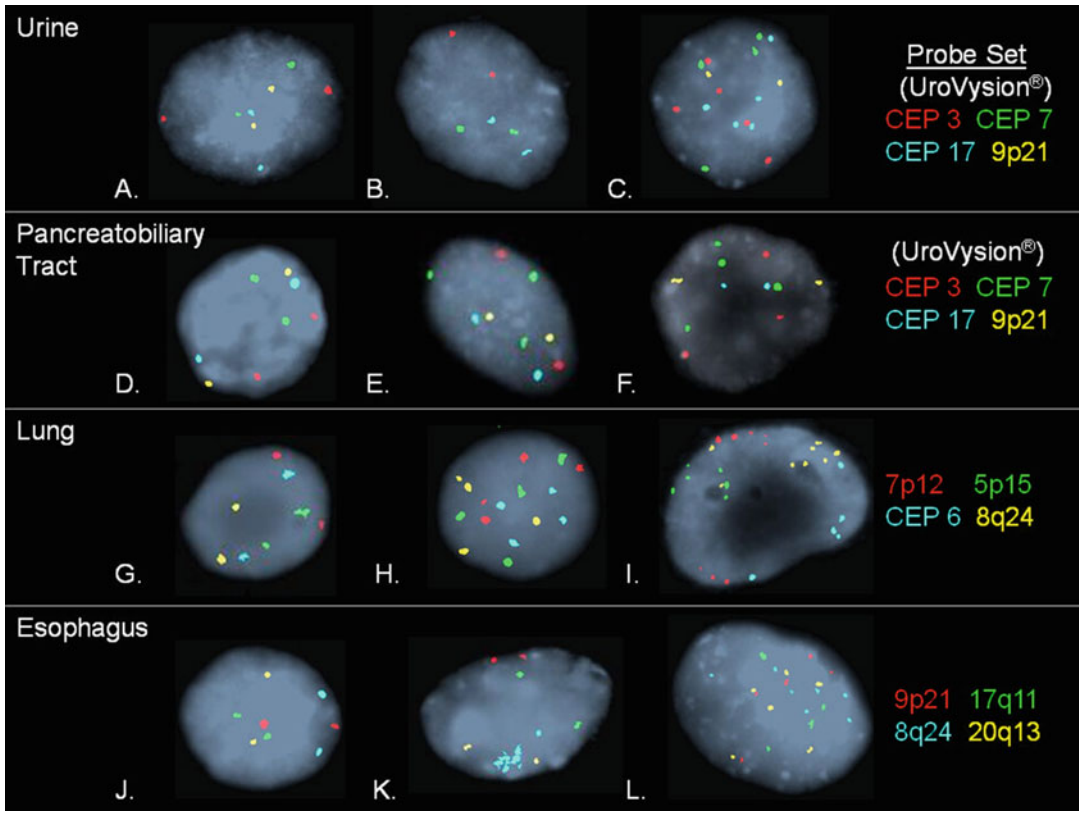

Fig. 1.1 Representative examples of FISH signal patterns observed in cytologic specimens from urine and brushing specimens from bile duct, lung, and esophagus. Normal (disomic) signal patterns (**a**, **d**, **g**, **j**), homozygous 9p21 loss (**b**), tetrasomy (**h**), polysomy (**c**, **f**, **i**, **l**), hypertetrasomy (**i**), trisomy 7 (**e**), and isolated gain (amplification) of 8q24 (**k**)

laboratory record for cases interpreted as normal, whereas at least two pictures be captured and documented for specimens with abnormal results.

Test Interpretation

Subsequent to specimen processing and hybridization, FISH signals are assessed using a fluorescence microscope equipped with the appropriate filters necessary to enumerate specific probe fluorophores. Non-neoplastic cells generally show two copies for each of the FISH probes since each probe targets the two alleles in an individual cell (Fig. 1.1). Occasionally, normal cells will show only one copy of a probe due to signal overlap or incomplete hybridization. Specimens are interpreted as abnormal when the number of cells demonstrating losses or gains of probes exceeds thresholds established in normal value studies. The scanning procedure used to identify aneuploid cells by FISH in cytologic specimens primarily focuses on assessing signal patterns in cells that appear morphologically abnormal (irregular nuclear shape, large nuclei, mottled nuclear staining) with the DAPI nuclear counterstain [55, 56]. Cells with abnormal appearing morphology by the DAPI counterstain are further assessed using the fluorescence microscope filters. Cells with abnormal FISH signal patterns are recorded and if the number of cells with these patterns exceeds predetermined cutoffs, the case is interpreted as abnormal. As illustrated in Fig. 1.1, numerous types of aneuploidy or chromosomal abnormalities are identified by FISH including polysomy (gains of two or more of the four probes in a cell), tetrasomy (four signals for all four probes), trisomy or single gain (gain of a single probe with two or fewer copies of the other probes), and homozygous loss (complete loss of both probes from an individual target). Each of these types of abnormalities can be identified with different FISH probe sets.

Thresholds for different types of chromosomal abnormality depend on the body site and probe set used [55]. In urine specimens that are being evaluated for bladder cancer with UroVysion, two primary types of chromosomal abnormalities are observed, polysomy and homozygous 9p21 (Fig. 1.1). The finding of polysomy generally correlates with the presence of a high-grade tumor, whereas homozygous 9p21 loss often suggests the presence of a low-grade papillary tumor [57]. There are potential pitfalls that one must avoid when interpreting urine specimens by FISH. Urine specimens can contain a wide variety of nonmalignant entities that can impede interpretation such as inflammatory cells, bacteria, proteinaceous debris, sperm, crystals, and lubricant [56]. It is generally still possible to interpret the FISH signals when one or more of these entities are present. However, there are instances where these entities obscure a majority of the epithelial cells causing the specimen to be uninterpretable. These specimens should be interpreted as nondiagnostic. Caution should also be used when evaluating urine specimens from patients receiving immunosuppressive therapy to prevent kidney rejection following transplantation, since these patients can harbor BK virus infected epithelial cells. Data from our group show that BK virus infection can be a rare cause of false-positive FISH results in patients with extremely high titers of the BK virus in their urine [58].

Although biliary and urinary tract specimens are both evaluated with the UroVysion probe set, there are differences in the types of FISH abnormalities that are observed for these two body sites. A large fraction of pancreatobiliary specimens with abnormality demonstrate trisomy 7 (three CEP 7 probes without gains in the other three probes; Fig. 1.1), while this is a rare finding in specimens from the urinary tract. A trisomy 7 result on pancreatobiliary tract specimens is considered an equivocal diagnosis, since only about 50 % of these patients will be diagnosed with malignancy on clinicopathologic follow-up [23]. Additionally, polysomic cells from urine often demonstrate high level gains of individual chromosomes (i.e., up to eight copies for each probe), while polysomic cells from the biliary tract cells infrequently demonstrate more than four or five signals for each of the four probes. However, independent of the degree of chromosomal gains observed, a polysomic result in either specimen is highly specific for malignancy [11, 23].

There are two main types of chromosomal abnormality observed in specimens being analyzed for lung cancer, hypertetrasomy and tetrasomy (Fig. 1.1). Hypertetrasomy refers to cells that show three or more copies in at least two of the four probes, with one or more of the probes exhibiting at least five copies. Tetrasomy is defined as four (or possibly three due to signal overlap) copies of the probe set for each of the probes. This distinction is important because a previous study suggests that 88, 53, and 37 % of patients with a hypertetrasomy, tetrasomy, and negative FISH result, respectively, were found to have lung cancer when FISH was performed on specimens diagnosed as negative or equivocal by routine cytology [40]. These data suggest that a hypertetrasomy FISH result is more specific and therefore more suggestive of lung cancer than a tetrasomy FISH result.

The methodology used to assess esophageal brushing cytology specimens by FISH, and the respective chromosomal abnormalities, is different than what has been described for other body sites. Multiple different chromosomal abnormalities may be observed in esophageal specimens including 9p21 chromosomal loss, single chromosome gain, amplification, and polysomy (Fig. 1.1). A previous report shows that microscopic FISH analysis of esophageal brushing specimens can be carried out by enumerating 100 non-squamous epithelial cells and if the percentage of cells demonstrating chromosomal loss or gain exceeds normal value cutoffs, the case is interpreted as abnormal. However, if no abnormalities are observed in the 100 cell enumeration or if the only abnormality that is observed is 9p21 loss or single gain, then the remainder of the slide should be scanned for polysomic cells. If $\geq$4 polysomic cells are observed in that additional scan, the specimen should be diagnosed as abnormal [49]. Patient's whose samples exhibit polysomy are likely to have high-grade dysplasia or cancer, while patients with hemizygous or homozygous 9p21 loss alone are likely to have Barrett's esophagus without dysplasia or Barrett's esophagus with low-grade dysplasia [49]. In summary, analysis of specimens by FISH is different based on the probe set used, the diagnostic cutoff values, and the body site analyzed.

How the Test(s) Have Changed Medical Practice

Aneuploidy detection by FISH has revolutionized how we detect tumor cells in cytologic specimens. Nearly all studies to date have suggested that FISH has a significantly higher sensitivity than conventional cytology for the detection of tumor cells in most specimen types. The improved ability to detect tumor offers the possibility of providing therapy at earlier more treatable stages and can reduce healthcare costs by reducing the amount of clinical evaluation required to arrive at a diagnosis. FISH is more time consuming and expensive to perform than conventional cytology and therefore has not replaced cytology. But FISH has become an extremely important ancillary tool for diagnosing selected specimens in cytopathology and cytogenetic laboratories.

Future Directions

We have summarized the success of FISH for detecting aneuploidy in solid tumors and have discussed the role FISH has played in changing clinical practice. As scientists and clinicians forge ahead in the genome era, the technologies used to detect aneuploidy will likely change, and aneuploidy assessment of tumors will likely be just part of large-scale genome analyses of individual tumors including DNA and RNA mutations analyses and epigenetic interrogation. The role that aneuploidy plays in carcinogenesis will also continue to be an active area of research. A recent review article by Gordon et al. [59] discuss this topic in depth and highlight the importance of aneuploidy in cancer development. More importantly, aneuploidy has become a promising target for future cancer therapies. These therapies may target mechanisms involved in tumor cell chromosomal instability (e.g., mutations that trigger instability), target cellular responses to aneuploidy (e.g., targeting specific pathways such as ubiquitin–proteasome pathway), or target specific cancer cells with genetic dependencies owing to recurrent chromosomal gains or losses [59]. No matter what the strategy, future studies

uncovering the genetic and epigenetic mysteries of solid tumor will transform what we know about these tumors and will hopefully shed light on how to cure and reduce the mortality associated with cancer.

Disclosure Drs. Kipp and Halling and the Mayo Clinic have patents and receive royalties from the sale of some of the FISH probes discussed in this study. They also receive grant funding from Abbott Molecular, Inc. to develop FISH assays for the detection of neoplastic cells in cytologic specimens.

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