Value of FISH Testing in Cancer Care

Within the clinical practice of molecular pathology, our understanding of deoxyribonucleic acid (DNA) and its functions is one of the most important discoveries of the last century. Our knowledge of DNA structure and function aids in understanding hereditary factors within disease, assessing risk factors in advance of disease development, and developing medications to treat several genetic diseases, including cancer.

Among the numerous, medically beneficial technical tools available to decipher DNA changes is fluorescence in situ hybridization (FISH). FISH is a powerful technique used in diagnostic, prognostic, and predictive cancer testing (see SIDEBAR). The key element of this technology is the utilization of fluorescent probes to detect DNA sequences of interest. A cancer diagnosis is most often based on pathologic interpretation of the hematoxylin and eosin (H&E) stained slide and immunohistochemical stains. Once the determination of malignancy has been made, FISH testing is utilized to determine if a targeted therapeutic drug is applicable.

Paraffin FISH

Pathologists use paraffin FISH testing—the application of the FISH methodology to formalin-fixed, paraffin-embedded (FFPE) sections—to confirm or exclude a histologic diagnosis, or as a tool to determine appropriateness of targeted therapy in cancer patients. There are several benefits of paraffin FISH testing, including the ability to perform retrospective studies, and the ability to identify the cells of interest morphologically and score the area of interest. However, as compared to FISH testing on conventional suspension samples, paraffin FISH can be labor intensive and variable due to differing fixation times between samples and referring histology labs, and the interpretation must be carefully reviewed and the assay vigilantly controlled due to truncation of signal and overlapping cells. Despite these challenges, paraffin FISH is advantageous to cancer testing given the multitude of targeted therapies that are now available.

Paraffin FISH must be considered separately from the conventional suspension FISH method, and it must be noted that due to the use of interphase nuclei, a prior knowledge of the anomaly of interest is required (see FIGURE 1).

FIGURE 1
How does FISH work?

<table>
<thead>
<tr>
<th>Paraffin</th>
<th>Cytospin/Fresh Prep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>Denaturation</td>
</tr>
<tr>
<td>Probe Denaturation</td>
<td>Hybridization</td>
</tr>
<tr>
<td>Overnight</td>
<td>Post Hybridization Wash</td>
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SIDEBAR

Cancer Diagnosis, Prognosis, and Predictive Therapy

Diagnosis

Some cancers develop as a result of chromosomal changes, such as translocations, and these changes are linked to specific types of cancer. Translocations occur when a section of DNA moves from one chromosome to another chromosome. The resulting DNA sequence may cause a chimeric protein to be produced, which in turn could be the genetic event that causes carcinogenesis. Translocation FISH probes can be used in the diagnosis of this type of cancer.

Prognosis

Other cancers may develop as part of a multiple-hit theory of carcinogenesis. In the multiple-hit theory, more than one genetic mutation is required for the development of cancer. In these types of cancer, multiple FISH probes may be used.

Predictive or Targeted Therapy

FISH testing for solid tumor oncology is often performed after the patient has been diagnosed with cancer. For example, following a breast cancer diagnosis based on an H&E examination, FISH studies can be ordered to determine if amplification of the HER2/neu gene is present. If amplification is present, the patient may be eligible for the drug Herceptin.
FISH

Methods and Tissue Preparation
The two FISH methods commonly utilized in clinical laboratories differ in their provenance. The sample type determines which FISH method will be used. The initial method was developed in cytogenetics laboratories that first performed FISH on samples, such as blood and bone marrow. This is known as metaphase FISH because the cells are arrested during the metaphase portion of the cell cycle. The more recently developed method is the previously mentioned paraffin method, which is referred to as the histologic method due to its derivation from methods typically used in immunohistochemistry (IHC) laboratories. This method is also known as interphase FISH because the cells are in their natural cell cycle state within the tissue and have not been arrested in metaphase. Regardless of the method utilized, superior technical skill and strict quality control are necessary for the procedure to be reproducible in the clinical lab (see FIGURE 2).

Metaphase and Interphase FISH
Metaphase FISH—most commonly used with blood for hematologic oncology cases—is performed in cells that have been arrested in the metaphase portion of the cell cycle while DNA is coiled into recognizable chromosomes. Interphase FISH is used when the DNA is unwound and is most often performed using FFPE tissue, which double-stranded DNA is essentially unzipped, or opened, using a combination of heat and chemical treatment. Both the DNA denaturation, also called DNA melting, is the process by which double-stranded DNA is essentially unzipped, or opened, using a combination of heat and chemical treatment. Both the DNA and the DNA probe must be denatured so that DNA denaturation, also called DNA melting, is the process by which double-stranded DNA is essentially unzipped, or opened, using a combination of heat and chemical treatment. Both the DNA and the DNA probe must be denatured so that...
when favorable conditions are applied, the probe can hybridize to the tissue. Under favorable conditions, hybridization occurs when the fluorescently labeled probe binds to the DNA in the tissue according to the specific sequence.

**Probes in Use**

Genetic aberrations are detected by FISH based on the type of probe correlated to the type of aberration. For example, amplification probes are used to determine if a gene is amplified within a cell. Normally, a cell has two copies of a particular gene, but amplification indicates that more than two copies per cell are present. HER2/neu is an example of an amplification probe.

Alternatively, deletion probes are used to determine if a gene has been deleted and whether the deletion is homozygous (both copies of the gene are deleted) or hemizygous (one copy of the gene is deleted). Examples of deletion probes include PTEN and 9p21. A third type, the translocation probe, is used to determine if a gene or portion of a gene has translocated to another chromosome. This type of probe is usually a break-apart probe, which has a fluorescent signal on both ends. If the DNA is separated due to a translocation, the signals will appear separated (hence the break-apart terminology). Examples of this type of probe include EWSR1 found in Ewing sarcoma and SYT found in synovial sarcoma.

Keep in mind that FISH probes can be expensive, and specialty equipment and experienced technologists and pathologists are required for proper testing to be performed effectively. As such, FISH testing is not inexpensive; nevertheless, it is significantly less expensive than an errant course of chemotherapy. Using the FISH results, clinicians can be confident in their chemotherapy selection.

**Necessary Equipment**

Cytogenetics laboratories usually perform FISH on metaphase slides and, therefore, they typically have the necessary equipment to perform FISH assays with the possible exception of the microscope. IHC laboratories should have the necessary equipment to perform FISH assays as well, with the possible exception of a fluorescent microscope. If the IHC laboratory performs immunofluorescence assays, a fluorescent microscope would be available. Beyond this, basic equipment necessary for FISH include a pH meter, balance, stir plate, microcentrifuge, refrigerator/freezer, waterbath, and slide drying oven.

**Value of Accurate Testing**

The basic premise of FISH testing involves establishing the area of interest on the H&E slide, then transferring this area to an unstained paraffin slide, which is then pretreated, probed, and co-denatured using FISH methodology. Crucial to paraffin-based analysis is the assessment of signal patterns in the cells. For this reason, robust internal and external quality control slides are required for diagnostic paraffin FISH testing in order to decrease the likelihood of an incorrect result due to an analysis error.

There are a number of variations to the basic FISH method that can be used in cancer treatment depending on the nature and number of samples being processed and the type of technology in use. On a related note, reimbursement for FISH testing has a good history, and new CPT codes are developed frequently to account for new types of FISH being performed.

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