FISH for Cutaneous Melanoma

Background

Melanoma is the deadliest form of skin cancer. In 2013, it is estimated that 76,690 new cases of melanoma will be diagnosed with 9,480 deaths.[1]

Although the majority of melanomas can be distinguished from benign nevi on the basis of histologic criteria, a significant subset of melanocytic proliferations present conflicting features that preclude a definitive diagnosis for even the most expert pathologists. An unacceptably high number of lesions cannot be precisely and reproducibly classified as either entirely benign or malignant by routine histologic and immunohistochemical techniques. The morphologic limitations in the diagnosis of these histologically borderline lesions lead to both under- and over-diagnosis of melanoma. Over-diagnosis of melanoma can lead to inappropriate therapy and psychologic burdens, whereas under-diagnosis can lead to inadequate treatment of a potentially deadly cancer.

The fluorescence *in situ* hybridization (FISH) molecular assay helps to distinguish these borderline cases by looking for DNA abnormalities present in the vast majority (95%) of melanomas but typically absent in benign nevi. The identification of common chromosomal aberrations in melanomas that are typically absent in benign nevi has led to the use of FISH probes on chromosomes 6 and 11 that can aid in the diagnosis of melanoma in histologically ambiguous cases. The following 4 FISH probes targeting three loci – RREB1 (6p25), MYB (6q23) and CCND1 11q13, and one centomere on two chromosomes accurately distinguished melanoma from benign nevi with a high sensitivity and specificity. The melanoma FISH test can be an important tool in the diagnosis of melanoma in cases with ambiguous or borderline histological findings, and thereby significantly impact the management of patients by guiding the most appropriate treatment.

Clinical Indications

Although the clinical and pathologic diagnosis of some melanomas is clear-cut, the diagnosis of certain melanocytic proliferations remains one of the most challenging areas in pathology. The differential diagnosis could be an entirely benign lesion such as a Spitz nevus versus a melanoma with spitzoid morphologic features by conventional histopathological evaluation. The melanoma FISH test is an important tool in the diagnosis of melanoma in cases with ambiguous or borderline microscopic findings. It has increased sensitivity and specificity compared to other ancillary techniques such as immunohistochemistry. This assay is not to be used as a screening test for obvious melanoma and should always be ordered as a part of a dermatologic pathology consult and interpreted in a working context.

Methodology

The test can be performed on routinely processed formalin-fixed paraffin-embedded tissue, and utilizes DNA probe hybridization, fluorescence microscopy and signal enumeration. The test is similar to other diagnostic FISH assays.

Short DNA fragments (FISH probes) are hybridized to a 5 μm–thick, formalin-fixed, paraffin-embedded section of a tumor of interest. Overlapping wavelength spectrums of the currently available fluorochromes limit the maximum to four probes that can be concurrently hybridized on a single slide. Nonbound, fluorescently labeled DNA is washed away. The section on a slide is then examined under a fluorescence microscope and demonstrates specific signals enumeration.

Interpretation

A distinct fluorescent signal indicates a single copy of the gene or centromere. Ideally, each diploid nucleus will display two signals for each analytical chromosomal locus probed, subject to truncation artifact. Identification of three or more signals for a DNA region of interest is consistent with a gain at that locus. If a DNA region of interest is deleted, less than 2 (1 or 0) signals will be identified. In a typical 4 to 6 μm formalin-fixed, paraffin-embedded (FFPE) section, nuclear truncation occurs. Therefore, for many perfectly normal diploid cells, hybridized probes will be outside the plane of section or overlapping, resulting in less than 2 signals per nucleus. False-positive or false-negative signals can result from imperfect hybridization. Flourescence microscopy can then be used to ascertain the number of signals per probed locus in each cell nucleus detected by specific fluoresence. Because of the inherent variability of FISH signals, each experiment requires the examination of an adequate number of cells and the implementation of strict quality control measures. The collected data are presented as a percentage of nuclei containing more than 2 copies of a particular locus

or as a percentage of cells showing an increase or loss of signals in comparison to a probe directed against a centromere region of a chromosome. With appropriate controls, the signal cutoffs (a percentage of cells that must fulfill a particular FISH criterion for a result to be considered abnormal) have been determined and validated for each probe. Rigorously interrogated cutoff values are critical to the robustness of a FISH assay.

At Cleveland Clinic Laboratories, our analyses are performed by an experienced molecular technologist working closely with the molecular pathologist to ensure signal enumeration is performed for the appropriate melanocytic target cells. Final interpretation is performed by a board-certified dermatopathologist.

Limitations of the Assay

For pathologists and clinicians contemplating incorporating melanoma FISH in their practice, it is critical to understand nuances of the FISH technique, the potential pitfalls, and the ancillary role for this test in the pathologic evaluation of melancytic lesions. Thus, cutaneous melanoma FISH must not be used as a stand-alone test and must be considered as a diagnostic adjunct to routine pathologic examination of tissue and clinicopathologic correlation.

References

1. American Cancer Society. Cancer Facts and Figures 2013. http://www.cancer.org/acs/groups/content/@epidemiologysurveilance/documents/document/acspc-036845.pdf. Accessed Feb. 11, 2013.
2. Song, J, Mooi, WJ, Petronic-Rosic, V, Shea, CR, Stricker, T, Krausz, T. Nevus versus melanoma: to FISH or not to FISH. *Adv Anat Pathol*. 2011, May; Vol. 18, No. 3; 229-234.
3. Gerami P, Mafee M, Lurtsbarapa T, Guitart J Haghighat Z, Newman M. Sensitivity of fluorescence in situ hybridization for melanoma diagnosis using RREB1, MYB, Cep6, and 11q13 probes in melanoma subtypes. *Arch Dermatol*. 146(3):273-278.
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Test Overview

Test Name	FISH for Melanoma
Ordering Mnemonic	CMFISH
Methodology	Short DNA fragments (FISH probes) are hybridized to a 5 μm–thick, formalin-fixed, paraffin-embedded section of a tumor of interest.
Specimen Requirements	Formalin-fixed paraffin-embedded tissue 5um thick
Billing Code	89257
CPT Codes	88368x2

Technical Information Contact:

Wendy Nedlik
216.444.8410
nedlikw@ccf.org

Scientific Information Contacts:

Steven Billings, MD
216 444.2826
billins@ccf.org

Roger Klein, MD, JD
216.445.0776
kleinr3@ccf.org