Fluorescence In Situ Hybridization

Amy Y.-Y. Chen¹ and Andrew Chen²

Journal of Investigative Dermatology (2013) 133, e8. doi:10.1038/jid.2013.120

Fluorescence in situ hybridization (FISH) is a cytogenetic technique used to detect the presence or absence and location of specific gene sequences. It can visualize specific cytogenetic abnormalities (copy number aberrations) such as chromosomal deletion, amplification, and translocation. FISH has been used in prenatal diagnosis and has served both as a diagnostic and as a prognostic marker for various sarcomas. More recently, FISH entered the field of dermatology in aiding the evaluation of ambiguous melanocytic lesions. This article will discuss the concept of FISH, its application, and its advantages and limitations in dermatology, with an emphasis on melanoma.

HOW IS FISH PERFORMED?

FISH involves the binding, or annealing, of fluorescence-labeled, target-specific nucleic acid probes to their complementary DNA or RNA sequences and the subsequent visualization of these probes within cells in the tissue of interest. The tissue of interest can either be formalin-fixed, paraffin-embedded sections or fresh-frozen tissue.

First, the DNA or RNA sequences from the tissue of interest are allowed to denature to become single stranded. Next, a FISH probe is selected and applied. The selection of an appropriate FISH probe is a critical step for enhancing its value as a diagnostic test because FISH only detects those chromosomal abnormalities that are specifically targeted by the probes used. Different probes are used depending on the diseases or malignancies under investigation. For example, a large number of recurrent cytogenetic abnormalities have been found in melanomas by comparative genomic hybridization (CGH); these recurrent abnormalities serve as excellent candidates for FISH probes (Song et al., 2011).

Once the probe is selected, the fluorescence labeling of the probe can be done either directly or indirectly. In direct fluorescence labeling, the fluorochrome(s) to be detected by the fluorescence microscope is directly bound to the probe DNA. In indirect labeling, a hapten, which is not visible under a fluorescence microscope, is incorporated into the probe DNA. The hapten is then detected immunohistochemically by a fluorophore-tagged antibody directed against the hapten. Next, the fluorescent-labeled probe and the target DNA or RNA sequences are brought together in the hybridization process, during which the fluorescent-labeled probe anneals to the targeted sequences. Posthybridization washings remove excessive unbound probe. The slides are then examined. Principles of FISH are illustrated in Figure 1.

Because FISH can be performed on formalin-fixed, paraffin-embedded tissue, it is possible for a pathologist to select a specific area or areas of tumors to be examined by FISH. This enables correlation between FISH results and tumor morphology under conventional light microscopy. For more details about FISH, readers are referred to the Fluorescence In Situ Hybridization (FISH)—Application Guide (Liehr, 2009).

INTERPRETATION OF FISH

Each fluorescently labeled probe that hybridizes to a cell nucleus in the tissue of interest appears as a distinct fluorescent dot. Each dot identifies a single copy of the chromosomal locus with a homologous DNA sequence. Diploid nuclei will have two dots. If there is duplication in the region of interest,

WHAT FISH DOES

• FISH is used to visualize specific cytogenetic abnormalities.
• It can serve as a supplementary diagnostic tool in pigmented lesions. However, it should not be used as a stand-alone test.
• FISH cannot replace traditional histopathologic analysis.
• FISH must correlate clinical, pathologic, and molecular information.

LIMITATIONS

• Probe design requires knowledge of specific chromosomal abnormalities to be studied.
• Cutoff signals may differ among laboratories.
• Processing errors, imperfect hybridization, nonspecific binding, photobleaching, interobserver variability, and false-positive and -negative results are possible.

¹Department of Dermatology, Boston University School of Medicine, Boston, Massachusetts, USA and ²Division of Plastic and Reconstructive Surgery, Department of Surgery, Henry Ford Health System and Wayne State University, Detroit, Michigan, USA

Correspondence: Amy Y.-Y. Chen, Department of Dermatology, Boston University School of Medicine, Boston, Massachusetts 02118, USA.
E-mail: ayyen@alum.mit.edu
CGH typically requires paraffin block preparation. In addition, copy number changes must be present in at least 30–50% of the cells for them to be evident on CGH analysis, whereas FISH requires only 20–30 well-visualized cells to provide an accurate count of fluorescence signals. As a result, FISH can be used both in large bulky tumors and in tumors in which the malignant component only contributes to a small proportion of the overall cellular populations. Last, FISH probes can demonstrate balanced translocations that are not detectable at CGH resolution (Gerami and Zembowicz, 2011).

**FISHING IN DERMATOLOGY: MELANOMA AND MORE**

In contrast to melanoma and a subset of Spitz nevi, melanocytic nevi do not show chromosomal aberrations after karyotyping or CGH. These cytogenetic differences have been exploited to aid in the diagnosis of ambiguous melanocytic lesions. A landmark study to identify the most accurate FISH probes for melanoma was carried out in 2009 (Gerami et al., 2009a). Fourteen candidate cytogenetic abnormalities detected on CGH in prior studies were tested through FISH on 148 melanomas and 153 nevi (including 17 Spitz nevi and 30

---

**WHAT FISH CANNOT DO: COMPARISON BETWEEN FISH AND CGH**

To perform FISH, one must know what one is “FISHing” for. The FISH result is only positive or negative in relation to the interrogated chromosomal region. This is in contrast to CGH, in which copy number aberrations of the entire genome in a tissue of interest are interrogated in a single experiment. Compared with FISH, CGH is more expensive and has a longer turnaround time. Because cells must be microdissected,
The majority of Spitz nevi have a normal chromosomal analysis; however, a subset of Spitz nevi have been found to have increased copy number of chromosome 11p (Figure 2) (Bastian et al., 1999). The increase in 11p has not been found in melanoma, allowing for possible differentiation in difficult-to-diagnose spitzoid melanocytic neoplasms.

FISH may play a future role in determining prognosis and identifying tumors with greater metastatic potential, although the clinical utility of these have yet to be determined. For example, FISH analysis for monosomy 3 has helped confirm the diagnosis of metastatic uveal melanoma (Gerami et al., 2009a). These four probes are now commercially available and have been tested in a number of subsequent studies to aid in the diagnosis of ambiguous melanocytic lesions. FISH has been used to distinguish nevus from mitotically active nevi (Gerami et al., 2009b).

Although FISH can serve as an adjunct in diagnosis of melanocytic lesions, there are intrinsic limitations to its results such as processing errors, imperfect hybridization, nonspecific binding, photobleaching caused by prolonged light exposure, and interobserver variability, as well as false-negative and false-positive (tetraploidy) results. Furthermore, because melanomas are genetically heterogeneous, different genetic aberrations may be seen in different sections of the same tumor, underscoring the importance of selecting the most appropriate area(s) for FISH analysis.

Use of the FISH technique in other areas of clinical dermatology has not been as well established. For example, although FISH is not routinely used in the diagnosis of cutaneous lymphomas, recent publications investigating specific gene rearrangement, deletion, or translocation have shown the potential for future applications in cutaneous T-cell lymphoma to provide a biologic basis for possible gene-directed therapy as well as prognosis (Marty et al., 2008; Pham-Ledard et al., 2010). Figures 3 and 4 show the use of FISH to identify IFN regulatory factor 4 gene (IRF4) translocation in certain cases of cutaneous T-cell lymphoma. Figure 3 shows in schematic fashion how break-apart, dual-color fluorophore-labeled probes can be used to demonstrate translocations, as well as extra copies of the locus of interest. IRF4 gene probes directed against the 5′ and 3′ ends were differentially labeled (one red, one green) and hybridized. Figure 4 shows a comparison of binding to normal and diseased tissues; the FISH pattern in Figure 4d is normal, with dual color (red and green). In Figure 4h, the signal pattern is split (one locus is dual color, but the other red and green are split), which is consistent with a translocation. Figure 4l demonstrates an extra signal, which is consistent with an extra copy of the IRF4 locus. More recently, FISH has been utilized to assess clonality in bone marrow and skin infiltrates in patients with neutrophilic dermatoses and myeloid malignancy (Sujobert et al., 2012).

Figure 3. IRF4 locus-specific fluorescence in situ hybridization (FISH) strategy. (a) Schematic representation of all BAC clones hybridizing to the 6p25 region used in this study. RP11-119L15, CTD-2317K17, and CTD-3052J12 BAC clones, which showed nonspecific hybridization on chromosome 16, were discarded. The break-apart BAC probe strategy used CTD-2308G5 as the 5′ IRF4 BAC probe and RP11-164H16 as the 3′ IRF4 BAC probe. (b) The FISH signal pattern expected in interphase nuclei samples. Normal nuclei would exhibit a two-fusion (2F) signal pattern corresponding to the juxtaposition of BAC clones probes. Nuclei with an IRF4 locus break point, suggesting translocation, would show a split signal pattern (1F–1R–1G). Nuclei with extra copies of a nonrearranged IRF4 locus should exhibit more than a 2F signal pattern. Reprinted with permission from Pham-Ledard et al., 2010.

Figure 4. Histological, immunophenotypical, and fluorescence in situ hybridization (FISH) aspects of three typical cases with and without IRF4 locus rearrangement. Left: Lymph node section of cutaneous anaplastic large-cell lymphoma (c-ALCL) without IRF4 locus rearrangement. (a) Hematoxylin–eosin and safran (HES). Bar = 20 μm. (b) Positivity of CD30 immunostaining. (c) Immunostaining shows expression of multiple myeloma antigen 1 (MUM1) by more than 85% of tumor cells. (d) Normal FISH signal pattern (2F). Bar = 5 μm. Middle: Skin section of case 6 with C-ALCL and IRF4 rearrangement. (e) HES, ×400. (f) Positivity of CD30 immunostaining. (g) Immunostaining shows MUM1 expression by more than 85% of tumor cells. (h) Split FISH signal pattern (1F–1R–1G). Bar = 5 μm. Right: Skin section of case 8 with transformed mycosis fungoides and IRF4 locus rearrangement. (i) HES, ×400. (j) Positivity of CD30 immunostaining. (k) Immunostaining shows MUM1 expression by 10–50% of large tumor cells. (l) FISH signal pattern shows an extra signal of SpectrumGreen-labeled RP11-164H16 (2F+1G extra signal), indicating that the break point maps to the 6p25 region in the genomic area corresponding to the RP11-164H16 sequence. Bar = 5 μm. Reprinted with permission from Pham-Ledard et al., 2010.
SUMMARY
FISH is a powerful technique used to visualize specific cytogenetic abnormalities. Its most significant role in dermatology to date lies in its ability to aid in the diagnosis and management of ambiguous melanocytic lesions. Although recent studies have suggested that FISH can be used as a supplementary diagnostic tool in pigmented lesions, FISH must not be used as a stand-alone test, and it cannot replace traditional histopathologic analysis. One must correlate clinical, pathologic, and molecular information. “FISHing” in dermatology continues to evolve, and we look forward to future studies to further delineate its roles in various dermatologic diseases.

CONFLICT OF INTEREST
The authors state no conflict of interest.

ACKNOWLEDGMENT
We thank Pedram Gerami for his critical review.

SUPPLEMENTARY MATERIAL
Answers and a PowerPoint slide presentation appropriate for journal club or other teaching exercises are available at http://dx.doi.org/10.1038/jid.2013.120.

REFERENCES
Bastian BC, Wesselmann U, Pinkel D et al. (1999) Molecular cytogenetic analysis of Spitz nevi shows clear differences to melanoma. J Invest Dermatol 113:1065–9
Busam KJ, Fang Y, Jhanwar S et al. (2012) Diagnosis of blue nevus-like metastatic uveal melanoma confirmed by fluorescence in situ hybridization (FISH) for monosomy 3. J Cutan Pathol 39:621–5
Gerami P, Zembowicz A (2011) Update on fluorescence in situ hybridization in melanoma: state of the art. Arch Pathol Lab Med 135:830–7
Gerami P, Jewell SS, Morrison LE et al. (2009a) Fluorescence in situ hybridization (FISH) as an ancillary diagnostic tool in the diagnosis of melanoma. Am J Surg Pathol 33:1146–56
Gerami P, Wass A, Mafee M et al. (2009b) Fluorescence in situ hybridization for distinguishing nevoid melanomas from mitotically active nevi. Am J Surg Pathol 33:1783–8
Liehr T (2009) Fluorescence In Situ Hybridization (FISH)—Application Guide. Springer: Berlin, Germany
Marty M, Prochazkova M, Laharanne E et al. (2008) Primary cutaneous T-cell lymphomas do not show specific NAV3 gene deletion or translocation. J Invest Dermatol 128:2458–66
O’Connor C (2008) Fluorescence in situ hybridization (FISH). Nat Educ 1(1)
Pham-Ledard A, Prochazkova-Carlotti M, Laharanne E et al. (2010) IRF4 gene rearrangements define a subgroup of CD30-positive cutaneous T-cell lymphoma: a study of 54 cases. J Invest Dermatol 130:816–25
Song J, Mooi WJ, Petronic-Rosic V et al. (2011) Nevus versus melanoma: to FISH, or not to FISH. Adv Anat Pathol 18:229–34
Sujbert P, Cucuconi W, Vignon-Pennamen D et al. (2012) Evidence of differentiation in myeloid malignancies associated neutrophilic dermatosis: a fluorescent in situ hybridization study of 14 patients. J Invest Dermatol 133:1111–1114