Clinical Application and Limitations of the Fluorescence In Situ Hybridization (FISH) Assay in the Diagnosis and Management of Melanocytic Lesions: A Report of 3 Cases

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Histopathologic examination is the gold standard for the diagnosis of melanocytic lesions, including melanoma, and guides management options and disease prognosis based on the depth of invasion. Although most melanomas can be readily distinguished from benign nevi, some pigmented lesions are more ambiguous and can be challenging to interpret as truly benign or truly malignant. Unfortunately, misclassification can render severe consequences for the patient, making it imperative to explore further analysis to determine the true nature of an ambiguous lesion. A relatively new technique known as fluorescence in situ hybridization (FISH) has become prevalent in dermatopathology for distinguishing between benign and malignant pigmented lesions; however, there are few reports on the application of FISH results in the clinical setting. We present 3 cases in which a FISH assay was utilized to assist in the diagnosis and management of ambiguous pigmented lesions. We also provide a review of the most recent literature regarding this diagnostic modality.

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Histopathologic examination currently is the gold standard for the diagnosis of melanocytic lesions, including melanoma. Although most melanomas can be readily distinguished from benign nevi, some pigmented lesions can be more challenging to diagnose. Even among expert dermatopathologists, the diagnostic concordance is quite low when it comes to ambiguous melanocytic lesions[1]; in fact, variable interpretation can occur even in lesions that are not considered to be morphologically ambiguous.[2,3] Unfortunately, misclassification can render severe consequences for patients including mortality due to underdiagnosis or considerable morbidity from overdiagnosis that can lead to unnecessary procedures such as wide local reexcision, lymph node biopsy or dissection, or aggressive chemotherapy.

If a dermatopathologist encounters a difficult-to-interpret melanocytic lesion, the case may be discussed in a conferencelike setting or slides may be sent to peers for consultation after additional staining. A second opinion from an expert dermatopathologist has been shown to improve patient care in 27% of cases involving difficult melanocytic lesions[4]; however, disagreement among expert dermatopathologists can occur in up to 25% of cases.[5-7] The thickness of the lesion can sometimes assist in determining appropriate management options,[8] but because of the potential legal ramifications associated with missed diagnoses, ambiguous lesions may be incorrectly classified as melanomas.[9] As a result, new technologies are in development that aim to unequivocally diagnose and confirm melanomas. The need for such tools is clear; however, it is

imperative that clinicians always make a correlation between clinical and pathologic findings.

We present 3 cases in which the fluorescence in situ hybridization (FISH) assay was used to diagnose and guide the management of ambiguous pigmented lesions. We also provide a review of the most recent literature regarding this diagnostic modality. In all 3 cases, FISH analysis was performed by a specialist trained in cytogenetics. Criteria for FISH positivity have been provided by Gerami et al.[9] For a specimen to be considered positive, it had to demonstrate 1 of the following criteria: (A) gain in RREB1 (ras responsive element binding protein 1) relative to CEP6 (chromosome 6 centromere) greater than 55%; (B) gain in RREB1 greater than 29%; (C) loss of MYB (myeloblastosis oncogene) relative to CEP6 greater than 40%; or (D) gain in CCDN1 (cyclin D1) greater than 38%.[9] After obtaining FISH results, each case was extensively discussed in a multidisciplinary tumor conference to determine the most favorable management approach for each patient.

Case Reports

Patient 1—A 21-year-old woman with Fitzpatrick skin type I and no personal or family history of melanoma presented with a progressively enlarging symmetric, well-circumscribed, 5-mm dark black papule on her left cheek of approximately 2 months' duration without evidence of bleeding, ulceration, or irregular borders. The patient had no palpable lymphadenopathy. Her medical history was notable for 2 dysplastic nevi that had been excised 5 years prior. The patient denied frequent use of tanning salons or sunbathing, but she reported occasional sunburns throughout her childhood.

An excision biopsy with 1-mm margins was performed for histopathologic diagnosis, which revealed features suggestive of a deep penetrating nevus with congenital features; however, the analysis also revealed features of substantial concern such as extreme cellularity, crowded nests, absence of compelling maturations, extension of melanocytes at the periphery, and scattered mitoses (Figure 1). Based on these conflicting features, 4 expert dermatopathologists were unable to differentiate the lesion as a peculiar congenital nevus or a malignant melanoma with a thickness of 2 mm. Because of the ambiguous nature of the lesion, ancillary molecular studies were recommended; thus a FISH assay was performed.

The FISH analysis revealed an RREB1 gain of 53% and a CCND1 gain of 67%, meeting 2 criteria for FISH positivity and thus supporting the diagnosis of malignant melanoma. After a lengthy discussion of the case in a multidisciplinary melanoma conference, wide local reexcision with 1-cm margins and sentinel lymph node biopsy were performed with no evidence of advanced disease. Twenty-four months following the

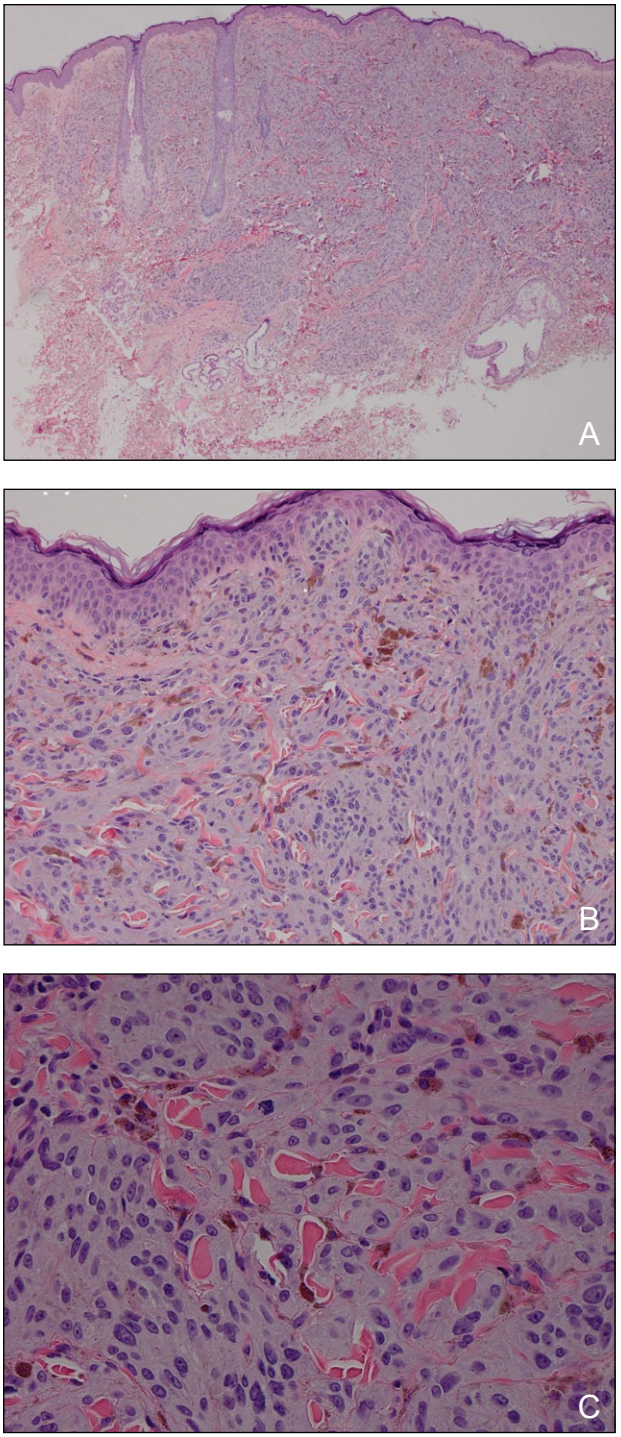

Figure 1. Excision biopsy in patient 1 revealed features suggestive of a deep penetrating nevus with congenital features but also with features of substantial concern such as extreme cellularity, crowded nests, absence of compelling maturations, extension of melanocytes at the periphery, and scattered mitoses including near the base of the specimen (A, B, and C)(H&E; original magnifications ×4, ×10, and ×20, respectively).

initial diagnosis, the patient has shown no evidence of recurrence and continues to return for follow-up. In this time, she has had 1 severely atypical nevus on her abdomen that was conventionally excised.

Patient 2—A 56-year-old woman with Fitzpatrick skin type II and a history of melanoma in situ of the lower back that was diagnosed 10 years prior presented to an outside dermatologist with a 2-mm blue papule on her upper back. The dermatologist did not note the lesion during a full-body skin examination that had been performed 6 months prior to presentation. An initial shave biopsy was performed by the outside dermatologist to rule out a vascular proliferation and revealed ambiguous histopathology of a nodular melanocytic lesion in the dermis associated with a scar (Figure 2). Irregular nests with lack of maturation were observed as well as occasional enlarged hyperchromatic nuclei and rare mitotic figures that were suggestive of an atypical melanocytic tumor or possibly a nevoid melanoma.

Interestingly, results of the FISH analysis did not meet any of the criteria for melanoma; however, its association with a scar without prior biopsy performed at the site and no connection to the epidermis led to a decision to pursue aggressive treatment of the lesion with complete reexcision using 1-cm margins because of the persistent uncertain biologic potential of the lesion. Given the banal clinical impression of the lesion on initial evaluation, reexcision without sentinel lymph node mapping was chosen as the treatment modality. Although the FISH analysis was not indicative of melanoma, aggressive management was recommended by the multidisciplinary team based on the worrisome histopathology and abrupt onset of the lesion.

Patient 3—A 76-year-old woman with Fitzpatrick skin type I was referred for evaluation of a "dark mole" on her right leg of more than 20 years' duration that measured 6×8 mm. The patient denied any notable changes to the lesion over the last 20 years, but given its appearance as a dark brown papule atop an irregular brown macule, a biopsy was performed. Initial histopathology revealed atypical epithelioid melanocytic proliferation arising in a compound dysplastic nevus with moderate to severe atypia of epithelioid dermal melanocytes (Figure 3). The degree of atypia warranted FISH analysis to rule out a subtle early melanoma arising in association with the nevus. None of the FISH criteria for melanoma were met, and a final diagnosis of an irritated nevus with atypical features was favored; however, because the lesion extended to the edges of the biopsy specimen, reexcision with 4-mm margins was recommended by the multidisciplinary team. The negative FISH analysis in addition to the unchanging lesion over multiple decades helped to reassure the patient and the medical team of the final diagnosis and management plan.

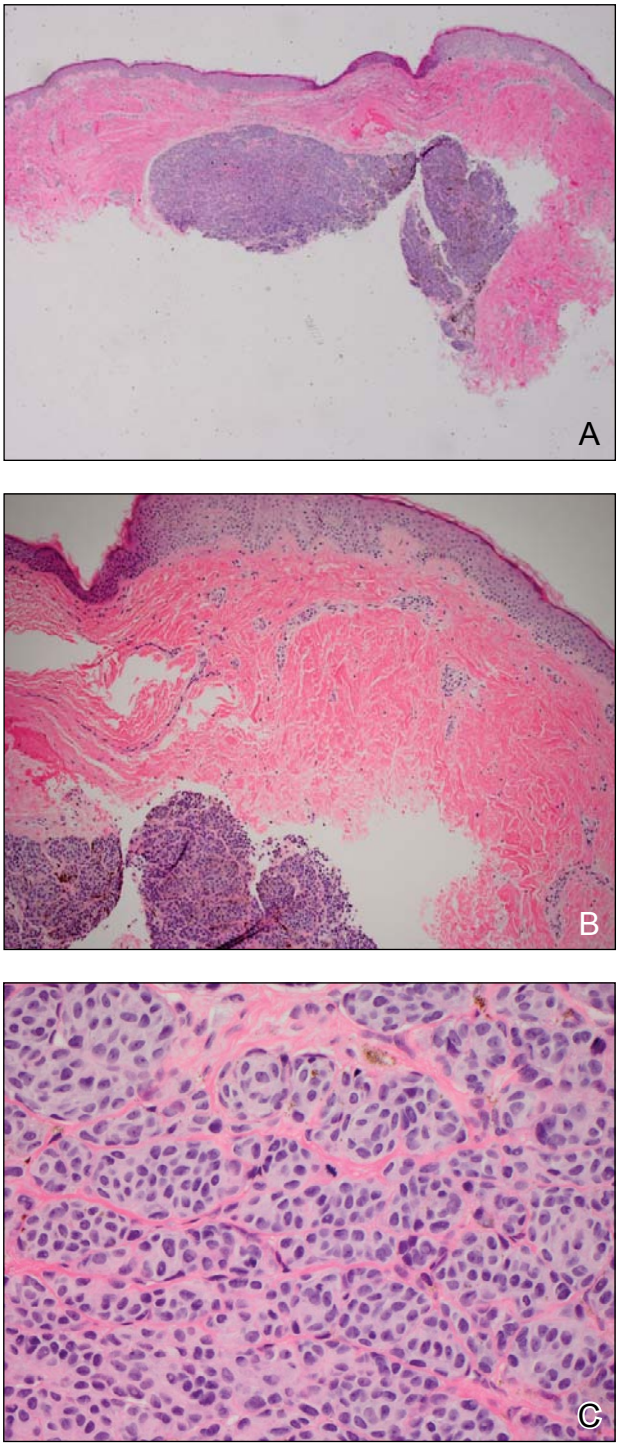

Figure 2. An initial shave biopsy of a blue papule in patient 2 showed irregular nests with lack of maturation, occasional enlarged hyperchromatic nuclei, and rare mitotic figures that were suggestive of an atypical melanocytic tumor or possibly a nevoid melanoma (A, B, and C)(H&E; original magnifications ×4, ×10, and ×20, respectively).

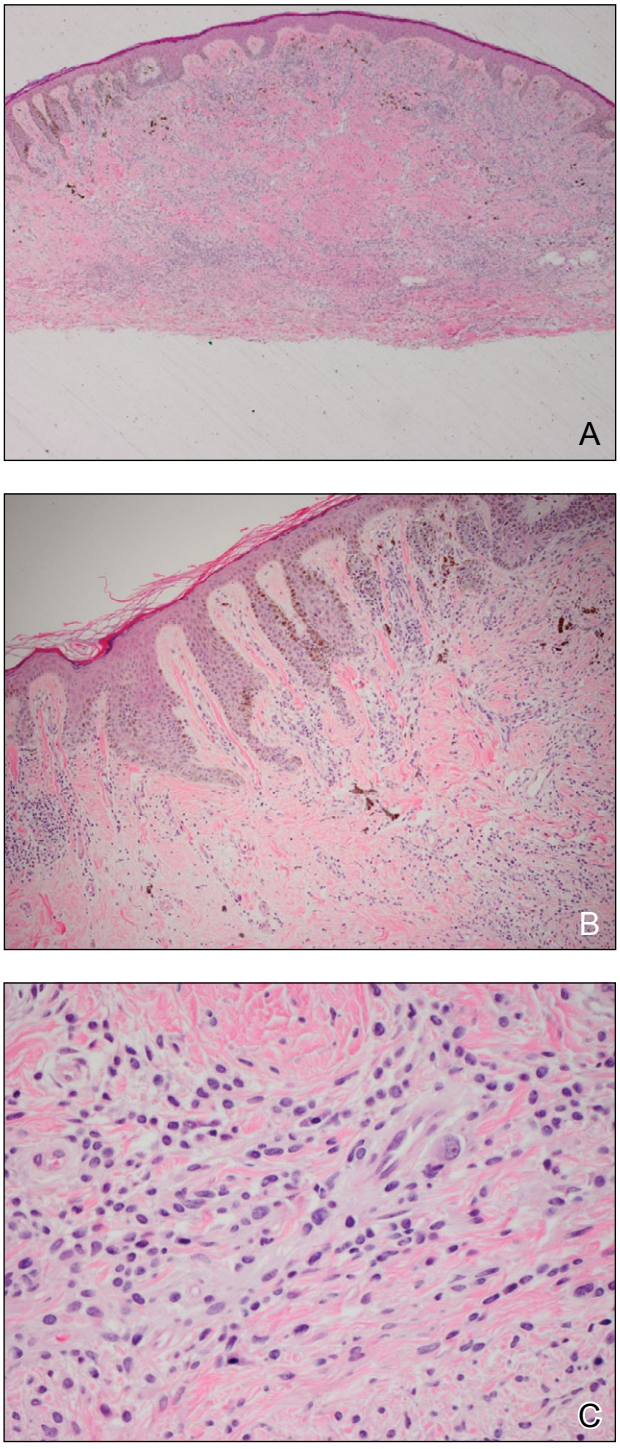

Figure 3. Histopathology results for patient 3 revealed an atypical epithelioid melanocytic proliferation arising in a compound dysplastic nevus with moderate to severe atypia of epithelioid dermal melanocytes (A, B, and C)(H&E; original magnifications ×4, ×10, and ×20, respectively).

Comment

Melanomas and melanocytic nevi may demonstrate overlapping mutations (eg, *BRAF* or N-*ras* mutations),[10-12] but these pigmented lesions are notably different regarding the presence or absence of clonal chromosomal aberrations.[9] Melanomas display recurrent patterns of chromosomal alterations, with copy number increases in chromosome arms 1q, 6p, 7, 8q, 17q, and 20q, and the loss of chromosome arms 6q, 8p, 9p, and 10q. In contrast, melanocytic nevi, except for Spitz nevi, show no evidence of chromosomal aberrations after karyotyping or comparative genomic hybridization (CGH).[9,13-16] Spitz nevi have shown a gain of chromosome arm 11p in 20% of lesions, which is not seen in melanoma[17]; however, CGH is expensive, time consuming, and not based on a lesion's morphology, which arguably limits its routine clinical application.[16]

Because of these distinct clonal differences in chromosomes, FISH is an accessible ancillary molecular diagnostic study that offers the opportunity to assist in distinguishing benign nevi from melanoma in histologically ambiguous lesions. The FISH method helps to visualize chromosomal abnormalities such as amplification, deletion, and translocation.[18] Diagnostic studies using FISH analysis also have been found to be applicable in other cancers, such as those involving the lungs and bladder.[19,20]

Gerami et al[9] established a discriminatory algorithm from a training set of 301 tumors and validated it on an independent set of 169 unequivocal nevi and melanomas as well as 27 cases with ambiguous pathology using paraffin-embedded tissue samples. A combination of 4 probes (6p25 [RREB1], 11q13 [CCDN1], 6q23 [MYB], CEP6) were used to correctly classify melanoma with 86.7% sensitivity and 95.4% specificity. With these probes, the group was able to correctly diagnose 6 of 6 cases with ambiguous pathology that later metastasized as melanomas. There was a significant difference in the metastasis-free survival between test-positive and test-negative cases with ambiguous pathology (P=.003). This combination of 4 FISH probes targeting 3 loci on chromosome 6 and 1 locus on chromosome 11 demonstrated the most powerful discriminatory ability.[9]

Interestingly, the manufacturer of the Vysis line of DNA FISH probes (Abbott Laboratories)[21] has different criteria for FISH positivity than those outlined by Gerami et al.[9] The FISH analysis is considered positive if at least 1 of the following 4 criteria is fulfilled: (A) average CCND1 signals per nucleus greater than or equal to 2.5; (B) average MYB signals per nucleus greater than or equal to 2.5; (C) 31% or more nuclei with loss of MYB relative to CEP6; or (D) 63% or more nuclei abnormal (ie, less than or greater than 2 signals per nucleus) for RREB1. Recently, Kerl et al[22] found a false-negative rate of 30.7% and 25.8% using the Abbott[21] and Gerami et al[9] criteria,

respectively, in 163 histologically unambiguous melanomas and thus proposed combining both sets of criteria, which yielded a false-negative rate of 17.8%; however, the authors acknowledged that even with the combined criteria, false-negative cases remained and 33% (4/12) of false-negative cases examined using CGH were free of genetic aberrations.[22] The possibility of false-negative results should always be considered when interpreting FISH analyses, as was the case with patient 2 in our case series.

Fluorescence in situ hybridization analysis also has been shown to help distinguish mitotically active nevi from nevoid melanoma, which can be difficult to histologically differentiate given certain overlapping features. Gerami et al[23] evaluated 10 nevoid melanomas in comparison to 10 mitotically active nevi using FISH analysis and found that all 10 nevoid melanomas demonstrated chromosomal aberrations in either chromosome 6 or chromosome 11, which was not seen in any of the 10 mitotically active nevi. This finding also further confirmed that dermal mitoses alone in the absence of additional malignant features do not constitute a diagnosis of melanoma.[23] In melanomas that occur in association with nevi, Breslow thickness, which is important for both management and prognosis, can be difficult to assess solely by histologic examination.[24] One study found FISH analysis valuable in helping to determine the Breslow thickness in these difficult lesions, as it helped to delineate the transition from the melanoma to the nevus.[25]

In another study of 43 unequivocal melanomas and nevi using the same 4 probes, Vergier et al[26] were able to differentiate these lesions with 85% sensitivity, 90% specificity, 89.5% positive predictive value, and 86% negative predictive value. They also reviewed 95 ambiguous melanocytic tumors, and although histopathologic analysis alone yielded a specificity of 52% and FISH alone yielded a sensitivity of 43%, combining the histopathologic diagnosis with FISH results increased the sensitivity and specificity to 90% and 76%, respectively. In addition, survival analysis using the Kaplan-Meier method showed a trend for worse prognosis in FISH-positive patients. Vergier et al[26] recommended clinical use of the FISH analysis in ambiguous lesions as well as in lesions with discordant histopathologic analysis with the recommendation that FISH-positive cases should be managed as melanomas.

Spitz nevi, however, remain problematic when determining if they are benign or malignant. Based on their analysis of various Spitz nevi, Vergier et al[26] recommended that if a dermatopathologist favors malignancy, a FISH-negative result should not alter the dermatopathologist's initial interpretation; however, in a Spitz nevus that is favored as being benign, a FISH-negative result may lead to conservative excision, as also recommended by McCalmont.[27] Because Spitz nevi are a genetically heterogeneous group, use of FISH analysis may be limited in these lesions.[28] A recent study indicated that a 9p21 assay in addition to the standard melanoma FISH assay may play a role in spitzoid melanoma diagnosis.[29]

Fluorescence in situ hybridization analysis using the same 4 probes also has been studied in distinguishing 12 cellular blue nevi from 5 blue nevus–like melanomas with 100% sensitivity and 100% specificity.[30] Other studies have demonstrated the utility of FISH analysis in helping to differentiate between benign lesions and melanomas.[16,31-38] The FISH method also may play a crucial role in determining prognosis and identifying tumors with greater metastatic potential.[39] Recently, FISH analysis for monosomy 3 has helped confirm the diagnosis of metastatic uveal melanoma[40,41] and also may play a role in determining prognosis,[42] though its clinical utility has yet to be determined.[43]

Although use of the FISH assay can assist in the diagnosis and further management of ambiguous melanocytic lesions, there are limitations to its results, including false negativity. Interobserver variability also exists as well as processing errors. Additionally, because melanomas are not genetically homogenous, they may exhibit different features in different sections of the tumor,[44] and genetic aberrations may only be seen in certain subsets of melanoma.[45] Reproducibility also may be challenging.[16] The sensitivity of FISH also may be lower in certain subsets of melanomas, such as desmoplastic melanomas.[46] Given its limitations, the precise clinical application of FISH has yet to be defined; however, we have found this method to be helpful in determining both immediate and long-term management techniques for ambiguous melanocytic lesions in our patients.

Conclusion

Pigmented lesions can be challenging to differentiate histologically as being truly benign or malignant. Fluorescence in situ hybridization assays can assist in the diagnosis and further management of these indefinite lesions, especially in cosmetically sensitive areas where misclassification can result in severe consequences for the patient. Additionally, FISH analysis may play a pivotal role in helping to determine prognostic expectations as well as appropriate follow-up techniques in histologically ambiguous lesions. It is important to note, however, that false-negative results can occur even in metastatic melanomas and complete reliance should not

be placed on cytogenetic results alone.[47] Although recent studies conclude that FISH should be used as a supplementary diagnostic tool in the management of pigmented lesions, emphasis should be placed on correlating clinical, molecular, and pathologic findings, as was done in our patients. Future studies are needed to better elucidate the full utility of the FISH assay in diagnostic, prognostic, and management practices.

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