Molecular Melanoma Diagnosis Update

Gene Fusion, Genomic Hybridization, and Massively Parallel Short-Read Sequencing

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KEYWORDS

• Melanoma • Spitz nevus • BAP-1 inactivated spitzoid nevus • Kinase gene fusion • Molecular • Comparative genomic hybridization

KEY POINTS

- Molecular evaluation of melanocytic tumors can be diagnostically useful to confirm malignancy or benignancy.
- Molecular tools are ancillary and supplemental to histopathologic evaluation and do not replace conventional microscopy.
- Immunohistochemistry, fluorescence in situ hybridization (FISH), array comparative genomic hybridization (aCGH), and massively parallel short-read sequencing, often referred to as next-generation sequencing (NGS), each provide varied (and often incomplete) additional information, and careful planning is necessary if tissue is limited.

Melanocytic tumor diagnosis remains a challenging area — if not the most challenging and controversial area — in dermatopathology, and analysis by conventional microscopy has limitations in defining entities precisely and in establishing biologic potential. Additionally, diagnostic criteria and diagnostic approaches vary considerably across the field, and because of fear of underdiagnosis, the diagnosis of melanoma is commonly, readily, and perhaps too easily rendered. Fortunately, molecular tools are available as ancillary techniques and hold the potential to provide some measure of diagnostic uniformity and insight in the evaluation of controversial tumors; additionally, these techniques provide the potential to unveil new oncogenic pathways that may disrupt existing morphology-based diagnostic conclusions and

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Clin Lab Med 37 (2017) 473–484
http://dx.doi.org/10.1016/j.cll.2017.06.002 **labmed.theclinics.com**

methods. There are various methods to probe the underlying genetic changes present in tumors, including immunohistochemistry, FISH,[1,2] aCGH,[3,4] and massively parallel short-read or NGS.[5] Each technique provides slightly different information with advantages and disadvantages. Herein, these techniques and how they can supplement conventional assessment are briefly described.

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The use of immunohistochemistry to evaluate the presence or absence of specific proteins constitutes a well established, widely available, nonmolecular approach, but selected stains can provide surrogate molecular information (**Table 1**). In conventional immunostaining, Melan-A, S100, and SOX-10 represent the most widely used reagents applied to melanocytic tumors in diagnostic dermatopathology. By contrast to traditional or mainstream approaches used to confirm lineage or define distribution, both p16 and BAP-1 are primarily used to provide a surrogate view of underlying molecular status.

At a cellular level, the importance of *CDKN2A* locus is its expression of tumor suppressors p16(Ink4a) and p19(Arf). A major function of p16 is through its suppression of cyclin-dependent kinase (CDK) 4 to inhibit cell cycle progression, while p19 functions through direct binding to the *MDM2* protein, blocking degradation of p53.[6] Given its central importance in critical cellular pathways, p16 immunohistochemical expression has been extensively evaluated.[7–10] The underlying assumption is that initiating driver mutations that induce melanocyte proliferation (ie, mutation in *BRAF*) are maintained in a nonproliferative state by p16 activity. With *CDKN2A* loss or mutation, the affected melanocytes are then allowed to bypass this G1 checkpoint with an increased potential of malignancy. Although this is an oversimplification of the pathway, comprehensive studies examining the genomic alterations in advanced cutaneous melanoma have found up to 70% of cases having mutation, deletion, or methylation of *CDKN2A*.[11] Therefore, the complete

Table 1
Surrogate molecular information provided by selected immunostains

Determinant	Reactivity with Melanoma	Reactivity with Melanocytic Nevi	Comment
p16 (protein product of *CDKN2A*)	Potential loss (as a surrogate for *CDKN2A* loss)	Retained	High false-negative rate
BAP-1	Loss in blue nevus-like melanoma and ocular melanoma	Retained; loss in BAPoma	Expression loss corresponds to BAP-1 loss or chromosome 3p loss
ALK	• Positive if gene fusion present • Positive if ALK alternative transcription initiation present	Positive if gene fusion present	Kinase gene fusion common in a subset of Spitz nevi and Spitz tumors
NTRK1	• Strongly positive if NTRK1 gene fusion present • May cross-react with NTRK3 fusion	• Strongly positive if NTRK1 gene fusion present • May cross-react with NTRK3 fusion	Kinase gene fusion occurs in a subset of Spitz nevi and Spitz tumors