Assessing Genetic Expression Profiles in Melanoma Diagnosis

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KEYWORDS

• GEP • RT-PCR • Genetic expression profiling • Diagnostic test • Melanoma

KEY POINTS

- Genetic expression profiling (GEP) is an emerging diagnostic tool used to assist in discrimination between benign nevi and malignant melanomas.
- GEP evaluates a panel of genes with reverse transcription quantitative polymerase chain reaction to determine if mRNA expression is more consistent with a benign or malignant pattern.
- GEP tests face challenges with respect to demonstration of improvement upon the current pathologic "gold standard" for diagnosis of melanoma.
- National Comprehensive Cancer Network Guidelines do not recommend use of GEP diagnostic assays for routine clinical care.
- Like fluorescent in situ hybridization and array comparative genomic hybridization technologies, GEP may supply additional, independent information regarding challenging or ambiguous lesions; results should be considered in the context of the histopathologic findings, on a case-by-case basis.

INTRODUCTION

Accurate diagnosis of melanoma is critical. Thin melanomas have a better prognosis than thick melanomas,[1] presumably because they represent earlier lesions that do not have the biological capacity to metastasize. Thus, if appropriately treated, most thin melanomas are curable. In contrast, most advanced melanomas are lethal, despite recent advances in targeted immunotherapies. Most melanocytic tumors can be readily categorized as benign or malignant with traditional histopathology. However, there is a subset of ambiguous/equivocal melanocytic tumors with the potential to metastasize that defy classification with conventional histologic criteria. The interobserver reproducibility among pathologists in the evaluation of challenging melanocytic neoplasms is also poor.[2–5] Thus, the "gold-standard" histologic evaluation has the potential to miss a subset of lethal melanomas.

The consequences of missing these ambiguous, yet lethal melanomas are dire and frequently result

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in more aggressive, morbid, costly procedures and treatments, and death in some cases. In addition, the medicolegal risk has reinforced the need for providers to err on the side of caution.[6,7] For these reasons, there has been a drift in the diagnostic criteria that may increase sensitivity of melanoma detection, at the expense of specificity.[8–12] This shift toward cautious behavior may protect the patient and providers from misdiagnosis, but may also lead to overtreatment and a disproportionate increase in melanoma incidence rates relative to mortalities.[13] Ideally, a diagnostic test that exceeds the current gold standard would be more objective and increase reproducibility and specificity without compromising sensitivity.

MOLECULAR DIAGNOSTIC TESTS FOR MELANOMA

One candidate diagnostic tool is genetic expression profiling (GEP; **Table 1**). GEP is a relatively recent addition to the diagnostic armamentarium for melanoma and differs from DNA-based testing such as fluorescent in situ hybridization (FISH) and comparative genomic hybridization (CGH) (tumor cytogenetics). Over the past decade, ambiguous melanocytic tumors have been increasingly evaluated using FISH and/or CGH (see **Table 1**). FISH has the advantage of evaluating for large chromosomal deletions, duplications, and translocations in a subset of chromosomal loci that have a well-established association with melanoma.[14] However, only a limited number of chromosomal loci are evaluated; the interpretation remains subjective, and specialized expertise is required.[15] CGH overcomes some, but not all, of the subjectivity of FISH and allows evaluation of large chromosomal aberrations across the entire genome.[16,17] However, CGH requires relatively large quantities of tissue and is limited by tumor heterogeneity, making it less reliable in thinner neoplasms.[17] Although FISH and CGH are the most commonly used molecular diagnostic tests to date, GEP is an alternative molecular technology that could improve diagnostic accuracy, by increasing objectivity, reducing variability, and taking advantage of changes in the tumor's biology. **Table 1** summarizes the most salient features of the various diagnostic tests for melanocytic lesions.

GENETIC EXPRESSION PROFILING METHODOLOGY

Tissue Collection

GEP is a relatively straightforward method used to detect abnormal expression of messenger RNA (mRNA). Ideally, the assay will follow Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) Guidelines.[36] GEP uses reverse transcription in combination with quantitative polymerase chain reaction (RT-qPCR) technology (**Fig. 1**). The whole tumor (including the surrounding stromal microenvironment) or a dissected or a laser-captured component can be tested, using fresh, frozen, or formalin-fixed and paraffin-embedded tissue. Microdissection allows an enriched collection of malignant cells and improves the sensitivity by reducing contamination with benign cells. Conversely, use of the undissected tumor has the advantage of capturing expression changes induced in the tumor microenvironment, for example, normal stromal and inflammatory cells.

Reverse Transcription of Messenger RNA to complementary DNA

Once tumor tissue is obtained, RNA is extracted and complementary DNA (cDNA) is created from mRNA using RT. Multiple factors can affect this step, including the source of RT, variability in the quantity and quality of the tumor RNA, mRNA priming methods, and even reaction protocols. Therefore, using standardized reagents and protocols and mandating quality assurance of the tissues and tested RNA is paramount. Otherwise, process variability may exceed the differences observed in gene expression between benign and malignant lesions, leading to an invalid test. Testing the quality and quantity of cDNA is also important because accurate quantification of gene expression requires RT and qPCR reactions to occur in a linear range.

Quantitative Expression Analysis

After sufficient cDNA is obtained, genes selected for the expression profile are amplified by using gene-specific primers, designed to amplify the genes of interest with qPCR (MIQE Guidelines suggest the primer sequence, or at least the region of the gene being amplified, to be made available in publications). The amplification process is measured in real time, and through comparison to a set of coamplified control genes, determines how much mRNA from each gene was present in the original sample. Internal control genes and standardized RNA extraction kits, primer construction, and PCR equipment have reduced variability in this step. Reliable analytical methods exist for quantifying expression; however, detailed information regarding the testing protocol remains critical. In some cases, protocol details are considered proprietary, making independent validation of the test challenging.