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Gene Expression Analysis Differentiates Melanomas from Spitz Nevi

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ABSTRACT

Introduction: Pediatric Spitz nevi can pose significant diagnostic challenges to both clinicians and dermatopathologists when the current image-recognition based gold standard is employed. PRAME (preferentially expressed antigen in melanoma) and/or LINC (long intergeneic non-coding RNA 518) gene expression in adult patients in samples obtained non-invasively via adhesive patches differentiates primary melanomas from atypical nevi and other pigmented lesions with a NPV of over 99%, a sensitivity of 91%, and a specificity of 69%, to help clinicians rule out melanoma and the need for surgical biopsies of atypial pigmented lesions with suspicion for melanoma. Surgically obtained melanomas from adult patients show the same gene expression pattern.

Methods: In this study, we investigate gene expression patterns of pigmented lesions from FFPE tissue block samples (n=23, 9 male, 14 female patients, median age 12) with a focus on differentiating Spitz nevi from melanomas in children and young adults.

Results: PRAME levels were significantly (P<0.001) increased based on normalized Ct cycle counts (lower cycle counts indicate higher expression levels) in melanomas (mean Ct 33.83 ± 0.54, 95% CI 32.85-34.80) when compared to Spitz nevi (mean Ct 37.21 ± 0.98, 95% CI 35.41-39.01) or common nevi (mean Ct 36.94 ± 0.80, 95% CI 35.47-38.40), respectively. LINC and 4 control genes showed similar expression levels in all 3 pigmented lesion groups investigated. Clinically and histopathologically complex pediatric Spitz nevi demonstrated gene expression signatures almost identical to gene expression signatures of common pediatric nevi but different from melanomas in children and young adults.

Discussion: PRAME but not LINC gene expression can be a valuable molecular aid to differentiate melanomas from Spitz nevi, groups of pigmented lesions that can be particularly difficult to assess in children and young adults.

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INTRODUCTION

The accurate assessment of pigmented skin lesions, especially in pediatric settings, is challenging even for pigmented lesion experts.[1] The current gold standard of visual assessment paired with histopathology combines two diagnostic modalities that both rely on image and pattern recognition. Inherently, image and pattern recognition are subjective leading to inter and intra-observer discrepancies that may impact how pigmented skin lesions are managed and how plans for treatment and follow-up are devised. Improved diagnostic aides and options are desirable.[2-4] Furthermore, it is increasingly recognized that the way a pigmented lesion looks either clinically or under the microscope, is heavily influenced by changes in gene expression patterns that themselves are caused by mutations (in case of pigmented lesions most often acquired in a series of events as a consequence of UV damage).[5] Even intricate assessments of morphological features by dermoscopy or confocal microscopy, while without doubt beneficial in the hands of trained experts, are indirect and preceded by changes that may provide superior assessment if easy ways can be found to determine their nature.[6] Our team recently validated a molecular dermatology gene expression platform using adhesive patches to non-invasively collect skin samples. LINC (long intergeneic non-coding RNA 518) and/or PRAME (preferentially expressed antigen in melanoma) gene expression in adult patients differentiates primary melanomas from atypical nevi and other pigmented lesions with a NPV of over 99%, a sensitivity of 91% and a specificity of 69% to support clinicians with the management of difficult to assess pigmented lesions.[2] Board certified dermatologists and pigmented lesion experts who use LINC/PRAME gene expression to help guide the management of pigmented lesions, surgically biopsy about half as often while missing fewer melanomas.[3]

The study presented here addresses frequent questions from clinicians who use the test described above and who wanted to know, how Spitz nevi in children and young adults, a group of pigmented skin lesions frequently very similar in morphological appearance to primary cutaneous melanomas and therefore notoriously difficult to assess,[1] compare to melanomas and pediatric common nevi at the gene expression level.

MATERIALS AND METHODS

Study Subjects and Tissue Block Samples

The 23 subjects in this archival study were male (n=9) and female (n=14) patients (median age 12) with histopathologically confirmed pigmented lesions (pediatric melanomas and

melanomas in young adults, n=6 (3 were melanomas in situ and 3 were Stage 1 invasive melanomas); pediatric Spitz nevi, n=9; pediatric common nevi, n=8) who met the inclusion and exclusion criteria defined in a Central Institutional Review Board (WCG, WIRB-Copernicus Group, Princeton, NJ) approved protocol. Eight micrometer microtome sections from FFPE tissue blocks of histopathologically confirmed samples from the respective patients were prepared and mounted on glass slides. To best mimic standard of care daily practice scenarios, the histopathologic diagnosis was based on a singe read of a board certified dermatopathologist (DH). For processing, the sections were removed from glass slides, paraffin was dissolved, and RNA was isolated from the tissue sections using PureLink FFPE total RNA isolation kits (Invitrogen, Cat. K1560-02, Carlsbad, CA) following the manufacturer's instructions.

Gene Expression Analyses

Total RNA was reverse transcribed to complementary DNA (cDNA) using SuperScript® VILO™ cDNA Synthesis Kits. The resulting cDNA was subsequently used for target gene expression analysis with qRT-PCR on an ABI7900 PCR system (Life Technologies). Each qRT-PCR reaction used 3pg of total RNA, in duplicate, in 20uL volume on 384-well PCR reaction plates using pre-designed gene-specific TaqMan probe chemistries (Life Technologies). An averaged cycle threshold (Ct) value of the duplicate measurements was used in the analysis. Gene expression was considered detected if the qPCR reaction yielded an amplification curve and a measurable Ct value, or not detected if the reaction yielded an 'undetermined' Ct value (amplification curve never above detection threshold). In addition to the 2 target genes LINC and PRAME, ACTB (beta-Actin) was used for normalization of LINC and PRAME gene expression data and of 3 additional control genes. The control genes represent genes with high (ACTB and B2M, beta-2 microglobulin), medium (PPIA, peptidylpropyl isomerase A) and low (CMIP, c-Maf inducing protein) expression levels in human tissues.

Statistical Analysis

All statistical analyses and gene expression comparisons were performed using Microsoft Excel. All 'undetermined' Ct counts from qPCR were assigned a value of 40 (the maximal thermal cycling number in a given qPCR reaction).

RESULTS

As observed in adult pigmented lesion patients,[2-3] PRAME levels were significantly increased based on normalized Ct cycle counts in pediatric melanomas and melanomas in young adults (mean Ct 33.83 ± 0.54, 95% CI 32.85-34.80) when compared to pediatric Spitz nevi (mean Ct 37.21 ± 0.98, 95% CI 35.41-39.01, $P<0.001$). Lower cycle counts indicate higher expression levels (Figure 1A). Pediatric common nevi demonstrated PRAME gene expression levels almost indistinguishable from Spitz nevi (mean Ct 36.94 ± 0.80, 95% CI 35.47-38.40). LINC and 4 control genes (ACTB, B2M, CMIP and PPIA) showed similar expression levels in all 3 pigmented lesion groups studied (Figure 1B). ACTB was used for normalization of LINC, PRAME, B2M, CMIP, and PPIA qPCR input levels therefore, its Ct count histograms are not separately depicted in Figure 1B.

FIGURE 1. Gene expression analysis of the target genes LINC and PRAME (A) and of control genes (B) in FFPE tissue block samples of pediatric melanomas, pediatric common nevi and pediatric Spitz nevi. All values are normalized against ACTB. Lower Ct values represent higher gene expression. PRAME gene expression in melanomas is statistically significantly higher in melanomas compared to Spitz nevi or common nevi ($P<0.001$).

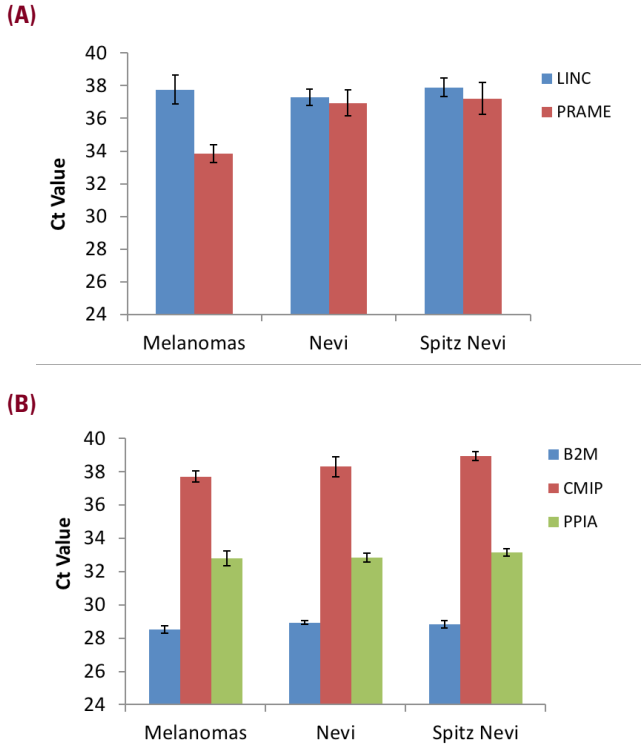
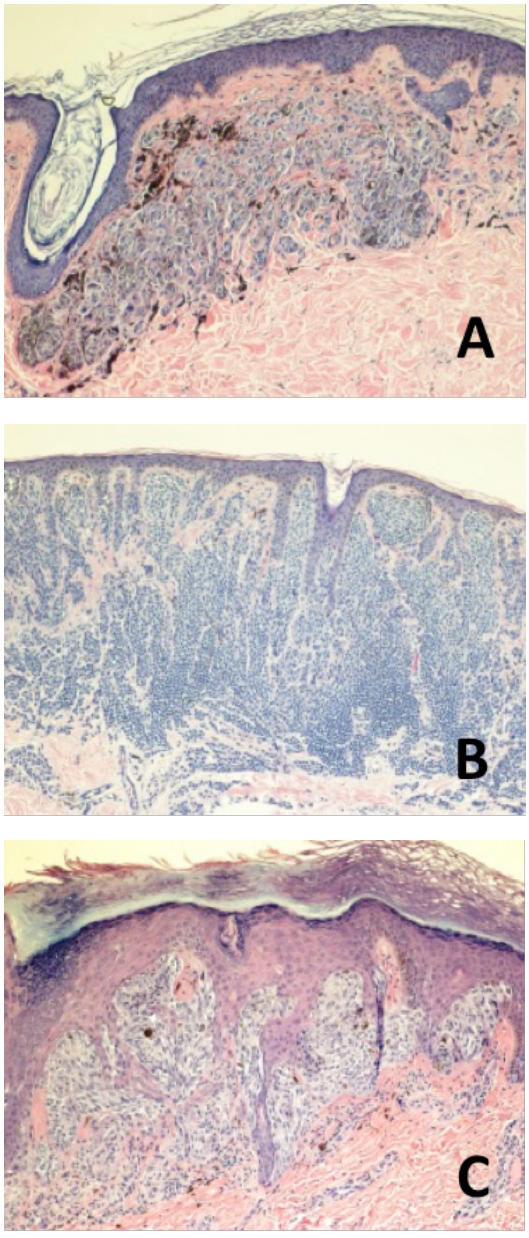


Figure 2 shows representative examples of histopathological analyses of the analyzed pigmented lesion cases using routinely processed H&E slides from the same FFPE tissue blocks also used for gene expression testing. Examples for a melanoma, common nevus and Spitz nevus case (Figure 2A-C, respectively) are depicted.

DISCUSSION

While the current morphology-based gold standard of assessing primary pigmented skin lesions can be challenging in older patients leading to biopsy ratios of up to and greater than 30 benign lesions for every 1 melanoma detected,[7] additional complexities exist in children and young adults. Efforts to address some of these complexities have led to proposed sentinel lymph node biopsies as a diagnostic method for estimation of

FIGURE 2. Representative examples of histopathological analyses of pediatric pigmented lesion cases (H&E stained slides from FFPE tissue blocks) also assessed for gene expression. (A) pediatric melanoma (200x magnification, (B) pediatric common nevus (100x magnification), (C) pediatric Spitz nevus (200x magnification).



the malignant potential of particularly difficult to categorize Spitz tumors.[1] While recent analyses showed no prognostic benefit,[1] the concept highlights the clear need to find better and ideally non-invasive options. We recently validated such a non-invasive option that differentiates primary cutaneous melanomas from non-melanoma skin lesions including atypical nevi based on LINC and/or PRAME gene expression in 398 adult patients with a high sensitivity (91%), high specificity (69%), and a negative predictive value above 99%.[2] Board certified dermatologists who use the test, surgically biopsy suspicious pigmented lesions about half as often while missing fewer melanomas.[3] This test is currently available commercially in the US. It is used by a growing number of over 500 dermatologists many of whom were curious what LINC and PRAME gene expression patterns in Spitz nevi and melanomas in children and young adults might look like. This study attempts to find first answers. While Spitz nevi frequently resemble melanomas morphologically, we found that they resemble common nevi with their low PRAME gene expression level. Larger studies will be needed to determine what role, if any, LINC might play in this patient population. Data sets summarized in Figure 1 suggest that LINC is expressed at comparable levels in all 3 pigmented lesion types studied here. These findings indicate that LINC might be expressed at proportionally higher levels in pediatric nevi compared to nevi in adults,[2] possibly limiting its potential to help categorize pigmented lesions in young patients. PRAME, on the other hand, appears to be a gene expression candidate well suited to differentiate Spitz nevi from melanomas in children and young adults.

DISCLOSURES

Burkhard Jansen, Maesa Hanhan, and Zuxu Yao are employees of DermTech. Doyle Hansen and Ronald Moy are members of DermTech's Scientific Advisory Board.

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