Melanoma Mimic

A Case of Multiple Pagetoid Spitz Nevi

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Background: Differentiating Spitz nevi from melanoma can be difficult. Pagetoid spread of melanocytes is among the features making diagnosis difficult. Rare reports of isolated pagetoid Spitz nevi exist.

Observations: We present a unique case of multiple pagetoid Spitz nevi initially diagnosed as multiple in situ melanomas. Germline karyotyping, CDK4 and CDKN2A sequencing, and comparative genomic hybridization of HRAS, BRAF, KRAS, RAF1, CDKN2A, Rb1, MAP2K1, MAP2K2, PTEN, and PTPN11 genes did not identify mutations in this case. Germline and somatic sequencing of BRAF exon 15 revealed no mutations at V600D/E/K. In addition, single-nucleotide polymorphism microarray analysis (330K) on lesional and normal skin revealed no genome-wide copy number changes or loss of heterozygosity.

Conclusions: Clinicians should be aware of the occurrence of multiple pagetoid Spitz nevi to avoid morbidity associated with the misdiagnosis of multiple melanomas. The genetic mechanisms of pagetoid spread of melanocytes are not fully understood.


The Spitz nevus often poses a diagnostic dilemma for the pathologist because of histologic features that overlap with those of malignant melanoma. Multiple variants of Spitz nevi have been described, including the rarely reported pagetoid Spitz nevus.1,2 The pagetoid Spitz nevus consists of primarily an intraepidermal pagetoid proliferation of epithelioid melanocytes, and its histopathologic characteristics can be easily confused with those of melanoma in situ. Because of the diagnostic dilemma posed by some Spitz nevi, recent research3-6 has focused on genetic alterations that may help differentiate Spitz nevi from melanoma when results of routine histologic testing prove indecisive.

We present, to our knowledge, the first case of a patient with multiple pagetoid Spitz nevi. We provide the results of germline genetic analyses, including peripheral blood karyotyping, comparative genomic hybridization (CGH), and specific melanoma-associated gene sequencing to assess the patient for germline and somatic genetic alterations. In addition, we performed a 330K single-nucleotide polymorphism microarray analysis on lesional and normal skin, looking for somatic alterations in the patient’s multiple pagetoid Spitz nevi.

REPORT OF A CASE

A 34-year-old healthy white woman presented to her primary care physician with concerns about a pigmented macule on her left leg that she found unsightly. She had no personal or family history of melanoma. The lesion was biopsied and diagnosed as melanoma in situ, lentigo maligna type. She was referred to a dermatologist (J.P.) for excision of the melanoma in situ. She was referred to a dermatologist (J.P.) for excision of the melanoma in situ. The lesion was excised with standard 5-mm margins.

Because of the melanoma diagnosis, a full-body skin examination was completed and 2 additional pigmented macules were biopsied. Both of the lesions were also diagnosed as melanoma in situ. Concerned about 3 melanomas in situ in a young healthy woman, the dermatologist had the specimens reviewed by a dermatopathologist who concurred with the diagnoses, and these lesions were excised with 5-mm margins.

The dermatologist continued to be skeptical that the patient had 3 melanomas in situ; therefore, 6 additional bland-
appearing pigmented macules on the bilateral lower extremities, head, and upper extremity were biopsied at random to determine what was “normal” for the patient. When all 6 lesions were diagnosed on the basis of histologic analysis as melanoma in situ, lentigo maligna type, or premalignant melanocytic dysplasia with significant pagetoid melanocytic spread, the patient was referred to the University of Utah Huntsman Cancer Institute. In the interim, the 6 lesions were excised, with conservative margins.

On physical examination, the patient had more than 50 bland-appearing tan-to-brown macules scattered on her head, neck, trunk and extremities, without features clinically suggestive of melanoma, as shown in Figure 1. Hematoxylin-eosin–stained slides of all biopsied and excised melanocytic lesions were reviewed by dermatopathologists (including S.R.F.) at our institution. All slides showed symmetric, incompletely circumscribed, junctional melanocytic proliferations composed of epithelioid melanocytes, as shown in Figure 2. Melanocytes were distributed mainly in single-cell array with pagetoid scatter to the upper reaches of the epidermis. A few melanocytic theques and rare Kamino bodies were identified. Histologically, these lesions were fairly consistent with the limited reports1,2 of pagetoid Spitz nevi, that is, circumscribed melanocytic lesions composed of few junctional nests and extensive pagetoid spread of epithelioid melanocytes singly and in nests with mild cytologic atypia and minimal architectural disorder. To our knowledge, this is the first reported case of multiple pagetoid Spitz nevi.

This patient’s history of multiple examples of a rare type of lesion suggests that she may have an underlying genetic susceptibility leading to this pattern and provided an opportunity to discover a molecular cause for this atypical pattern of melanocytic proliferation. Therefore, we were anxious to investigate possible germline and somatic genetic etiologies that might be related to the unusual melanocytic lesions. Understanding why this patient’s “signature lesion” was a pagetoid Spitz nevus might lead to better understanding of pagetoid spread of melanocytes more broadly, including those found in malignant melanoma.

Laboratory investigations included peripheral blood karyotyping to rule out large germline translocations or rearrangements. Germline sequencing was performed for melanoma predisposition genes, including CDK4 (OMIM *123829) and CDKN2A (OMIM *600160). A CGH array was used to analyze germline DNA for smaller gene defects known to be associated with melanocytic disease, including HRAS (OMIM *190020), BRAF (OMIM *164757), KRAS (OMIM *190070), RAF1 (OMIM *164760), CDKN2A, RB1 (OMIM *614041), MAP2K2 (OMIM *601263), MAP2K1 (OMIM *176872), PTEN (OMIM *601728), and PTPN11 (OMIM *176876). Both germline and lesional DNA sequencing of BRAF exon 15 was performed to determine whether BRAF V600D/E/K mutations were present. A 330K single-nucleotide polymorphism analysis was performed on lesional and normal skin to evaluate copy number and loss of heterozygosity.7-10

METHODS

All investigations were approved under the guidance of the institutional review board at the University of Utah. After obtaining written informed consent from the patient and her parents, whole blood specimens were obtained. The parents were

Figure 1. Clinical photographs of the patient’s nevi show bland-appearing brown macules scattered on the trunk and extremities.
reported to be phenotypically normal. Their blood was collected so they could be genotyped to help determine the parental origin of a genetic alteration if one was detected in the patient. Whole blood from the patient was sent for G-band karyotyping at the 550-band level (ARUP Laboratories, Salt Lake City, Utah). Genomic DNA from the patient’s blood and nevi was amplified by polymerase chain reaction (PCR) for analysis of exon 15 of the \textit{BRAF} gene (which contains the common V600E mutation). The primer sequences for PCR were as follows:

\begin{align*}
\text{BRAF\_ex15 F} & \quad 5'\text{TCATAATGCTTGCTCTGATAGGA} \\
\text{BRAF\_ex15 R} & \quad 5'\text{GGCCAAAAATTTAATCAGTGGA}
\end{align*}

One nanogram of DNA was amplified using Qiagen SYBR master mix and primers at a final concentration of 0.4µM in a PCR cycler (Roto Gene Q; Qiagen, Valencia, California) under the following run conditions: 95°C for 5 minutes; 40 cycles (95°C for 10 seconds, 57°C for 20 seconds, and 72°C for 20 seconds). The bidirectional DNA sequence was then obtained, analyzed, and compared with the published \textit{BRAF} gene sequence.\textsuperscript{11}

Peripheral blood DNA from the patient and her parents was sent to GeneDx, Gaithersburg, Maryland, where \textit{CDK4} and \textit{CDKN2A} gene sequencing and ExonArray-level CGH analyses for melanoma-associated genes were performed. The patient’s peripheral blood DNA was amplified by PCR for analysis of exons 1 to 3 of the \textit{CDKN2A} gene and the relevant coding region (exon 2) of the \textit{CDK4} gene with flanking splice sites. The bidirectional DNA sequence was obtained, analyzed, and compared with the published gene sequence.\textsuperscript{11} The methods used by GeneDx are expected to be greater than 99% sensitive in detecting mutations identifiable by sequencing. In addition, genomic DNA from the patient’s blood specimen was examined by array-based CGH, using a custom-designed oligonucleotide array (ExonArrayDx, version 1.0; GeneDx). Probe spacing for the array is between 100 and 200 bp within exons and 3 probes per intron regardless of size. The genes analyzed in the array included \textit{HRAS}, \textit{BRAF}, \textit{KRAS}, \textit{RAF1}, \textit{CDKN2A}, \textit{Rb1}, \textit{MAP2K1}, \textit{MAP2K2}, \textit{PTEN}, and \textit{PTPN11}. Hybridization data were analyzed with DNA Analytics software (Agilent Technologies, Santa Clara, California) to evaluate copy number at the exon level. The ExonArrayDx is designed to detect most single-exon deletions and duplications. Probe sequences and locations are from the human genome build (hg18). The genes \textit{HRAS}, \textit{BRAF}, \textit{KRAS}, \textit{RAF1}, \textit{CDKN2A}, \textit{Rb1}, \textit{MAP2K1}, \textit{MAP2K2}, \textit{PTEN}, and \textit{PTPN11} were chosen from those available for CGH testing at GeneDx because of their association with melanocytic disease.\textsuperscript{7-10}

In addition, DNA was extracted from formalin-fixed paraffin-embedded (FFPE) pagetoid Spitz nevi (n=3) and normal tissue (n=1) (RecoverAll; Ambion, Inc, Austin, Texas) from the patient’s biopsy and excision specimens. The lesional samples were selected by the dermatopathologist (S.R.F.) for their prominent pagetoid spread and large melanocytic involvement of the tissue block. Low quantification results required us to pool 2 of the low-yield nevi samples, and a third sample with sufficient genomic DNA was run on its own. Three samples were run on OncoScan FFPE Express (Affymetrix, Santa Clara, California), which is a 330K single-nucleotide polymorphism microarray platform that uses molecular inversion probe technology to provide copy number and loss of heterozygosity data for genome-wide and cancer-enriched loci. The assay was run as previously described and has been demonstrated\textsuperscript{12-14} to provide high-quality results on clinically archived FFPE specimens. Results were analyzed using Nexus Copy Number 3.1
Peripheral karyotyping revealed a normal female 46,XX chromosome profile without any evidence of deletion, duplication, translocation, or rearrangement. Results of CGH analysis of HRAS, BRAF, KRA5, RAF1, CDKN2A, Rb1, MAP2K1, MAP2K2, PTEN, and PTPN11 genes were also normal, with no detectable deletions or duplications. Germline CDK4 and CDKN2A genetic sequencing and germline BRAF exon 15 mutation analysis did not show any mutations. A D594N BRAF mutation was detected in a single nevus specimen, but no other somatic exon 15 mutations were detected (including no BRAF V600D/E/K mutations). No significant copy number changes were detected in the samples. Detectable regions of loss of heterozygosity found in the tumor sample were present in the normal skin tissue as well and ranged from 1 to 6 megabases, which is within the range of normal expected germline homozygosity.

To our knowledge, clinical description and genetic analysis of multiple pagetoid Spitz nevi have not previously been reported. Surprisingly, we did not find any germline genetic etiologic factors for our patient’s multiple pagetoid Spitz nevi. The lack of detectable copy number alterations or pathogenic loss of heterozygosity appears to indicate a benign tissue type and is consistent with the current treatment plan of careful observation. Although none of the patient’s lesions has been malignant, her condition merits continued close clinical observation to watch for any evolving malignant lesions.

In general, Spitz nevi show little to no genetic mutation, whereas melanoma frequently shows significant genetic mutation. Takata et al recently found that 23 of 24 melanomas examined showed at least 1 genetic or epigenetic alteration, and none of the 12 typical Spitz nevi or 16 atypical Spitz nevi showed significant genetic alterations. Of note, the study by Takata et al did not include pagetoid Spitz nevi. Boone et al performed fluorescence in situ hybridization (FISH) targeting cytogenetic abnormalities associated with melanoma (6p25 [RREB1], 6q23 [MYB], Cep6 [centromeric portion], and 11q13 [cycin D1]) on 2 cases of multiple Spitz nevi. One case showed balanced tetraploidy and the other showed no abnormality. Neither of these cases was pagetoid Spitz nevi.

Gerami et al examined 24 cases of superficial neoplasms with prominent pagetoid melanocytosis using FISH probes targeting the same cytogenetic abnormalities as Boone et al. Gerami et al found that 5 of 7 unequivocal melanomas had positive FISH results; none of 6 unequivocal benign lesions was positive. Two of 11 indeterminate cases were FISH positive. One of the FISH-positive indeterminate cases, on re-excision, was unequivocally diagnosed as melanoma using routine light histologic examination. Gerami et al suggested that FISH may be helpful in determining benign vs malignant superficial lesions with prominent pagetoid melanocytosis. Interestingly, they found that lesions in younger women were more likely to be benign, as seen in the present case.

Little is understood about the mechanism of pagetoid spread of melanocytes in benign or malignant entities. Vioros et al showed an association between somatic BRAF exon 15 (which includes region V600E) mutations and pagetoid spread of melanocytes in malignant melanoma. Furthermore, they showed that BRAF mutations were more common in younger patients who had increased survival. From the correlation with young age, the authors extrapolated that melanomas with BRAF mutations may have different, less deadly metastatic patterns.

Of note, benign nevi have a high rate of BRAF mutations while generally lacking pagetoid spread of melanocytes. Spitz nevi, which have rarely been found to have BRAF mutations, more often contain pagetoid spread of melanocytes. Using CGH and DNA sequencing of BRAF exon 15, we did not find germline BRAF mutations in this case of multiple pagetoid Spitz nevi. However, 1 of the 3 atypical spitzoid lesions from the patient demonstrated a BRAF mutation at D594N. There was no appreciable difference clinically or histologically in this nevus. In a panel of 105 biopsy-derived melanoma cell lines reported by Ugurel et al, only 1 cell line carried a BRAF D594N mutation. Thus, the relevance of this specific mutation remains unclear. Taken together, these findings suggest a more complicated molecular pathway to melanocytic pagetoid spread than BRAF mutations alone.

We also did not find somatic copy number alterations or loss of heterozygosity in the lesional tissue (using genome-wide microarray analysis), suggesting a genomic stability not often seen in melanoma.

There are limitations to our study. We performed testing only on germline and 3 lesional skin specimens. With the exception of the genes fully sequenced, we cannot rule out point mutation, small deletion, or small insertion in the germline genes analyzed. It is possible that tissue from other pagetoid Spitz nevi from our patient would have shown a different genetic profile.

Dermatologists should be aware of pagetoid Spitz nevi, including multiple pagetoid Spitz nevi, to avoid morbidity associated with the misdiagnosis of melanoma in situ and multiple scarring excisions. Genetic analysis can be helpful in differentiating Spitz nevi from melanoma, with Spitz nevi generally lacking extensive genetic alteration. Furthermore, this unique case raises interesting questions regarding the overlap in behavior of benign and malignant melanocytes. For instance, why is this patient’s signature nevus composed primarily of melanocytes in the epidermis, a feature more commonly seen in melanoma? More investigation into the molecular pathogenesis of pagetoid spread of melanocytes in pagetoid Spitz nevi is warranted, with the goal of better diagnosing, treat-
ing, and preventing melanoma while recognizing potential mimics.

Accepted for Publication: August 18, 2011.

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Author Contributions: Drs Harris and Leachman had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Harris and Leachman. Acquisition of data: Harris, Florell, Papenfuss, Jahromi, Kohlmann, Schiffman, Quackenbush, Cassidy, and Leachman. Analysis and interpretation of data: Harris, Florell, Jahromi, Schiffman, Quackenbush, Cassidy, and Leachman. Drafting of the manuscript: Harris, Florell, Jahromi, Schiffman, Quackenbush, and Leachman. Critical revision of the manuscript for important intellectual content: Harris, Florell, Jahromi, Schiffman, Quackenbush, Cassidy, and Leachman. Obtained funding: Leachman. Administrative, technical, and material support: Harris, Florell, Papenfuss, Schiffman, Cassidy, and Leachman. Study supervision: Florell and Leachman.

Financial Disclosure: None reported.

Funding/Support: This study was supported in part by GeneDx (ExonArray CGH performance). The majority of funding for the study was through the Tom C. Mathews Familial Melanoma Research Clinic Endowment, the Huntsman Cancer Foundation, and Institutional Core support through Genetic Counseling Core (Huntsman Cancer Institute Cancer Center Support Grant P30CA042014).

Additional Contributions: We wish to thank Kristina Heintz, RN, BSN, for her clinical coordination with the patient; Brad V. Graham, BS, MS, for technical support with BRAF sequencing; and Sherri Bale, PhD, and her team at GeneDx, Gaithersburg, Maryland, for performing the ExonArrayDx analysis.