Genetic Similarities between Spitz Nevus and Spitzoid Melanoma in Children

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BACKGROUND. Melanoma in children is rare. Diagnosis of the subtype of melanoma known as Spitzoid melanoma can be extremely challenging in this age group. Spitzoid melanoma clinically and histopathologically resembles a benign melanocytic proliferation referred to as Spitz nevus. In some cases, distinction between the two is impossible. Initial misdiagnoses of Spitzoid melanomas as Spitz nevi, thus leading to fatal outcomes, have occurred. The genetic basis and biologic behavior of Spitzoid melanoma is unknown. Although melanoma specimens exhibit high rates of mutation in the B-*RAF* and N-*RAS* genes, the Spitzoid melanoma subtype has not been evaluated. Spitz nevi have been found to be associated with a low percentage of mutations in the H-*RAS* gene; however, the mutational profile of H-*RAS* in Spitzoid melanoma is unknown.

METHODS. The authors evaluated a unique series of melanomas occurring in prepubescent children that showed Spitz nevus–like histopathology (Spitzoid melanoma). All of the melanomas in the current series have metastasized to lymph nodes, confirming the diagnosis of melanoma. The authors examined these tumors, as well as age-matched Spitz nevi, for mutations in the B-*RAF,* N-*RAS,* and H-*RAS* genes.

RESULTS. Activating hotspot mutations in the B-*RAF,* N-*RAS,* and H-*RAS* genes were not identified in Spitzoid melanoma or Spitz nevus specimens.

CONCLUSIONS. There are genetic similarities with respect to the B-*RAF,* N-*RAS,* and H-*RAS* genes between Spitzoid melanoma and Spitz nevi. Such similarities further differentiate these two tumor types from other melanoma subtypes and from melanocytic nevi, respectively. However, mutation analysis of B-*RAF,* N-*RAS,* and H-*RAS* was not useful in differentiating between Spitzoid melanoma and Spitz nevus in children. ***Cancer* 2004;101:2636–40.** © *2004 American Cancer Society.*

KEYWORDS: Spitz nevus, melanoma, Spitzoid melanoma, B-*RAF*, *RAS*, mutation.

The gold standard for diagnosis of melanoma is histopathologic examination. There are a number of subtypes of melanoma, one of which resembles a benign melanocytic neoplasm called *Spitz nevus* and is known as *Spitzoid melanoma* or *melanoma with Spitz nevus–like features.*[1] Spitz nevi typically occur in children and adolescents, although they can occur in adults as well. In contrast, melanomas of all types, including Spitzoid melanoma, are rare in children and are typically observed in adults. Therefore, diagnosis of Spitzoid melanoma in children is extremely difficult, and misdiagnoses can occur.

Approximately 3–4% of all melanomas arise before age 20 years, and 0.4% develop before puberty.[2] Spitzoid melanomas, like Spitz nevi, present as solitary papules or nodules that may or may not exhibit pigmentation on clinical examination. The *ABCD rule* (i.e., asymmetry, border irregularity, color variation, and diameter $>$ 6 mm) for diagnosis of melanoma does not apply to these lesions,

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which therefore are often difficult to identify correctly.[3] Despite detailed characterization over the past few decades of the histopathologic features of these tumors, studies have revealed a lack of objective criteria for differentiating them from Spitz nevi, even when reviewed by expert pathologists.[4] Cases in which metastatic melanomas initially were misdiagnosed as Spitz nevi, thus leading to fatal outcomes, are well documented in the literature.[5] In contrast, cases in which lymph node metastases occurred without further disease progression have also been reported.[6] Due to the rarity of melanoma in children and adolescents and the difficulties associated with its diagnosis, these tumors are problematic for dermatologists, pathologists, and pediatric oncologists.

Approximately 90% of all melanomas are sporadic melanomas. A number of genes have been associated with sporadic melanoma, and low somatic mutation rates have been reported in these genes, which include p53,[7] *CDKN2A*,[8] N-*RAS*,[9] and *PTEN*.[10] Recently, the B-*RAF* oncogene was found to have a high rate of mutation (53–80%) in melanoma.[11] Soon after these findings became known, 70–90% of melanocytic nevi (junctional, compound, intradermal, congenital, and dysplastic nevi) also were found to have B-*RAF* mutations, suggesting a role for B-*RAF* in the early phase of melanocytic neoplasia.[12]

The *RAS* genes (N-*RAS*, K-*RAS*, and H-*RAS*) and B-*RAF* serve as key signal transducers in the RAS/RAF/MAPK signaling pathway, which regulates diverse physiologic processes including cell growth, differentiation, and apoptosis, and plays an important role in melanocytic neoplasia.[13] Activating hotspot mutations in the *RAS* genes (particularly N-*RAS*) have been found in some melanomas and frequently involve amino acids 12 and 13 in exon 2 or amino acid 61 in exon 3. K-*RAS* and H-*RAS* mutations are rare in melanoma.[9] It is noteworthy that a small proportion of Spitz nevi (approximately 8%) exhibit mutations in the H-*RAS* gene.[14] The activating mutations in B-*RAF* cluster in exons 11 and 15, with $>$ 90% involving amino acid 599 in exon 15.[11]

Spitz nevi are benign proliferations of melanocytes that are clinically and histopathologically distinct from other benign melanocytic growths. Some studies have reported the absence of hotspot B-*RAF* mutations in Spitz nevi, a finding that also distinguishes this lesion from other melanocytic nevi and suggests the involvement of different genes in its development.[15,16] Similarly, Spitzoid melanomas represent a clinically and histopathologically distinct subset of melanomas. In general, Spitzoid melanomas occur sporadically and arise in the absence of a family history of melanoma. The genetic basis of Spitzoid melanomas is unknown, and their biologic behavior is not well understood. In the current study, we evaluated Spitzoid melanomas in prepubescent children. By selecting this patient subpopulation, we were able to examine a unique subset of childhood melanoma that had not been studied previously with regard to somatic mutations in candidate genes for melanoma development. All melanomas in the current series had metastasized to lymph nodes, thus confirming the diagnosis of melanoma. We evaluated these tumors for hotspot mutations in the B-*RAF*, N-*RAS*, and H-*RAS* genes, all of which play a role in the RAS/RAF/MAPK signaling pathway and have been associated with either melanoma or Spitz nevi.

MATERIALS AND METHODS

Selection of Specimens

The current study was approved by the Western Institutional Review Board. Two groups of formalin-fixed, paraffin-embedded tumor specimens were selected for study. The first group consisted of 9 Spitzoid melanoma specimens obtained from children age $\leq$ 10 years; disease was confirmed by the presence of metastases. The second group consisted of 10 'typical' Spitz nevus specimens obtained from children age $\leq$ 10 years; the diagnostic criteria for these tumors have been described previously.[17,18]

Mutation Detection

Paraffin-embedded specimens were cut into 5 μm thick sections and stained with hematoxylin. Tumor cells were microdissected using a PixCell II Laser Capture microdissection system (Arcturus, Mountain View, CA). Exons 11 and 15 of the B-*RAF* gene, exons 2 and 3 of the N-*RAS* gene, and exons 1 and 2 of the H-*RAS* gene were amplified via the polymerase chain reaction (PCR), using forward and reverse primer sequences as described previously,[11,16] and then were directly sequenced using an ABI 310 sequencer system (Applied Biosystems, Foster City, CA). These results were confirmed by allele-specific PCR for hotspot B-*RAF* mutations, specifically for the V599E activating mutation.

RESULTS

Clinical and Histopathologic Features

The histopathologic features of a Spitzoid melanoma and a Spitz nevus from the current series are shown in Figure 1. The clinical and histopathologic features of 7 of the 9 Spitzoid melanomas (Patients 11–17) evaluated in the current study have been reported previously.[18] The Spitzoid melanomas in the current series were de novo melanomas occurring in prepubescent children (age $\leq$ 10 years) that exhibited Spitz nevus–

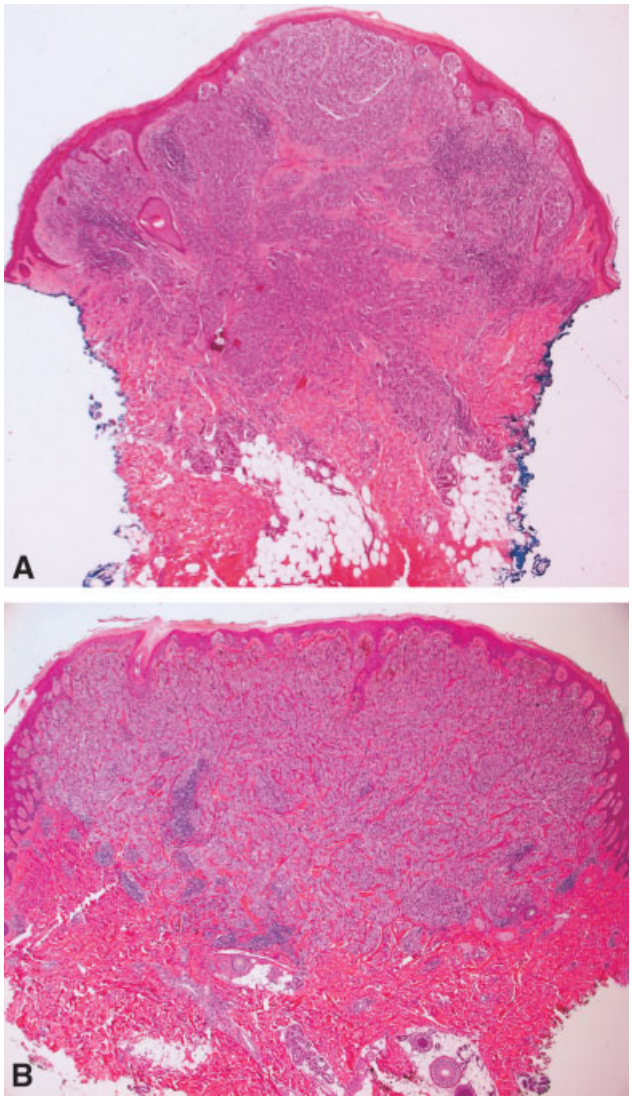

FIGURE 1. Histopathologic features of Spitz nevus and Spitzoid melanoma. (A) Histopathologic findings in a typical Spitz nevus (Patient 5). On low magnification, the lesion is well circumscribed and symmetric. The tumor is composed of melanocytes that form nests within the epidermis and dermis and 'mature' as they descend deep into the dermis. (B) Histopathologic findings in a Spitzoid melanoma (Patient 18) that has metastasized to the lymph nodes. This lesion possesses numerous similarities to a Spitz nevus on histopathologic examination and represents an example of a diagnostically challenging Spitz tumor. Original magnification ×35 (A); ×25 (B).

like histopathology. All patients except for one (for whom a diagnosis was not rendered) originally were diagnosed by the referring pathologist as having Spitz nevus, atypical Spitz nevus, or atypical Spitz nevus versus melanoma (Table 1). All specimens were reviewed independently by two dermatopathologists (D.N.S. and J.M.M) to verify the presence of Spitzoid or Spitz nevus–like histopathology. Patients were confirmed to have melanoma on the basis of the finding that the disease had metastasized. As summarized in Table 1, the median patient age was 6 years (range, 2–10 years). There were six males and three females. Six tumors were localized to the extremities, two to the head and neck, and one to the trunk. All neoplasms had metastasized to the regional lymph nodes (Stage III [T1–T4aN1–N3M0] disease[19]). No Spitzoid melanoma was associated with a congenital melanocytic nevus, xeroderma pigmentosum, systemic malignancy, immunosuppression, or in utero metastasis.

Mutation Analysis

We evaluated 9 specimens of Spitzoid melanoma and 10 age-matched specimens of Spitz nevus for mutations in the B-*RAF*, N-*RAS*, and H-*RAS* genes. Hotspot mutations in these candidate genes were not observed in any of the tumor specimens. A number of silent mutations, representing polymorphisms, were noted, primarily in the H-*RAS* gene and only rarely in the N-*RAS* gene (Table 1). The most common polymorphism, which has been reported previously, was the T→C mutation at nucleotide 81 of the H-*RAS* gene (CAT→CAC), which does not lead to a change in the encoded protein sequence (H27H). We found the same polymorphisms in DNA samples obtained from the overlying normal epithelium as well as in control DNA samples, suggesting that these represent germline sequence variations in the H-*RAS* and N-*RAS* genes. No sequence alterations were found in the B-*RAF* gene.

DISCUSSION

In 1948, Spitz[20] described a series of 13 cases of 'melanoma of childhood', which was characterized by certain histopathologic features and a benign clinical course. It was recognized subsequently that these lesions represented an entity distinct from melanoma, and thus the lesion was renamed *Spitz nevus*, or *spindle and epitheloid cell nevus*.[17] The classic Spitz nevus is a benign melanocytic tumor that typically occurs in childhood but can also be found in adults. A subset of melanoma, Spitzoid melanoma, mimics these neoplasms, both histopathologically and clinically. Ever since its first description, these tumors continue to pose a diagnostic challenge. Even in Spitz's original series, one child died of metastatic melanoma. It remains unclear as to whether Spitz nevus and Spitzoid melanoma reside at opposite ends of a biologic spectrum or represent two separate entities.

A number of histopathologic criteria have been established to differentiate between Spitz nevus and Spitzoid melanoma. In most cases, the diagnosis of a typical Spitz nevus can be achieved by histopathologic

TABLE 1
Summary of Clinical and Histopathologic data and Mutation Analysis

Patient no.	Diagnosis	Original diagnosis[a]	Breslow thickness (mm)	Age at diagnosis (yrs)	Gender	Anatomic site	Sequence variations					
							B-*RAF*		N-*RAS*		H-*RAS*	
							E11	E15	E2	E3	E2	E3
1	SN		1.0	4	F	Leg						
2	SN		0.8	9	M	Ear					H27H	
3	SN		0.7	2	M	Arm						G75G
4	SN		1.0	10	M	Arm					H27H	
5	SN		2.5	6	F	Arm						
6	SN		1.1[b]	5	F	Thigh						
7	SN		1.8	4	M	Knee					H27H	
8	SN		2.2[b]	4	F	Arm					H27H	
9	SN		0.9	10	F	Thigh					H27H	
10	SN		1.6[b]	5	M	Face					H27H	
11	SMM	None	9.0	2.5	F	Thigh				L79L	H27H	
12	SMM	ASN	7.0	8	M	Leg					H27H, V45V	
13	SMM	SN vs. MM	7.0	8	F	Arm						
14	SMM	SN	8.0	2	M	Face						S65S
15	SMM	SN	3.5[b]	2	M	Neck					H27H	
16	SMM	ASN vs. MM	9.3	10	M	Arm						
17	SMM	ASN	1.9[b]	10	F	Back					H27H	
18	SMM	ASN	8.0	6	M	Arm						
19	SMM	ASN	4.4	6	M	Leg						

SN: Spitz nevus; SMM: Spitzoid melanoma; ASN: atypical Spitz nevus; MM: melanoma; F: female; M: male.

[a] Histopathologic diagnosis at biopsy by the referring pathologist.

[b] This measurement represents the thickness of the lesion on initial biopsy, with the lesion showing extension to the deep margin of the specimen. A measurement of the entire lesion is not available for these patients.

examination. In a subset of cases, however, it is nearly impossible to differentiate between the two. Over the years, researchers have attempted to develop ancillary diagnostic techniques that can differentiate between Spitz nevus and Spitzoid melanoma. A number of investigators have suggested using MIB-1 or S100A6 immunoreactivity for this purpose.[21,22] Bastian et al.[14] explored the use of comparative genomic hybridization to identify chromosomal aberrations in melanocytic neoplasms. Those investigators demonstrated that melanomas have high chromosomal alteration rates (~96%), whereas most Spitz nevi do not. In addition, they observed a copy number increase in 11p, which has been associated with mutations in the H-*RAS* gene, in a small proportion of Spitz nevi (~12%).[14] Most previous analyses are not age matched and compare Spitz nevi with conventional melanomas, rather than with the Spitzoid melanoma subtype. Moreover, to our knowledge, none of these ancillary diagnostic techniques have been shown to unequivocally diagnose borderline cores.

It is not known whether the genetic and environmental factors implicated in the development of melanoma in adults also play a role in childhood melanoma. Because of the rarity of childhood melanomas, these tumors have not been studied sufficiently, and they have not been assessed for genetic alterations in candidate genes for melanoma development. The prepubescent Spitzoid melanomas evaluated in the current study comprise a unique subset of childhood melanomas that have not previously been investigated at the molecular level. We found that Spitzoid melanomas of childhood, like Spitz nevi, do not harbor activating hotspot mutations in B-*RAF*, N-*RAS*, or H-*RAS*. These results further differentiate these two tumor types from other subtypes of melanoma. In addition, the observed clinical, histopathologic, and genetic similarities suggest that Spitz nevus and Spitzoid melanoma may represent opposite ends of the spectrum for a single tumor entity. Nonetheless, divergent (but as yet unknown) genetic events must occur to account for differences in the clinical behavior of these two tumor types.

In general, melanoma outcomes appear to be similar for children and adults and are dependent on the initial stage of the tumor.[2] However, the prognosis of Spitzoid melanomas in children remains controversial, due to the rarity of these tumors and the absence of studies with long-term follow-up. A number of cases of Spitz tumors with lymph node involvement

and without subsequent disease progression have been documented.[1,6,23] Nonetheless, cases with lymph node involvement leading to widespread metastasis and death also are well documented.[1,3,20,24–26] All Spitzoid melanomas examined in the current study exhibited lymph node metastasis without visceral involvement. The clinical follow-up of these cases currently is limited to 2–78 months; therefore, prognosis cannot be evaluated at present, and long-term follow-up is necessary.[18]

In summary, we conclude that mutation analysis of B-*RAF*, N-*RAS*, and H-*RAS* is not useful in differentiating between Spitzoid melanoma and Spitz nevus in children. Based on previous clinical observations and the results of the current study, there are clinical, histopathologic, and genetic similarities between Spitzoid melanomas and Spitz nevi. Such similarities differentiate these two tumor types from other melanoma subtypes and from melanocytic nevi, respectively. Although additional studies are needed, it is reasonable to speculate that Spitzoid melanomas may be biologically different from conventional (i.e., non-Spitzoid) melanomas. The genes and signaling pathways involved in the pathogenesis of Spitzoid melanomas and Spitz nevi are currently unknown. Future work will be directed toward uncovering the molecular basis and biologic characteristics of these tumors.

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