Lack of BRAF Mutations in Spitz Nevi

To the Editor:

Spitz nevi are benign melanocytic neoplasms that usually occur in childhood and adolescence. Although the diagnosis of classical Spitz nevus can be reliably achieved by conventional histopathologic criteria, there is a subset of cases in which it is difficult or impossible to differentiate Spitz nevi from melanoma with Spitz-like features (Barnhill *et al*, 1999). There are many cases described in the literature of Spitz nevi misdiagnosed as melanoma or vice versa. There is good documentation of histologically atypical Spitz nevi or lesions originally misdiagnosed as Spitz nevi, metastasizing to regional lymph nodes and leading to widespread metastasis and fatal outcome (Ghorbani *et al*, 1996; Barnhill *et al*, 1999; Lohmann *et al*, 2002).

BRAF belongs to RAF family of proteins (RAF1, ARAF, BRAF) that encode serine-threonine kinases and act in the RAS/RAF/MAPK pathway to transduce regulatory signals from Ras to MEK1/2 (Peyssonnaux and Eychene, 2001). Recently, mutations in BRAF have been identified in 59–80% of melanoma samples (Brose *et al*, 2002; Davies *et al*, 2002; Dong *et al*, 2003). More than 90% of BRAF mutations in melanoma involve codon 599 in exon 15, V599E amino acid substitution being the most common (Brose *et al*, 2002). Soon after the identification of BRAF mutations in melanoma, common melanocytic nevi were also shown to harbor sequence alterations in BRAF, suggesting that deregulation of the RAS/RAF/MAPK pathway is an early event in melanocytic neoplasia. A high frequency of V599 codon substitution (73–82%) has been reported in a variety of common melanocytic nevi including compound, junctional, intradermal, congenital, and dyplastic nevi (Pollock *et al*, 2003; Uribe *et al*, 2003). To investigate whether Spitz nevi, like other melanocytic nevi, have sequence alterations in BRAF, we analyzed Spitz nevi and melanoma samples for mutations in exon 15 of BRAF.

This study has been approved by Western Institutional Review Board (WIRB). Formalin-fixed, paraffin-embedded 30 Spitz nevi and 23 melanoma specimens were retrieved from the archives of Dermatopathology Laboratory at Columbia University. Histologic evaluation was performed by a dermatopathologist (D.N.S.), and two groups of tumor specimens were selected for this study: (1) "typical", unequivocal Spitz nevi and (2) vertical growth phase melanoma (invasive melanoma). The diagnostic criteria for these tumors have been described previously (Paniago-Pereira *et al*, 1978).

Paraffin-embedded specimens were cut in 5 μm thickness and stained with hematoxylin. Tumor cells were microdissected using a PixCell II Laser Capture Microdissection System (Arcturus, Mountain View, California). Exon 15 of BRAF was amplified by PCR using forward and reverse primer sequences as described previously (Davies *et al*, 2002), and directly sequenced with an ABI 310 Sequencer System (Applied Biosystems, Foster City, California). Mutation confirmation was performed by sequencing with the reverse strand primer, as well as PCR amplification and sequencing with a different primer set.

A total of 53 specimens, 30 Spitz nevi and 23 melanoma tumor samples, were analyzed in this study. The age of patients with Spitz nevi ranged from two to 35 (average of 16 y), there were 14 female and 16 males in the group, and all were histologically categorized as "typical" Spitz nevi without unusual features. The melanoma cases selected were all invasive melanomas, with an average Breslow tumor thickness of 3.0 mm.

Mutations in BRAF exon 15 were detected in 13 of 23 (57%) of melanoma samples; however, sequence alterations were not noted in 30 Spitz nevi (Table I). The most frequent mutation was the T1796A substitution, resulting in the V599E amino acid change in BRAF exon 15. This mutation was detected in eight of 23 (35%) melanoma specimens. In one sample, both V599E and T598I (C1793T) mutations were observed (Fig 1*A*). Tandem bp substitutions, GT1795-96AG and GT1795-96AA, which encode V599R and V599K amino acid changes, respectively, were detected in five melanoma tissues (Fig 1*B*, *C*).

BRAF mutation analyses reported to date have mostly been on cell lines rather than primary melanoma samples. Recently, V599 alterations have been reported in 14 of 25 (56%) primary melanoma tissues in one study (Uribe *et al*, 2003), and 28 of 97 (29%) melanoma specimens in another study (Yazdi *et al*, 2003). We found mutation rate of BRAF (exon 15) to be 57% in 23 primary melanomas. The most common mutation in our series was V599E, accounting for eight of 13 (62%) mutations detected. All of the 13 mutations identified affected the activation domain of BRAF and led to V599 amino acid substitution. Our results are consistent with the previous studies that activating BRAF mutations are common in melanoma.

Common melanocytic nevi have also been shown to harbor a high frequency of sequence alterations in BRAF. In one study, 63 of 77 (82%) melanocytic nevi (including congenital, intradermal, compound, dysplastic nevi) were found to have V599E mutation (Pollock *et al*, 2003). Similarly, V599 codon substitution has been reported in 16 of 22 (73%) of melanocytic nevi consisting of compound, junctional and intradermal nevi (Uribe *et al*, 2003). These data implicate the involvement of BRAF in the early phases of melanocytic neoplasia. Spitz nevi are benign melanocytic proliferations that are clinically and histologically distinct from other benign melanocytic nevi. In an attempt to investigate the role of BRAF in the development of Spitz

Table I. Mutation frequencies of Spitz nevi and melanoma in BRAF exon 15

Tumor type	Mutation frequency in BRAF Exon 15	Mutation data (number of cases)
Spitz nevus	0/30 (0%)	
Melanoma	13/23 (57%)	V599E (8)
		V599K (4)
		V599R (1)
		T598I (1)

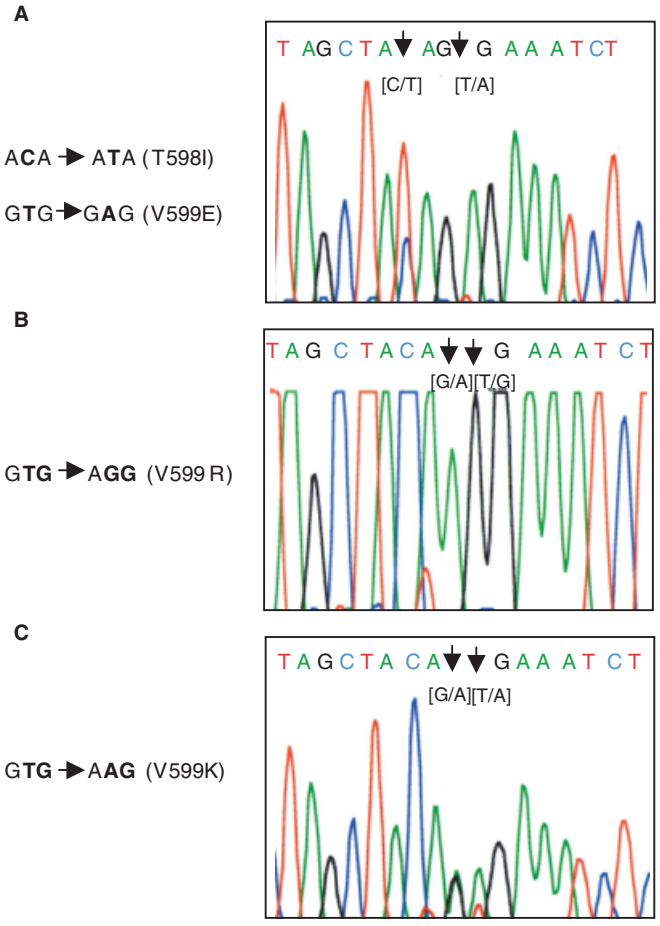

Figure 1
BRAF exon 15 mutations in melanoma. (*A*) Two point mutations, C1793T and T1796A substitution resulting in T598I and V599E amino acid change, respectively. (*B*) GT1795-96GG, encoding the V599R amino acid change. (*C*) GT1795-96AG, encoding the V599K missense mutation.

nevi, we examined 30 Spitz nevi for mutations in BRAF exon 15. Interestingly, we did not find any sequence alterations. During the preparation of our manuscript, Yazdi *et al* also reported the absence of V599E mutation in 69 Spitz nevi (Yazdi *et al*, 2003). The lack of common exon 15 BRAF mutation in Spitz nevi further differentiates this tumor from other common melanocytic nevi and suggests the involvement of different gene/s in its development.

Spitz nevi are benign melanocytic proliferations that can resemble melanoma on histologic examination. There is a subset of cases in which it is difficult or impossible to distinguish from melanoma. Over the years researchers attempted to develop ancillary diagnostic techniques with which one can differentiate Spitz nevus from melanoma. For example, a significant difference in the frequencies and types of chromosomal aberrations between melanomas and Spitz nevi exist, and can be useful in differentiating these tumors in some cases (Bastian *et al*, 1999; Bastian *et al*, 2003). But techniques that are able to unequivocally discriminate among borderline cases, and a large series confirming their usefulness are lacking (Su *et al*, 2003). The lack of exon 15 BRAF mutation in Spitz nevi may be useful in distinguishing these tumors from melanoma with Spitz-like features. The mutational profile of BRAF in melanoma with Spitz-like features, however, is unknown. Evaluation of a series of this subset of melanoma will help to address this issue and determine if BRAF mutations can help to distinguish Spitz nevi from melanoma with Spitz-like features.

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