Chromosomal Aberrations by 4-Color Fluorescence In Situ Hybridization Not Detected in Spitz Nevi of Older Individuals

Basil A. Horst, MD, DrMed; Yuqiang Fang, MD; David N. Silvers, MD; Klaus J. Busam, MD

Objective: To investigate whether Spitz nevi with typical histopathological features in older patients demonstrate chromosomal aberrations by 4-color fluorescence in situ hybridization (FISH).

Design: Retrospective medical record review, with prospective masked histopathological and cytogenetic analyses.

Setting: University-affiliated dermatology and dermal pathology setting.

Patients: Twenty-five patients 50 years or older with melanocytic nevi showing histopathological features typical of Spitz nevi.

Main Outcome Measures: Three dermatopathologists masked to the patients’ ages reviewed histopathological sections of melanocytic lesions for features typical of Spitz nevi. FISH was performed on samples with typical histopathological features by a 4-color FISH probe set used for the evaluation of malignant melanocytic neoplasms.

Results: None of the study cases showing histopathological features typical of Spitz nevi had detectable chromosomal abnormalities by FISH.

Conclusions: Spitz nevi in older patients demonstrate molecular features similar to those of Spitz nevi in younger age groups. The findings of normal karyotypes in combination with typical histopathological features are reassuring of Spitz nevus diagnoses in older patients and suggest no correlation of increased malignant potential with advanced age per se.


CLASSIFICATION SYSTEMS of melanocytic neoplasms that combine histomorphological findings with molecular genetic characteristics added a new dimension to the traditional categorization based on clinical, histopathological, and immunohistochemical features. From classification studies of melanocytic tumors based on DNA copy number changes, chromosomal aberrations that can be detected using a 4-color fluorescence in situ hybridization (FISH) probe set were shown to correlate with malignant melanocytic neoplasms. Spitz nevi were originally described by Spitz in 1948 as “benign juvenile melanomas.” These lesions show a higher prevalence among younger age groups, with most occurring in children younger than 15 years. In clinical practice, the term Spitz tumor is sometimes used to express uncertainty about a tumor's biological characteristics and clinical behavior in adult patients, in some cases despite typical histomorphological features. Whereas several genomic aberrations that frequently occur in melanoma and are absent from most common nevi were identified by comparative genomic hybridization, correlation of histopathological features with certain genetic characteristics is not well studied in Spitz nevi or Spitz tumors. Polyploidy is found in up to 10% of Spitz nevi,7 and 15% to 20% of Spitz nevi show isolated gains in chromosome 11p, which has not been described as a characteristic feature of melanoma.28

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In the present study, we investigated whether Spitz nevi in older patients demonstrate chromosomal aberrations by a 4-color FISH probe set used for the evaluation of malignant melanocytic neoplasms. All the lesions showed histopathological features that are typical of Spitz nevi in children or adolescents. So-called Spitz tumors, which exhibit problematic histopathological features, were not the focus of this study.
**Table. Clinical and Histopathological Features of Spitz Nevi in 25 Patients 50 Years or Older**

<table>
<thead>
<tr>
<th>Age Group, y</th>
<th>Anatomic Site, No.</th>
<th>Histopathological Type, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upper Extremities</td>
<td>Trunk</td>
</tr>
<tr>
<td></td>
<td>(n = 9)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>50-59 (n = 19)</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>60-69 (n = 3)</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>70-79 (n = 1)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>80-85 (n = 2)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Compound</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>Dermal</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

The study population comprised 18 women and 7 men (mean age, 57.8 years; median age, 55 years).

**METHODS**

**SELECTION OF STUDY PATIENTS**

The study protocol was approved by the institutional review board at Columbia University, New York, New York. In a computer-assisted search of medical records between July 1, 2000, and June 30, 2010, a total of 38 patients 50 years or older for whom the diagnostic pathology report included the term Spitz nevus were identified from the archives of the Dermatopathology Laboratory at Columbia University, New York, New York. Twelve consecutive cases of patients with a diagnosis of Spitz nevus served as controls, including 8 adults aged 25 to 45 years and 4 children aged 7 to 13 years.

**HISTOPATHOLOGICAL ASSESSMENT**

Lesions were independently evaluated by 3 of us (B.A.H., D.N.S., and K.J.B.) who were blinded to the patients' ages. Only lesions for which all 3 dermatopathologists agreed that the histopathological features were typical of Spitz nevi were included in the study. These features included the following: epidermal hyperplasia, a symmetric dome-shaped silhouette, the presence of lamino bodies (acellular pink globular structures of basement membrane material), a well-demarcated lesion (ending in a nest at the periphery), and monomorphic, epithelioid, and spindle-shaped melanocytes, with clefing around nests at the dermoepidermal junction. In addition, for compound and dermal lesions, typical features included maturation with descent (diminishing cell size and nest size) and the presence of mature thick collagen bundles. These criteria are well established and were previously described. Lesions lacking sufficient residual material and lesions with atypical histopathological features, as interpreted by 1 or more investigators, were excluded. Histopathological features considered atypical in junctional and compound lesions included a lack of circumscript, prominent pagetoid scatter, asymmetry of the silhouette, and marked cytological atypia or pleomorphism; in addition, atypical histopathological features in compound and dermal lesions included atypical mitoses, deep extension into subcutaneous fat, and mitoses in the deep dermal component.

**FLUORESCENCE IN SITU HYBRIDIZATION**

For FISH, (5-µm thick) sections were cut from 10% formalin-fixed and paraffin-embedded tissue blocks and placed on positively charged slides (ThermoShandon; Thermo Fisher Scientific Inc). FISH was performed using a panel of 4 probes, each labeled with a different spectrally distinct fluorophore. The 4 probes targeted (1) ras-responsive element binding protein 1 (Vysis LSI RREB1-Spectrum Red) on chromosome 6p25, (2) myeloblastosis (Vysis LSI MYB-Spectrum Gold) on chromosome 6q23, (3) cyclin D1 (Vysis LSI CCND1-Spectrum Green) on chromosome 11q13, and (4) a centromeric control probe for chromosome 6 (Vysis LSI CEP6-Spectrum Aqua) (all from Abbott Molecular Inc). A lesion was considered to have a positive FISH result if any of the following criteria were met: (1) gain in chromosome 6p25 (RREB1) relative to CEP6 greater than 35%, (2) gain in chromosome 6p25 (RREB1) greater than 29%, (3) loss of chromosome 6q23 (MYB) relative to CEP6 greater than 40%, or (4) gain in chromosome 11q13 (CCND1) greater than 35%. Probe enumeration was performed by 2 of us (Y.F. and K.J.B.) with extensive experience in FISH and cytogenetic analyses who were masked to the patients' ages.

**RESULTS**

**CHARACTERISTICS OF THE STUDY AND CONTROL POPULATIONS**

In total, 38 patients 50 years or older for whom the diagnostic pathology report included the term Spitz nevus were included in the age-masked evaluation for typical histopathological features. Eight lesions showed atypical histopathological features as interpreted by at least 1 dermatopathologist reviewer and were excluded from further analysis. Five additional lesions showed insufficient residual material for cytogenetic analysis. Therefore, a total of 25 lesions were analyzed. The mean age of the study population was 57.8 years (age range, 50-85 years), with a median age of 55 years. The cohort included 7 men and 18 women, with a 1:2.5 ratio of men to women. The lower extremities represented the most common anatomic site overall (10 of 25 lesions [40%]) and for women (9 of 18 lesions [50%]); the most common anatomic site for men were the upper extremities (3 of 7 lesions [43%]), followed by the back (2 of 7 lesions [29%]). The most common histopathological type was compound (14 of 25 lesions [56%], of which were predominantly dermal with a small junctional component), followed by junctional (9 of 25 lesions [36%]) and dermal (2 lesions [8%]). The distributions of anatomic sites and histopathological types by age group are summarized in the Table.

All 12 control subjects (8 adults aged 25-45 years and 4 children aged 7-13 years) had lesions with histopathological features of typical Spitz nevi as judged by all 3 dermatopathologists blinded to the patients' ages (see the “Histopathological Assessment” subsection of the “Methods” section). Among them, the anatomic site distribution for the adults was 4 lesions on the upper extremities, 3 on the lower extremities, and 1 on the trunk, while...
the distribution for the children was 1 lesion each on the head or neck (ear), upper extremities, lower extremities, and trunk. Therefore, the overall distribution of anatomic sites in the control group was similar to that of the study population 50 years or older. No lesions in the study population had developed on severely sun-damaged skin, and solar elastosis was not a significant finding in any of the cases studied; therefore, the possible effect of solar elastosis as a confounder in the masked evaluation was limited. A representative Spitz nevus occurring on the thigh of a 57-year-old woman with histopathological features typical of Spitz nevi in children or adolescents is shown in Figure 1.

Figure 1. Spitz nevus occurring on the thigh of a 57-year-old woman. A, Note epidermal hyperplasia, circumscribed symmetric proliferation of nests, and fascicles of melanocytes (hematoxylin-eosin composite, original magnification ×40). B, Nests of epithelioid melanocytes and Kamino bodies (arrow) (hematoxylin-eosin, original magnification ×100). C, Fluorescence in situ hybridization showing representative nuclei with normal (diploid) karyotype for all 4 chromosomal loci tested. Green indicates CCND1; red, RREB1; gold, MYB; and aqua, CEP6 (original magnification ×630).

We performed cytogenetic analyses on 25 typical Spitz nevi in patients 50 years or older and on 12 control lesions, masked to the patients’ ages. Four-color FISH enumeration showed normal karyotypes in all 25 study lesions, with none of 4 probe signals reaching threshold criteria for melanoma in any of the lesions tested, according to previously published scoring criteria. A negative (diploid) FISH result from a representative Spitz nevus in a 57-year-old woman is shown in Figure 1C. Negative FISH results were also obtained in 11 control cases of Spitz nevus from 4 children and 7 adults. One 44-year-old female control subject formally met the criteria for a positive FISH result showing balanced gains in 3 to 4 signals per nucleus for all 4 probes analyzed (chromosomes 6p25, 6q23, 11q13, and CEP6) (Figure 2), which indicates polyploidy and should not be interpreted as indicative of a malignant neoplasm (see the “Comment” section). Overall, chromosomal aberrations indicating malignant melanocytic neoplasms were not detected in Spitz nevi with typical histopathological features, independent of the age of patients.

**FLUORESCENCE IN SITU HYBRIDIZATION**

Spitz nevi are thought to occur mostly in children and adolescents, although some larger studies found higher incidence rates in adults. Pathologists generally agree on classic or typical examples of Spitz nevi, especially in younger patient populations. Diagnostic controversy is usually associated with Spitz nevi or tumors having atypical features. Because a rare atypical Spitz tumor may eventually manifest as a malignant melanoma by adverse outcome and because this scenario is more likely in adults than in children, a pathologist’s fear of misinterpreting such a Spitz nevus–like or Spitz tumor–like melanoma increases with the age of the patient. Hence, many pathologists hesitate to render a Spitz nevus diagnosis in older patients, and extreme caution before assigning such a diagnosis in older individuals has been proposed in the literature.

Therefore, we investigated whether Spitz nevi with typical histopathological features in older adults show molecular biological features associated with malignant melanocytic neoplasms and may differ from lesions observed in younger patients, which would reinforce caution in diagnosis. Patients with diagnostically controversial lesions exhibiting ambiguous features were excluded from the study population herein.

Within our collective of 25 typical Spitz nevi in patients 50 years or older, we observed a clear female predominance. Previous studies on the age distribution of Spitz nevi in adults describe a preponderance among women, mainly in the third decade of life, and a ratio of men to women that is close to 1 in children and in adults 40 years or older. Therefore, our patient group 50 years or older differed somewhat in sex distribution from that in the previous studies. The most prevalent histopathological type of Spitz nevus herein was compound, fol-
owed by junctional, which was similar to that described for younger patients in the previous studies.

To test for possible malignant neoplasm–related karyotype abnormalities in these lesions, we used a 4-color FISH probe set currently in use for the assessment of aggressive potential in melanocytic neoplasms, according to previously published criteria.10 For melanomas in general, sensitivity and specificity for this test are approximately 85% and 95%, respectively.3 Therefore, in ambiguous melanocytic neoplasms, FISH results can be helpful in the clinical decision-making process regarding additional treatment options (eg, wide local reexcision or possible sentinel lymph node biopsy). In all 25 lesions from patients aged 50 to 85 years, probe enumeration for RREB1, MYB, CCND1, and CEP6 did not meet threshold criteria for malignant melanocytic neoplasms as previously defined, with the findings of normal karyotypes based on the probe set used. Previous studies14-16 among patient populations that included few older adults reported normal karyotypes in patients with Spitz nevi as assessed by the same probe set in 4 additional patients 50 years or older. Furthermore, the findings of normal karyotypes in patients up to age 57 years can be inferred from an additional study17 on polyploidy in Spitz nevi; however, the total number of patients in the age group was not specified.

FISH was also performed in a control population of Spitz nevi, from 8 adults (age range, 25-45 years) and 4 children (age range, 7-13 years). Similar to the study population, the anatomic site distribution in our control population (see the “Characteristics of the Study and Control Populations” subsection of the “Results” section) did not show a predominance of Spitz nevi on the face, which has been described in textbooks as the most common location, especially in children.3 However, our findings are consistent with those by Requena et al,12 who reported a facial location in 22% of children and 7% of adults in their large series of 349 Spitz nevi. As expected, most of the control subjects herein had a normal karyotype, except in the case of a 44-year-old woman who showed balanced gains in all 4 probes analyzed (3-4 signals per nucleus for each probe) (Figure 2C). A likely explanation for balanced gains across several probes is polyploidy, and indeed tetraploidy has been observed in up to 10% of Spitz nevi,2 as well as in a case of agminated Spitz nevi using the same FISH probe set.14 Awareness of this phenomenon is critical to avoid false-positive interpretation of FISH results formally meeting criteria for malignant melanocytic neoplasms in the event of balanced gains in the chromosomal loci targeted.

Although most Spitz nevi with typical histopathological features behave in a benign fashion, rare cases of metastasis even in children are documented.17 Therefore, and because of a higher a priori probability of melanoma in older patients,2 dermatopathologists and dermatologists hesitate to infer general benign behavior of Spitz nevi in adults. Although Spitz nevi may biologically represent a spectrum of entities, our findings are reassuring of Spitz nevus diagnoses in older patients because the results herein indicate the absence of 4-color FISH detectable malignant melanocytic neoplasm–related chromosomal alterations in typical Spitz nevi independent of the age of patients. These findings are expected in benign neoplasms; however, it is possible that in spitzoid lesions other alterations associated with malignant behavior occur that are not detected by the current FISH test.

To better define the spectrum of molecular aberrations in Spitz nevi across different age groups, further studies are needed such as comparative genomic hybridization to evaluate chromosomal imbalances across the entire genome. Such studies could identify potential targets for the development of additional FISH probe sets specifically for evaluating spitzoid neoplasms. Chromosomal aberrations in Spitz nevi were shown to differ from those found in melanomas by comparative genomic hybridization.
ization \(^8\),\(^9\) and a 2012 study \(^10\) on a possible extension of the standardized 4-color FISH probe set, to enhance sensitivity for the detection of spitzoid melanomas from 70% to 86%, may signify first advancements in this regard. Furthermore, because chromosome 11p gains are seen in some Spitz nevi and have not been reported as an isolated finding in melanoma, an additional probe targeting this chromosome is a potential tool for the confirmation of a Spitz nevus diagnosis. Probe sets for chromosome 11p described in the original studies \(^2\), \(^8\) are not in routine clinical use because of the lack of standardized targets and cutoff criteria and because positive findings occur in only 15% to 20% of cases. The objective of the present study was to focus on chromosomal abnormalities detected by the standardized 4-color FISH probe set currently in clinical application.

A limitation of our study is the lack of outcome data, and we cannot exclude with certainty that some histopathologically benign lesions recurred or eventually progressed. However, our close and long-term relationship with referring dermatologists would prompt us to speculate that it would have come to our attention if the included cases had progressed.

In summary, advanced age in patients with typical Spitz nevi is not accompanied by chromosomal aberrations as detected by a 4-color FISH probe set currently in use for the evaluation of malignant melanocytic neoplasms. Based solely on FISH results and histopathological criteria, these lesions resemble Spitz nevi that occur in younger age groups. Our results are reassuring and suggest that the molecular characteristics of classic Spitz nevi in young patients and in older patients are similar, lending no support for increased likelihood of aberrations typical of melanoma with advanced age per se. However, studies targeting additional chromosomal loci are warranted to further determine the biological potential of Spitz nevi across different age groups.

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Correspondence: Basil A. Horst, MD, DrMed, Department of Dermatology, Columbia University Medical Center, Vanderbilt Clinic, Room 15-207, 630 W 168th St, New York, NY 10032 (bh2179@columbia.edu).

Author Contributions: Drs Horst and Busam 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: Horst, Silvers, and Busam. Acquisition of data: Horst, Fang, and Busam. Analysis and interpretation of data: Horst, Silvers, and Busam. Drafting of the manuscript: Horst and Busam. Critical revision of the manuscript for important intellectual content: Horst, Silvers, and Busam. Administrative, technical, and material support: Horst and Fang. Study supervision: Horst, Silvers, and Busam.

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REFERENCES