Immunoexpression of BAP1, ROS1, and ALK in Spitzoid Melanocytic Tumors

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Abstract

Background. Spitzoid tumors are a heterogeneous group of melanocytic neoplasms that frequently imposes diagnostic difficulties. Lately, several advances in molecular biology afforded significant discoveries on the pathogenesis of these tumors. BAP1 (BRCA-1 associated protein-1) inactivation and anomalous expression of kinase translocation-related proteins are among the main criteria launched by new classification proposals. Our aim was to systematically assess the immunoexpression of BAP1, ROS1 (receptor tyrosine kinase c-Ros onco gene 1), and ALK (anaplastic lymphoma receptor tyrosine kinase) proteins in an unpublished series of spitzoid tumors. Methods. Retrospective study based on 47 formalin-fixed paraffin-embedded tissue samples from 3 different institutions. BAP1, ROS1, and ALK immunostains were performed in all cases. We included 27 Spitz tumors without significant abnormality, 15 atypical spitzoid tumors, and 5 spitzoid melanomas. Results. We observed loss of BAP1 nuclear immunolabeling in 4.3% of evaluable cases (2/46), both of them atypical spitzoid tumors. The proportional frequency of BAP1-inactivated cases among atypical spitzoid tumors was 14.2% (2/14). No immunoexpression of ROS1 or ALK was found. Conclusions. Our study revealed 2 additional BAP1-inactivated cases and described its respective frequency. The absence of anomalous expression of translocation-related proteins ALK and ROS1 in this series, composed predominantly of low-grade/low-risk tumors, indicates that translocated spitzoid lesions may not be as prevalent as initially suggested, at least in some populations. Furthermore, our findings encourage additional investigation on unequal occurrence of such immunomarkers among different diagnostic categories of spitzoid neoplasms.

Keywords
spitzoid, melanocytic, BAP1, ROS1, ALK

Introduction

During the past few years, significant discoveries have largely changed the landscape of knowledge on the pathogenesis of spitzoid tumors. Consistent evidence indicates that it represents a biologically heterogeneous group of melanocytic neoplasms.1,2 BAP1 (BRCA-1 associated protein-1) inactivation and anomalous expression of kinase rearrangement-related proteins are among the main criteria introduced by new classification proposals. Our aim was to systematically assess the immunoexpression of BAP1, ROS1 (receptor tyrosine kinase c-Ros oncogene 1), and ALK (anaplastic lymphoma receptor tyrosine kinase) proteins in an unpublished series of spitzoid tumors.
is a reliable and cost-effective method to assess BAP1 status in melanocytic lesions.

In 2014, Wiesner et al demonstrated that chimerical proteins resulting from rearrangements of tyrosine kinases codifying genes represent key mechanisms in the genesis and the progression of a significant amount of spitzoid tumors. In spite of not being sufficient for malignant transformation, such alterations constitutionally activate signaling pathways associated to cellular proliferation. Receptor tyrosine kinase c-Ros oncogene 1 (ROS1) is a proto-oncogene highly expressed in several tumoral cell lines and belongs to a subfamily of insulin receptors that acts in response to growth or differentiation factors. Similarly, anaplastic lymphoma receptor tyrosine kinase (ALK) encodes a tyrosine kinase protein also belonging to a superfamily of insulin receptors. It is a widely known proto-oncogene that may be translocated, mutated, or amplified in various human cancers, but others may be affected. According to these authors, immunoexpression of anomalous proteins is frequent in translocated tumors. This special translocation-positive subgroup deserves clinical attention because part of its pathogenic alterations is potentially susceptible to commercially available kinase inhibitors that could be used in cases with aggressive biological behavior.

New classification proposals aiming to better reflect the biological complexity of spitzoid tumors have recently been introduced. While the use of molecular techniques is increasingly emerging on this field, we intended to investigate the role of immunohistochemistry on such a new scenario. In this article, we assessed the immunohistoexpression of BAP1, ROS1, and ALK proteins in an unpublished case series of spitzoid tumors.

**Material and Methods**

This was a retrospective study based on formalin-fixed paraffin-embedded samples obtained from the Surgical Pathology archives of 3 health care institutions in São Paulo, Brazil (Complexo SPDM/Hospital São Paulo, Instituto do Câncer do Estado, de São Paulo e Hospital do Câncer de Barretos). The samples were collected between 2000 and 2015 for health care purposes, mainly from General Dermatology Clinical Division and Adult Oncological Reference Centers. After the search for the descriptors Spitz nevus or Reed nevus or atypical spitzoid tumor or spitzoid melanoma in the respective internal systems, we found 80 cases from which 47 fulfilled the following inclusion criteria: (1) spitzoid tumor diagnosis (confirmed after research review by senior dermatopathologist; see criteria detailed below), (2) available paraffin block, and (3) sufficient amount of tumoral tissue remaining in the paraffin block. The main reason for exclusion was external review without available paraffin block (slide-only case review).

For each case, we selected 1 representative block and collected clinical and demographical data (age, sex, site, and follow-up), if available. Six whole tissue sections were performed in silanized slides using routine histologic techniques. The first section of each case was stained with hematoxylin-eosin. A 3-tier graduation/risk stratification system was applied: Spitz tumors without significant abnormality (SWA), AST, and spitzoid melanomas (MS). Inclusion criteria and graduation/stratification were based on widely accepted recommendations. We defined as spitzoid solely melanocytic lesions that showed fusiform and/or epithelioid cytomorphology, commonly with junctional nests and fascicles oriented vertically alongside interpapillary crista. Kamino bodies, epidermal hyperkeratosis, junctional clefting, and telangectasia were additional features, mainly observed in SWA and AST. Microscopic criteria for graduation/stratification are displayed on Table 1. Histomorphological diagnosis was properly performed by 2 pathologists, including a senior dermatopathologist (GL).

Immunohistochemical assays were performed according to parameters displayed on Table 2. We used primary antibodies against BAP1 (C4, 1:70; Santa Cruz), ALK (ALK1, RTU; Dako, and ROS1 (antiROS1, 1:100; Epitomics). Prior to immunostaining procedures, 9 densely pigmented cases, Reed nevi included (cases 4, 9, 11, 12, 19, 20, 41, 45, and 47), were submitted to melanin bleaching protocol with warm hydrogen peroxide, which is known not to reduce antigenicity. Adequate positive controls were selected following manufacturer’s recommendations and worked properly. In order to experimentally confirm that the bleaching method did not affect the antigenicity in our study, we performed control reactions both with and without melanin bleaching. BRAFV600E immunoassays (VE1, 1:200; Spring Bioscience) were performed in BAP1-inactivated lesions only.

Both histopathological and immunohistochemical features were interpreted by 2 pathologists blinded to clinical and demographic data. Discordant cases were reassessed to obtain a consensus result. One case (case 22) was excluded from the immunohistochemical assessment for BAP1 due to negative internal control.

This study was approved by the institutional board review (Comitê de Ética em Pesquisa da Universidade Federal de São Paulo) under the registry CAAE 49817115.6.0000.5505, according to ethical international standards stated in the Declaration of Helsinki.
Results

Among the 47 spitzoid lesions that compose this case series, we found 27 SWA, 15 AST, and 5 MS. Thirty-three patients were female and 14 were male (33 female and 14 male). The overall mean age at diagnosis was 21.2 years (1-65 years). The specific mean ages by diagnostic categories were 15.5 years for SWA, 23.9 years for AST, and 44.6 years for MS. In 2 cases, age was not available. Overall mean histological horizontal width was 5.7 mm. As others, we considered the histological horizontal width of the lesion and measured it directly on each slide using a microscopic ruler. The main clinical and pathological features are summarized in Table 3. Further access to clinicopathological data is possible through the supplementary material (available in the online version of the article).

We found loss of BAP1 nuclear immunoexpression in 4.3% of the total cases evaluated (2/46). Such cases were purely intradermal tumors composed solely of atypical epithelioid cells. Associated conventional melanocytic nevi were not found in any lesion. The proportional frequency of BAP1-inactivated cases was 14.2% (2/14) in the AST subgroup. It is worth highlighting the presence of perinuclear and membranous cytoplasmic immunostaining in BAP1-inactivated cases (cases 18 and 28). Such pattern of expression showed heterogeneous distribution and variable intensity, affecting up to half of neoplastic cells in each lesion. BAP1 representative immunohistochemical findings are shown in Figure 1. As previously mentioned, BAP1-negative tumors were further studied for BRAFV600E immunostains. Case 28 was VE1-positive and case 18 was VE1-negative. One lesion (case 28) showed increased tumor...
infiltrating lymphocytes. Follow-up data were available for patient 28 and revealed no evidence of disease after 5 years (Supplementary material, available in the online version of the article).

No immunolabeling for ROS1 or ALK proteins was found in any case (see Figure 2). Besides, there was absence of cases showing characteristic histological features of ALK-related tumors as previously reported in literature, such as plexiform dermal growth of intersecting fascicles and intercellular clefts.17,18

Discussion

Traditionally considered by some experts among the most troublesome entities of cutaneous melanocytic neoplasms, the spitzoid tumor group has recently become even more complex as a diagnostic category. Different studies have revealed new independent pathogenic mechanisms, reinforcing the hypothesis that spitzoid tumors represent a miscellaneous set of nonrelated biological entities.2,3,10 This possibility is quite attractive once it better fits to the highly variable biological behavior, long observed and feared by clinicians and pathologists.

The occurrence of BAP1-inactivated cases in our series adds to the emerging literature on this issue. Both of them were diagnosed as AST, showed heterogeneous cytoplasmic and membranous deposits of BAP1 protein, and presented characteristic histopathological features, as previously described in the literature.3,11 Our findings are in accordance to those of Gammon et al19 who reported a large subset of spitzoid tumors displaying loss of nuclear BAP1 immunolabeling but retaining clumped perinuclear staining pattern.19 In their series, composed of cases from research database files on melanocytic nevi with atypical epithelioid cell component, such labelling pattern was found in the majority of BAP1-related tumors. This aberrant BAP1 protein location has also been noted by others.3,13 Pathologists should be aware of this phenomenon in order to avoid misdiagnosis. Although not yet properly investigated, it has been postulated that different inactivating mechanisms might block the transport of the protein to its functional position in the nucleus.19

Due to its recent description, the known frequency of BAP1-associated lesions among spitzoid tumors is yet to be established. Before comparing our results with those previously reported in the literature, carrying out a critical analysis on the limits of a such comparative approach is important. As one could expect, previous studies presented different methodological designs and primary goals. As highlighted by Wiesner et al,11 most articles
have restricted the investigation of BAP1 to the AST diagnostic category. Others have expanded the search to a wider spectrum of melanocytic lesions (not spitzoid only) or were based on apparently biased research files.13,19,20 Basically, the points that unite them are the following: (1) the presence of spitzoid lesions and (2) BAP1 immunohistochemical assessment. We considered that these common aspects justify some comparison, even if limited.

We chose to consider only AST samples of each study because this category represented the totality of the BAP1-inactivated cases in our study and because it seems the most balanced comparative approach against other results found in the literature. As we can see from Table 4, the frequency of BAP1 inactivation in AST shows an exceptionally wide variation range. While samples obtained from Surgical Pathology files showed lower frequencies, case series composed of research files tended to show much higher rates. Our results indicate that BAP1 nuclear loss represents a minimal slice of spitzoid tumors, even when considering the AST group only. We support that the wide variation observed in literature is likely in part a consequence of different methodological approaches.

BAP1-related tumors commonly harbor BRAFV600E mutations.11 Monoclonal mutation-specific antibody VE1 is efficient to assess the expression of anomalous protein in formalin-fixed paraffin-embedded tissue samples.21 BAP1-inactivated and VE1-negative cases have been reported.12 In this study, 1 case (case 28) was VE1-positive and the other (case 18) was VE1-negative. We found no other BAP1-associated visceral malignances for both of them at the time of diagnosis. Patient 28 showed no evidence of disease after 5 years of follow-up.

The absence of immunoexpression of ROS1 and ALK in all cases is noteworthy. Despite the recent description and the incipient knowledge on its frequency, such unexpected results differ from previous studies and lead us to discuss its possible reasons. First, a striking feature on our set of cases was the asymmetrical distribution among SWA, AST, and MS, with predominance of the former. Although incipient, previous evidence shows that ALK-related tumors tend to be more frequent in AST-predominant series.17,18,22,23 Recently, Rand et al24 reported 2 cases of ALK-translocated spitzoid lesions showing 9p21 homozygous deletions. Both of them were AST.24 We considered that the relatively low AST frequency in our study might have influenced the negative results. Another point that further corroborates our findings was the absence of cases showing phenotypic features of ALK-translocated tumors, such as plexiform dermal growth of intersecting fascicles and intercellular clefts.17,18 Evidence for ROS1 immunoexpression in spitzoid tumors is scarcer. To the best of our knowledge, Wiesner et al2 were the only group to publish ROS1 immunohistochemical results in a spitzoid tumor only series. In a large molecular-based study composed of 140 cases, they found 24 ROS1-positive tumors.2 Due to the lack of molecular investigation in this study, further comparison is precluded.

The lack of investigation for genomic status through specific molecular methods (fluorescence in situ hybridization or sequencing) in this present study should be remarked. Although consistent evidence points to an association between kinase rearrangement and anomalous expression of related proteins, limitations on direct association between a given genomic translocation and its equivalent immunophenotype should not be ignored, as highlighted by others.2,23 However, our negative results obtained from a significant spitzoid tumor case series add to the incipient literature on this emerging interest theme.

Moreover, it is not possible to assume that spitzoid tumors in Brazilian population show the same profile observed in North American and European populations. Future research on variation in prevalence-by-population
is needed. Additional limitations of this study included the retrospective approach and the lack of full follow-up data. These aspects are intrinsically related and recurrent when studying rare skin lesions. Albeit challenging, prospective studies are imperative to better understanding spitzoid tumors.

In summary, this study revealed 2 additional BAP1-inactivated cases and described its respective frequency in an unpublished spitzoid tumor set of cases. The absence of anomalous expression of translocation-related proteins ALK and ROS1 in this case series, composed predominantly of low-grade/low-risk tumors, indicates that translocated spitzoid lesions may not be as prevalent as initially suggested, at least in some populations. Furthermore, our findings encourage additional investigation on unequal occurrence of such immunomarkers among different diagnostic categories of spitzoid neoplasms.

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Declaration of Conflicting Interests
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Ethical Approval
Not applicable, because this article does not contain any studies with human or animal subjects.

Informed Consent
Not applicable, because this article does not contain any studies with human or animal subjects.

Trial Registration
Not applicable, because this article does not contain any clinical trials.

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References

Table 4. BAP1-Related Atypical Spitzoid Tumors Case Series in the Literature.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of Cases</th>
<th>Molecular Investigation</th>
<th>BAP1 Results</th>
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<tbody>
<tr>
<td>Wiesner et al,11 2012</td>
<td>32</td>
<td>Yes</td>
<td>28.1% (9/32)</td>
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<tr>
<td>Gammon et al,19 2013</td>
<td>19</td>
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<td>78.9% (15/19)</td>
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<tr>
<td>Busam et al,20 2013</td>
<td>6</td>
<td>No</td>
<td>100% (6/6)</td>
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<tr>
<td>Piris et al,13 2015</td>
<td>3</td>
<td>Yes</td>
<td>100% (3/3)</td>
</tr>
<tr>
<td>Present study</td>
<td>14</td>
<td>No</td>
<td>14.2% (2/14)</td>
</tr>
</tbody>
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Informed Consent
Not applicable, because this article does not contain any studies with human or animal subjects.

Trial Registration
Not applicable, because this article does not contain any clinical trials.

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