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Ambiguous Melanocytic Tumors with loss of 3p21

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Abstract

Germline loss of function mutations in BAP1 are associated with the development of cutaneous melanocytic tumors with some histopathologic characteristics seen in Spitz nevi. Similar melanocytic tumors occurring in a sporadic setting have been demonstrated to have biallelic loss of BAP1. In some of these sporadic tumors, loss of BAP1 occurs through mutation of one allele and genomic loss of the other. We screened our database of comparative genomic hybridization profiles of ambiguous melanocytic tumors to identify cases with a single genomic event involving loss of the BAP1 locus. The prevalence of tumors with a single genomic event involving loss of BAP1 was 6.7% in our study population. We further characterized the BAP1 status in 17 of these tumors with available additional material, confirming loss of BAP1 in all cases. We describe BAP1 loss in a blue nevus like melanoma and further expand the histopathologic spectrum of spitzoid melanocytic neoplasms with BAP1 loss.

Introduction

Germline loss of function mutations in *BAP1* have recently been identified in families with increased incidence of uveal melanoma, mesothelioma, renal cell carcinoma, and other malignancies.[1–3] While *BAP1* mutations have been known to occur in rare cases of non-small cell lung cancer and breast cancer, more recently *BAP1* mutations have been identified in uveal melanoma, clear cell renal cell carcinoma, and myelodysplasia expanding the spectrum of neoplasia associated with loss of *BAP1*.[4–7]

BAP1 is an ubiquitin hydrolase whose functional roles are still being elucidated. BAP1 can deubiquitinate histone H2A and forms a complex with O-linked N-acetylgluycosamine transferase (OGT) and host cell factor-1 (HCF-1) that localizes to transcriptional start sites.[7–11] BAP1 appears to stabilize both HCF-1 and OGT by deubiquitination allowing for O-GlnNAcylation and activation of HCF-1 by OGT, thus affecting transcriptional regulation.[7] Loss of BAP1 sensitizes cells to ionizing radiation due to its role in homologous recombination.[12] *BAP1* biallelic loss in uveal melanoma and clear cell renal cell carcinoma is associated with poor prognosis and typically occurs as the result of somatic mutation of one allele and chromosomal loss of the other.[13,14]

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An increase in cutaneous melanocytic tumors has been observed in several families with germline *BAP1* loss of function mutations.[1,15] Clinically, these cutaneous tumors are tan-orange-pink elevated papules and plaques with a slightly translucent quality. Histopathologically, predominantly dermal melanocytes are arranged in nests or sheets, often forming a dermal nodule. The melanocytes have amphophilic to pale eosinophilic cytoplasm, well-defined nuclear membranes, and large vesicular nuclei with prominent nucleoli. While these melanocytic tumors are considered "spitzoid," due to their abundant amphophilic cytoplasm, other classical features of Spitz nevi, such as epidermal hyperplasia, clefting between melanocytes, dual epithelioid and spindle cell populations, and Kamino bodies are notably absent. A majority of these melanocytic neoplasms were considered to be benign by histopathologic criteria in the original report and often demonstrated a loss of function mutation of one *BAP1* allele and loss of the genetic material containing the other *BAP1* allele, loss of BAP1 expression, and activating *BRAF* mutations.[1]

Njauw and colleagues also identified families with germline *BAP1* mutations, but characterized their cutaneous melanocytic tumors as "severely atypical" and "reminiscent of nevoid melanoma," in many cases falling "short of frank malignancy though these lesions clearly lie within the spectrum of nevoid melanomas."[15] However, the images of their melanocytic tumors resemble those presented by Wiesner et al. as benign tumors.[1] The classification of melanocytic tumors with *BAP1* loss will likely evolve and our understanding of their biology will improve as our ability to distinguish them from other melanocytic tumors improves.

As the distinct cutaneous tumors seen in patients with germline *BAP1* mutations fell within the spectrum of what Wiesner and colleagues term "atypical Spitz tumors with epithelioid morphology" they screened a subset of these tumors encountered in two dermatopathology practices for loss of *BAP1*.[16] *BAP1* loss was identified in nearly one third of these cases, frequently in combination with a $BRAF^{V600E}$ mutation. Not surprisingly, these tumors with *BAP1* loss demonstrated similar features to those melanocytic tumors identified in patients with germline *BAP1* mutations. In the four cases with *BAP1* loss analyzed by array comparative genomic hybridization (CGH), 2 had no detectable copy number alterations, one demonstrated monosomy 3 and the one case demonstrated focal loss around 3p21 with an additional loss of part of chromosome 16.

Busam et al. recently reported a series of 6 combined "atypical spitz tumors" occurring in a sporadic setting in which the spitzoid component lacked BAP1 expression by immunohistochemistry while the conventional component retained BAP1 expression, and both components harbored $BRAF^{V600E}$ mutation.[17] The cases were selected due to the similarity of the spitzoid component to features of melanocytic tumors with *BAP1* loss in the familial setting.

To identify cases with *BAP1* loss and more fully assess the histopathologic spectrum of melanocytic tumors with *BAP1* loss, we identified cases of melanocytic neoplasms with genetic loss of *BAP1* (without additional genomic copy number aberrations) from our archive of array CGH performed for diagnostic purposes on borderline or ambiguous melanocytic tumors at the University of California, San Francisco. We discuss the

histopathologic commonalities between tumors with *BAP1* loss and expand the range of features that can be seen in these tumors.

Materials and Methods

We analyzed the array CGH profiles of 436 consecutive melanocytic lesions for which array CGH was performed as part of the diagnostic assessment at the University of California, San Francisco over a fourteen month period during 2010 and 2011. The study was approved by the UCSF Committee on Human Research. Of these 436 cases, 29 (7%) were routinely encountered cases, and the remaining 407 (93%) were consultation cases. For each case, microdissection of lesional tissue was performed, followed by DNA extraction as described previously.[18] Array CGH was performed on Agilent 4×180k microarrays (Agilent, Santa Clara, CA). The raw microarray images were processed with Agilent Feature Extraction software, and analyzed using Nexus Copy Number Software version 6.0 (Biodiscovery, El Segundo, CA). All cases with a loss encompassing the *BAP1* locus at 3p21 greater than1 Mb in length and without additional significant copy number aberrations on other chromosomes were selected for further study. We excluded cases with apparent loss at the *BAP1* locus that were less than 1 Mb in length as there is a documented copy number variation that spans this area.[19] We classified the loss at 3p21 as involving only part of the short arm of chromosome 3, the loss of the entire short arm of chromosome 3, or loss of the entire chromosome 3.

All cases were further evaluated when possible for mutations within the coding region of the remaining *BAP1* allele or loss of BAP1 staining. Mutation analysis of *BAP1* exons was performed on DNA isolated for array CGH analysis. Polymerase chain reaction (PCR) with primers for *BAP1* exonic regions and *BRAF* exon 15 and *NRAS* exons 1 and 2 was performed as described previously.[16,20] Sanger sequencing of PCR product was performed by Quintara Biosciences (Albany, CA). Mutations were identified using Sequencher software (Gene Codes, Ann Arbor, MI). Immunohistochemical staining for BAP1 was performed on 3-micron thick formalin-fixed paraffin embedded sections after 1 hour of antigen retrieval, using an autostainer (DAKO, Carpinteria, CA) and the BAP1 C-4 antibody (dilution 1:250, Santa Cruz Biotechnology, Inc., Santa Cruz, CA) with MACH 4 detection (Biocare Medical, Concord, CA). Absence of BAP1 staining in the nucleus was interpreted as negative staining with keratinocytes and stromal cells serving as internal positive controls.

The histopathologic features of all cases with loss of 3p21 were examined by IY and PEL when material was available. Unique features of the cases were identified and scored. We classified the cytologic features of the constituent melanocytes as spitzoid (enlarged cells with large vesicular nuclei and abundant homogeneous cytoplasm), conventional (small round melanocytes), blue nevus like (oval or spindled melanocytes with scant cytoplasm), deep penetrating nevus like (oval melanocytes with medium sized vacuolated nuclei and pale, vacuolated and lightly pigmented cytoplasm interspersed with melanophages).

Results

Genomic loss of greater than 1 megabase isolated to chromosome 3 spanning the *BAP1* locus was identified in 29 (6.7%) of the 436 cases. Of these 29 cases, 2 were routinely encountered cases and the remainder consultation cases. The clinical features, cytomorphology, type of genetic loss on chromosome 3, and clinical follow-up when available are summarized in Table 1. We identified a loss of function mutation in *BAP1* in 10 of 11 cases sequenced across *BAP1* coding regions. The case with wild-type *BAP1* by sequencing demonstrated loss of BAP1 by immunohistochemistry. Additionally, loss of function of *BAP1* was confirmed in 17 of these cases either by identification of a *BAP1* loss of function mutation or loss of BAP1 immunohistochemical staining (Table 2). In the remaining 11 cases, *BAP1* loss could not be confirmed due to a lack of case material. In no case with genetic loss of one copy of *BAP1* were we able to find preservation of BAP1 nuclear staining by immunohistochemistry. We were able to obtain clinical follow-up for nine patients, with an average length of follow-up of 16 months (median, 17 months). No recurrence was observed, and one patient underwent a sentinel lymph node biopsy, which was negative.

We limited our analysis of histologic features to the 17 cases with confirmed biallelic loss of *BAP1*. Of these 17 cases, 16 (94%) demonstrated spitzoid cytomorphology. The typical microscopic appearance was that of a dome-shaped and somewhat exophytic lesion, without a junctional component (Figure 1). Within the dermis, melanocytes were typically arranged in a loose nodular fashion, with small groups of cells separated by small amounts of stroma and interspersed lymphocytes. The melanocytes contained abundant, glassy, pale eosinophilic cytoplasm and demonstrated well-defined nuclear membranes with centrally or eccentrically placed oval to kidney bean shaped nuclei (Figure 1). Cellular size ranged from moderate to large, and a broad range of cell sizes were observed within individual tumors. A permeative lymphocytic infiltrate as can be seen in halo nevi was present in half of the cases with spitzoid morphology. Inflamed tumors tended to contain larger melanocytes with eccentric nuclei and a plasmacytoid appearance, apparently in contact with adjacent lymphocytes, suggesting they may be receiving a “kiss of death” (Figure 2).

One case demonstrated cellular blue nevus-like cytology and based on histopathologic features including high cellularity and mitotic activity was classified as a blue-nevus like melanoma (Figure 3). This case did not demonstrate inflammation.

Of the cases with spitzoid cytomorphology, five (31%) demonstrated small round melanocytes at the periphery of the lesion, often with small nests of similar or slightly larger, plump, oval melanocytes at the junction. These “combined” lesions included a junctional component, composed of only the common nevic component. The larger, spitzoid cells were intradermal, as in typical monophasic cases. In the combined cases, the lymphocytic infiltrate appeared to target only the spitzoid cells, ignoring the conventional component. The spitzoid portion was typically located in the center of the lesion, and flanked by the common nevi portion.

One case had a spitzoid junctional component consisting of nests of larger spitzoid melanocytes similar to the dermal cells, this case did not have a common nevus component (Figure 4). Two cases demonstrated large spitzoid melanocytes similar to those in the dermis in single cell array at the dermo-epidermal junction. One case contained an *NRAS*^{Q61R} mutation, and demonstrated similar spitzoid cytomorphology with marked pigment in melanophages and constituent melanocytes (Figure 5).

Discussion

The finding that in some families a germline mutation of *BAP1* predisposes to distinctive spitzoid melanocytic lesions established multiple such lesions as a marker for increased risk of cutaneous and uveal melanoma and mesothelioma.[1,2] This was soon followed by the recognition that identical lesions occur sporadically.[16]

Our study differs methodologically from previous ones in that the cases were culled from a large group of histopathologically difficult melanocytic tumors in which array comparative genomic hybridization (CGH) was performed for diagnosis as an ancillary technique. These were mostly cases in which slides were sent to our dermatopathology service for consultation, having been first seen by an outside pathologist or dermatopathologist, rather than case sent directly to our lab in formalin. The morphologic features of these cases varied widely, but all 436 were judged sufficiently difficult to warrant the use of CGH by a consultant dermatopathologist, or the slides and paraffin block arrived in our lab with a specific request for CGH that we believed reasonable to honor. We use CGH to assess copy number changes, with no aberrations favoring a benign or indeterminate interpretation (depending on the morphologic features), certain distinctive findings such as isolated gain of 7q or 11p favoring a Spitz nevus, and multiple aberrations involving chromosomes often involved in melanoma favoring that diagnosis. While the diagnostic problems that led to referral did not entirely revolve around the differential diagnosis of Spitz nevus-atypical Spitz tumor-spitzoid melanoma, these comprise the majority of cases assessed with CGH in our unit.

The finding that isolated losses involving chromosome 3 or 3p (either in whole or in part) were found in just over 6% of these cases shows that such cases are uncommon, but not exotically rare. In a majority of the cases, the losses were interstitial, involving a part of chromosome 3p, rather than a monosomy of chromosome 3 or loss of the entire short arm of the chromosome. The correlation between losses involving chromosome 3p21 and *BAP1* mutation was strong. We did not find cases in which a spitzoid morphology and chromosome 3 or 3p loss was present, yet BAP1 staining of nuclei was preserved. We also found that with the exception of a blue nevus-like melanoma with monosomy of chromosome 3, 3p21 loss was limited to spitzoid neoplasms. This makes it unlikely that other morphologic correlates of the loss of function of *BAP1* exist in significant numbers among ambiguous melanocytic neoplasms (e.g. cases in which nevoid or small cell melanoma, deep penetrating nevus-like melanoma, or desmoplastic melanoma are considerations).

We, like others noted the presence of a lymphocytic infiltrate targeting the larger spitzoid cells, and in lesions in which these were combined with small, round melanocytes, exclusively targeting the larger cells. The lymphocytic infiltrates can be dense, and can ascend to the dermo-epidermal junction. If one looks carefully, one can find lymphocytes in direct apposition to spitzoid melanocytes, with the latter showing morphologic evidence of apoptosis. The dying cells have eccentric pyknotic nuclei, and abundant, often ground glass-like cytoplasm. These features indicate an effective host response, and are equivalent to a partially developed halo reaction. In 1997, one of us (PEL) co-authored a paper on halo reactions in Spitz nevi.[21] Some of these cases had findings indistinguishable from spitzoid lesions with *BAP1* loss. We were able to re-review 7 cases from the 1997 study. Of these cases, 4 had features similar to those observed with *BAP1* biallelic loss, and 3 did not. It is notable that a clinical halo was not noted in the many lesions that occur in each patient with BAP1 mutations in a familial setting, and that the reported cases of sporadic lesions have not mentioned a halo, either. Hence, tumors with *BAP1* loss may represent a specific variant of "halo Spitz nevus."

We did not find morphologic evidence of melanoma supervening on the spitzoid populations in any of our cases. Two cases in patients with germline mutations in *BAP1* have been reported which sheets of anaplastic melanocytes were present adjacent to a lesion that otherwise resembled one of the multiple spitzoid neoplasms.[1,22] No such juxtaposition was present in any of our cases, nor can we recall seeing one in either our consultation or routine work. Clearly, given these two cases, and the role of BAP-1 mutation in cancer progression in other lineages, there may be an increased risk for melanoma to develop in a spitzoid lesion with *BAP1* mutation compared to a banal nevus, or even a dysplastic nevus. This tendency makes it desirable that these lesions be completely removed. The bulk of evidence is that they are benign. The initial report and subsequent descriptions have used the term "atypical Spitz tumor" (AST) in reference to these lesions. While there is undoubtedly a valid intermediate category between Spitz nevus and spitzoid melanomas, its boundaries can best be viewed as being still under construction. We would not be surprised if like other spitzoid neoplasms, cases with bialleleic *BAP1* loss but no features of melanoma will be shown to involve local lymph nodes (as evidenced by their positivity in sentinel lymph node biopsies). However, the families with germline mutations in *BAP1* did not include patients in whom melanoma presented with nodal involvement, in the absence of a preceding cutaneous melanoma. This suggests that Spitz tumors with *BAP1* loss without morphologic evidence of melanoma are best classified as nevi, even if they have features that imply partial transformation.

The advent of immunohistochemical staining for BAP1 has led to a different diagnostic paradigm from the one that we followed either when the acquisition of the cases in this study commenced, or just after the identification of the syndrome. If we see a lesion that has morphologic features of *BAP1* loss, we first perform immunohistochemical staining for BAP1. If there is loss of nuclear staining, and the lesion has features typical for this entity, without expansile-appearing aggregates or more atypical melanocytes, large sheets of cells, or increased numbers of mitoses we make the diagnosis of either Spitz nevus with features of *BAP1* loss, or combined Spitz nevus with features of *BAP1* loss. If the above-mentioned

atypical features are present, we supplement immunohistochemistry with CGH. The presence of additional chromosomal gains or losses would be worrisome for melanoma, depending on the specific genomic regions that are involved.

While previous studies of Spitz nevi with BAP1 loss only identified *BRAF*^{V600E} mutations in such tumors, we have identified one case with an activating NRAS mutation. This lesion demonstrated similar histopathologic features to the BRAF mutant tumors, although it was one of the more pigmented tumors in the series. Thus, this morphology is not isolated to BRAF mutant tumors, and the relative frequency of BRAF to NRAS mutations may be similar to the relative frequency of mutations in these genes in conventional nevi.

The blue nevus like lesion illustrated in Figure 3 also showed lack of staining for BAP1. Blue nevi and melanomas arising in them mostly have initiating mutations involving *GNAQ* or *GNA11*[23,24](p[11]), while Spitz nevi with *BAP1* loss lack such mutations, and instead have *BRAF*^{V600E} mutations. In a previous study, including colleagues from our dermatopathology section, multiple genomic aberrations were the most common correlate by CGH in blue nevus-like melanoma, with only one or two copy number changes in so-called atypical blue nevi.[25] While this case had only one chromomosomal copy number change, we believe that the sheet like growth, crowded nuclei and loss of staining for BAP1 warrants consideration as blue nevus-like melanoma. The significance of BAP1 loss in this setting is different. In uveal melanoma, lesions with BAP1 loss metastasize to the liver (the main site of metastasis in this neoplasm) at a much more frequent rate than do lesions lacking this mutation. As both blue nevi and uveal melanoma are driven by *GNAQ* or *GNA11* mutations, we hypothesize that *BAP1* mutations in blue nevus-like lesions are a marker for the acquisition of metastatic capacity.

In sum, our findings confirm that there is a consistent set of histopathologic findings in most melanocytic lesions with isolated chromosome 3 loss, and that cases with loss of function of *BAP1* make up the great majority of such cases. We did not find more anaplastic appearing lesions among these. Recurrence was not identified in the subset of patients for whom clinical follow-up was available. We believe that these lesions are best regarded as a distinctive form of Spitz nevus, overlapping with what has previously been reported as Spitz nevus with halo reaction. A minority of lesions with chromosome 3 loss is in the blue nevus series which includes blue nevus like melanoma.

Acknowledgments

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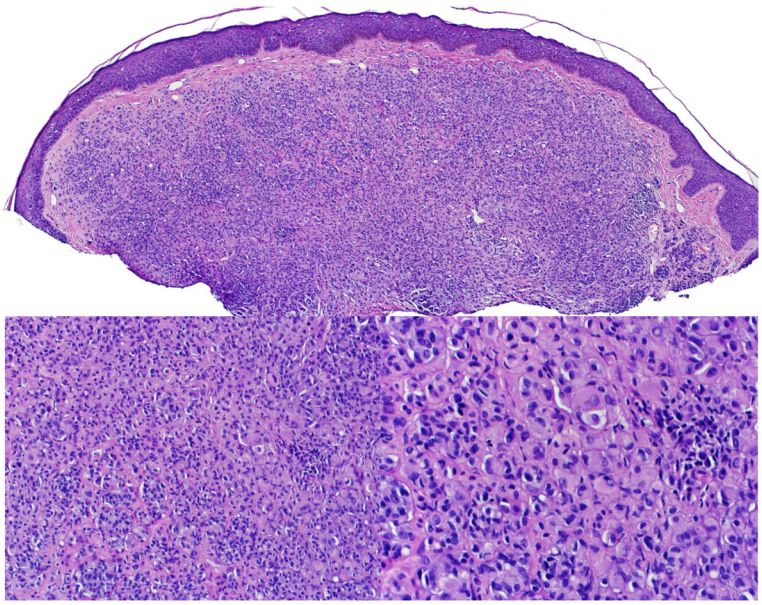

Figure 1.

A typical nevus with *BAP1* loss, case 13. This nevus is dome shaped, lacks a junctional component, and contains an infiltrative lymphocytic infiltrate. The constituent cells contain abundant glassy eosinophilic cytoplasm and have distinct cellular membranes. The melanocytes and their nuclei demonstrate a broad range of sizes, and the eccentric placement of the nucleus is more readily appreciated in larger melanocytes.

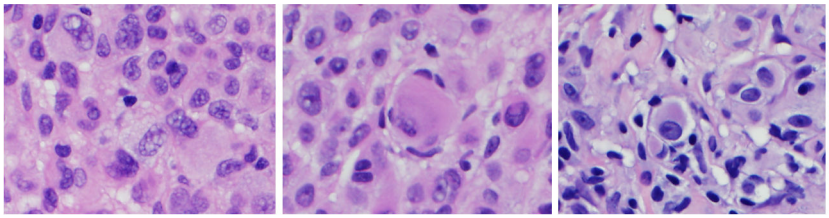

Figure 2.

Kiss of Death. The largest melanocytes within our cases often were observed to be in contact with neighboring lymphocytes. The melanocytes being “kissed” by lymphocytes demonstrated eccentric placement of their nucleus away from the adjacent lymphocyte, resulting in a plasmacytoid appearance.

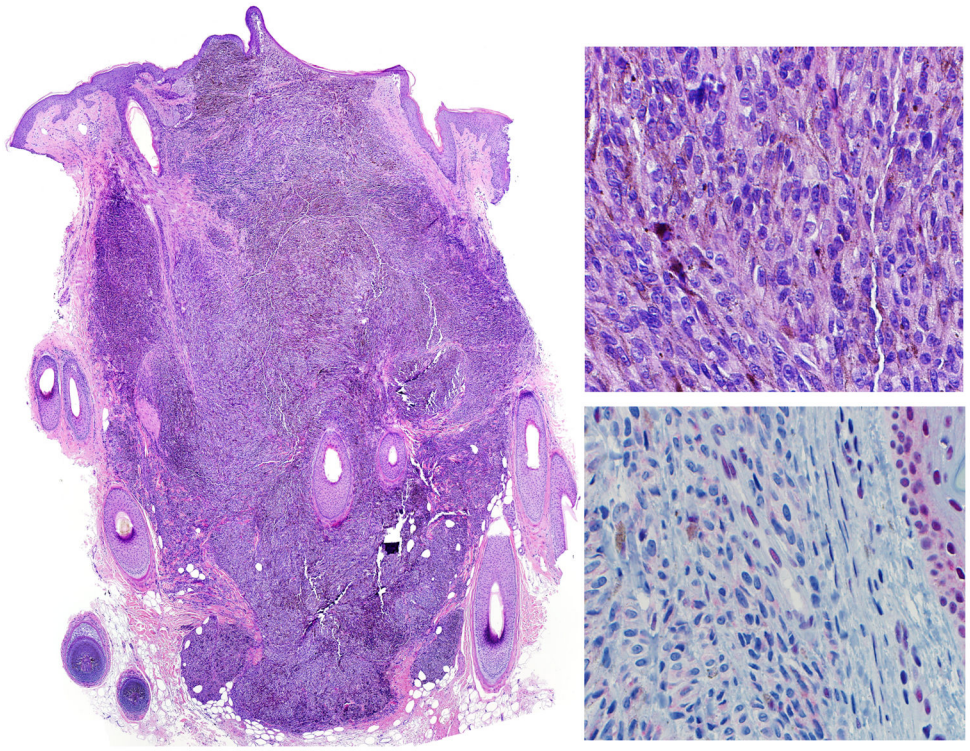

Figure 3.

Blue nevus like melanoma with *BAP1* loss and cellular blue nevus cytomorphology, case 15. Left. Low power view demonstrates the typical architecture of a blue nevus. Upper right. High power view demonstrates fusiform cells with scant cytoplasm. Lower right. BAP1 immunostaining demonstrates loss of BAP1 in the neoplastic melanocytes. BAP1 staining is preserved in the epithelium.

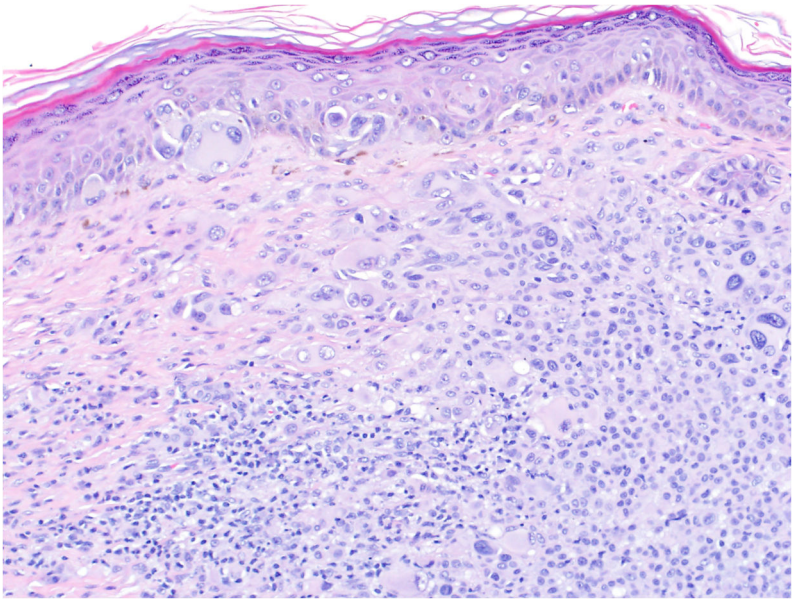

Figure 4.

A Spitz nevus with *BAP1* loss and a junctional component, case 4. Nests of large spitzoid melanocytes as seen in the dermis are present within an effaced epidermis.

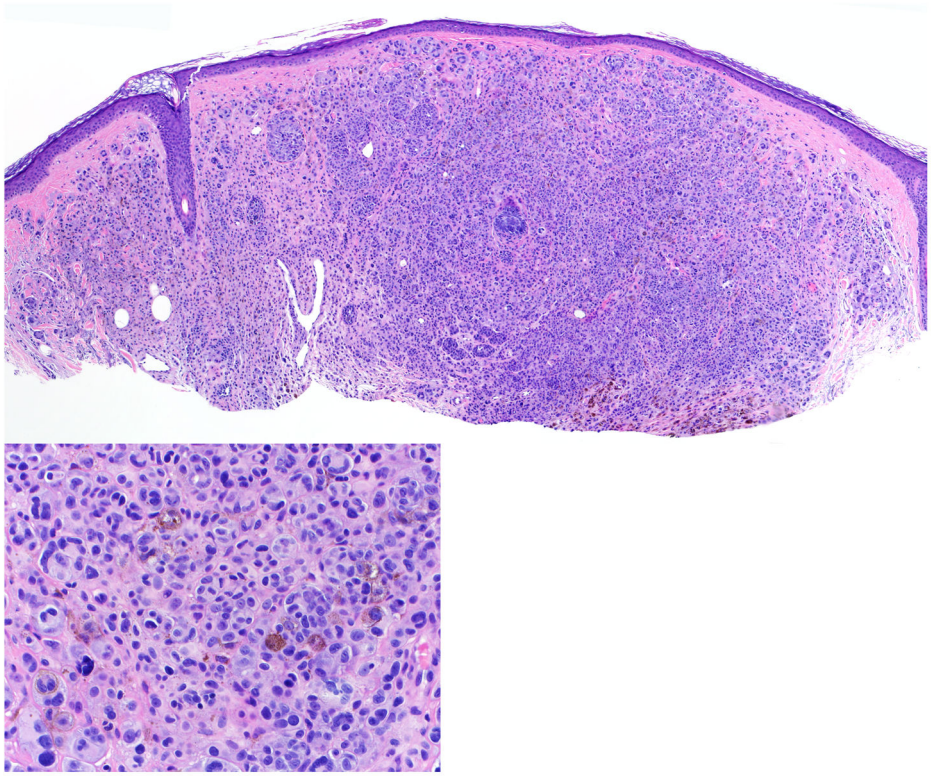

Figure 5.

NRAS^{Q61R} mutant Spitz nevus with *BAP1* loss, case 17. The tumor has a similar overall configuration to Spitz nevi with *BAP1* loss and *BRAF*^{V600E} mutation, with cells arranged in a dermal nodule. There is more pigmentation within this tumor than in the cases with *BRAF*^{V600E} mutation, not only in melanophages near the base of the lesion, but also within the constituent melanocytes.

Table 1

Clinical features of melanocytic tumors with isolated loss on chromosome 3 encompassing the *BAP1* locus. Biallelic loss of *BAP1* was confirmed by sequencing or immunohistochemistry for cases highlighted in bold. Follow-up was obtained for 9 patients, with an average follow-up period of 16 months, with no evidence of disease (NED) in all cases, and a negative sentinel lymph node biopsy (SLN-) in one case.

Case	Age	Gender	Location	Cytologic Features	Chromosome 3	Clinical follow-up
1	**8**	**M**	**not provided**	**spitzoid**	**partial loss**	**17 months, NED**
2	**14**	**F**	**back**	**spitzoid and conventional**	**partial loss**	
3	**15**	**F**	**scalp**	**spitzoid**	**partial loss**	
4	**16**	**M**	**ear**	**spitzoid**	**partial loss**	**22 months, NED**
5	**25**	**M**	**forearm**	**spitzoid and conventional**	**monosomy**	
6	**25**	**M**	**scalp**	**spitzoid**	**partial loss**	
7	**27**	**F**	**leg**	**spitzoid**	**partial loss**	**9 months, NED**
8	**30**	**F**	**shoulder**	**spitzoid**	**partial loss**	
9	**32**	**F**	**arm**	**spitzoid and conventional**	**partial loss**	
10	**34**	**F**	**neck**	**spitzoid**	**partial loss**	
11	**40**	**M**	**ear**	**spitzoid and conventional**	**partial loss**	
12	**41**	**F**	**cheek**	**spitzoid and conventional**	**partial loss**	
13	**45**	**F**	**scalp**	**spitzoid and conventional**	**monosomy**	
14	**50**	**M**	**ear**	**spitzoid**	**monosomy**	**24 months, NED**
15	**64**	**F**	**scalp**	**blue**	**monosomy**	**22 months, SLN-, NED**
16	**64**	**M**	**back**	**spitzoid and conventional**	**monosomy**	
17	**76**	**F**	**ear**	**spitzoid**	**monosomy**	**27 months, NED**
18	5	F	scalp	spitzoid	partial loss	
19	6	F	scalp	spitzoid	partial loss	4 months, NED
20	10	M	eyelid	spitzoid	partial loss	11 months, NED
21	11	M	cheek	spitzoid	partial loss	
22	13	F	back	spitzoid	partial loss	
23	23	F	back	spitzoid	partial loss	
24	30	M	chest	spitzoid	partial loss	
25	31	F	nose	spitzoid	monosomy	

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Case	Age	Gender	Location	Cytologic Features	Chromosome 3	Clinical follow-up
26	31	F	back	spitzoid	partial loss	
27	35	F	scalp	spitzoid	partial loss	
28	37	M	forearm	spitzoid and conventional	partial loss	12 months, NED
29	48	F	toe	conventional with features of ancient nevus	partial loss	

Table 2

BAP1 mutations for the 17 cases with confirmed *BAP1* loss. The *BAP1* mutation (with respect to RefSeq transcript NM_004656.2) and the functional consequence.

Case	*BAP1* mutation	Type of mutation	BAP1 immunohistochemistry	Oncogene Mutation
1	c.58_59insTG	Frameshift	NA	BRAF V600E
2	c.509_510insT	Frameshfit	NA	BRAF V600E
3	c.3G>A	Startsite, predicted N-terminal truncation	NA	BRAF V600E
4	c.2057-2delA	Frameshift	NA	BRAF V600E
5	not performed		BAP1 nucleus negative, cytoplasm positive	BRAF V600E
6	c.592G>T	Nonsense	NA	WT
7	not performed		BAP1 negative	WT
8	c.1392delC	Frameshift	NA	BRAF V600E
9	c.2023_2024insATA	Amino Acid insertion	NA	BRAF V600E
10	not performed		BAP1 negative	BRAF V600E
11	WT		BAP1 nucleus negative, cytoplasm positive	BRAF V600E
12	not performed		BAP1 negative	WT
13	c.1717delC	Frameshift	NA	BRAF V600E
14	c.506C>T	Missense	BAP1 negative	BRAF V600E
15	not performed		BAP1 negative	WT
16	not performed		BAP1 nucleus negative, cytoplasm positive	BRAF V600E
17	c.155G>A	Nonsense	NA	NRAS Q61R