Human Pathology (2019) **87**, 57–64

ELSEVIER

Human PATHOLOGY

www.elsevier.com/locate/humpath

Original contribution

Fusion of *ALK* to the melanophilin gene *MLPH* in pediatric Spitz nevi☆

Catherine T. Chung MD[a,b,*], Paula Marrano MLT[a], David Swanson MLT[c], Brendan C. Dickson MD, MSc[b,c], Paul Scott Thorner MD, PhD[b]

[a]*Division of Pathology, The Hospital for Sick Children, Toronto, M5G 1X8 Canada*
[b]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, M5S 1A8 Canada*
[c]*Department of Pathology and Laboratory Medicine, Mount, Sinai Hospital, Toronto, M5G 1X5 Canada*

Received 4 January 2019; revised 27 February 2019; accepted 1 March 2019

Keywords:
Spitz nevus;
ALK;
MLPH;
Melanophilin;
Kinase fusion;
FISH;
TruSight RNA Fusion Panel

Summary Spitzoid neoplasms typically affect young individuals and include Spitz nevus, atypical Spitz tumor, and Spitzoid melanoma. Spitz tumors can exhibit gene fusions involving the receptor tyrosine kinases *NTRK1*, *NTRK3*, *ALK*, *ROS1*, *RET*, or *MET*, or the serine-threonine kinase *BRAF*. Because most studies have been based on adult cases, we studied *ALK* fusions in Spitz nevi occurring in pediatric patients. Twenty-seven cases were screened for ALK expression by immunohistochemistry, and 6 positive cases were identified. These cases were studied further using the TruSight RNA Fusion Panel, and in 4 cases, exon 20 of the *ALK* gene was found to be fused to exon 14 of the *MLPH* (melanophilin) gene, a gene fusion that has only been reported in a Spitz nevus in an adult. The remaining 2 cases showed no fusion of *ALK* with any gene. The cases with the *MLPH-ALK* fusion showed a similar histology to that described for Spitz nevi with *ALK* fusions, with spindle-shaped and epithelioid melanocytes in fusiform nests with a plexiform growth pattern and infiltrative border. We created a breakapart fluorescence in situ hybridization assay for *MLPH*, and all 4 cases with the *MLPH-ALK* fusion were positive, whereas the other 23 cases in the study were negative. Thus, *ALK* and *MLPH* were fused only to each other in our series. Melanophilin is part of the melanosome trafficking apparatus together with MYO5a, TPM3, and RAB27a, all constitutively expressed in melanocytes. Kinase fusions involving *MYO5A* and *TPM3* have been reported in Spitz tumors, and our series adds *MLPH* to this group.

1. Introduction

Spitz tumors comprise a heterogeneous group of melanocytic neoplasms that typically affect young individuals and include Spitz nevus (benign), Spitzoid melanoma (malignant), and atypical Spitz tumor (intermediate histology and metastatic potential but overall good prognosis) [1-4]. Histopathologic criteria that can be used to differentiate nevi from melanoma may not be reliable for Spitz tumors [4], and even with

☆ Disclosures: The authors have no disclosures and no conflicts of interest. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

* Corresponding author at: Division of Pathology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8.
E-mail address: catherine.chung@sickkids.ca (C. T. Chung).

https://doi.org/10.1016/j.humpath.2019.03.002
0046-8177/

Spitzoid melanoma, the majority of childhood cases do not develop metastases beyond local lymph nodes [1]. Considerable effort has been made to validate cytogenetic and molecular markers that might help differentiate benign versus malignant. Although the majority of melanomas show numerous chromosomal aberrations (such as 6p gain, 6q loss, 9p loss, 11p gain), most Spitz nevi do not [2,5]. Isolated chromosomal copy number gains or losses have been found in Spitz (including atypical) nevi, but the changes are inconsistent [6-9]. Moreover, Spitz nevi typically lack activating mutations in *NRAS, KIT, GNAQ,* and *GNA11* that occur in melanoma [3,10]. Subsets of Spitz tumors show activating mutations in *HRAS* (15% of cases) associated with gain of 11p [11] or *BRAF* (5% of cases) associated with biallelic *BAP1* loss [12]. As well, *TERT* promoter mutations have been found in Spitz tumors with a malignant course but not in those with a benign outcome [4,13].

More recently, it has been found that Spitz tumors often exhibit specific gene fusions involving the receptor tyrosine kinases *NTRK1*, *NTRK3*, *ALK*, *ROS1*, *RET*, or *MET*, or the serine-threonine kinase *BRAF* [3-5,10,14-17], with each of these genes involved in 2%-17% of cases. However, these fusions occurred in a mutually exclusive pattern, with the resulting gene fusions occurring in about 50% of Spitz tumors overall. In these fusions, the 3′ portion of the kinase gene becomes linked to the 5′ portion of another gene, resulting in an in-frame mRNA transcript that encodes a chimeric protein with a constitutively activated kinase. The chimeric proteins activate oncogenic signaling pathways (such as the MAPK/ERK, PI3K/AKT/mTOR, and JAK-STAT), which induce cell proliferation and improve cell survival [3,10,16]. These translocations are found across the biologic spectrum of Spitz tumors, including Spitz nevi, atypical Spitz tumor, and Spitzoid melanoma, implying that these fusions are involved in the pathogenesis of Spitz tumors but are not sufficient for malignant transformation [3,5,16]. In fact, cases with gene fusions as the sole genetic anomaly tend to behave in a benign fashion without recurrence [4,10,18-20], whereas those that show malignant behavior demonstrate additional genetic changes such as *TERT* promoter mutation [4].

It is well known that pediatric melanocytic tumors behave differently from their adult counterparts [5]. Although several studies have noted that cases with gene fusions in Spitz tumors occur more often in younger patients [3,10,15,16,19], most of the studies have been based on adult cases [3,16] or a mixture of adult and pediatric cases [1,10,12,14,15,18-21], with very few studies based on pediatric patients only [4,17]. For this reason, we based our study on a series of pediatric cases, and in this study, we focused on *ALK* fusions in Spitz nevi.

In adult series, *ALK* fusions are found in 8%-11% of Spitz nevi, 5%-16% of atypical Spitz tumors, and 1%-3% of Spitzoid melanomas [1,3,14,16]. The frequency of *ALK* fusions in the pediatric population is unknown. Because Spitz tumors with *ALK* fusions overexpress ALK [1,3,10,14,16,19], this provided a ready starting point for us to screen a pediatric series of Spitz nevi. Cases that were found to be positive for ALK protein by immunohistochemistry were then analyzed for the partner gene fused to *ALK*. Previous studies have identified several genes that can partner with *ALK* in Spitz tumors, including *TPM3* (most commonly) but also *DCTN1, NPM1*, *TPR*, *CLIP1*, *GTF3C2, FBXO28, NPAS2,* and *PPFIBP1* [1,3,4,14,16,18,19]. Surprisingly, in our series, we found *ALK* was fused to the *MLPH* (melanophilin) gene, which has only been recently reported in a single adult case [22]. Moreover, in the Spitz nevi with an *ALK* fusion, the only partner gene detected was *MLPH*.

2. Materials and methods

2.1. Case selection

Cases coded under a diagnosis of "Spitz nevus" (which would include atypical Spitz nevus) were selected from the pathology archives at The Hospital for Sick Children, Toronto, Canada, between the years 2014 and 2017. All cases had been fixed in 10% formalin and embedded in paraffin (FFPE). All slides were reviewed by a pathologist (C. C.) to confirm the diagnosis and to ensure that lesional tissue was present for immunohistochemistry, fluorescence in situ hybridization (FISH), and RNA extraction. After review, 27 cases of Spitz nevi were included in the study (Table). The patients ranged from 9 months to 14 years old, with 14 males and 13 females.

2.2. Immunohistochemistry for ALK

FFPE tissue samples were cut at 4 μm, baked at 64°C for 1 hour, and dewaxed prior to staining. Immunostaining for ALK was performed with the Rabbit anti-ALK (D5F3) antibody (cat. # 3633; Cell Signaling, Beverly, MA) at 1:1000 dilution, preceded by epitope retrieval in 0. 01 mol/L citrate buffer (pH 6) in a pressure cooker. The antibody was detected with the IMMPRESS Anti-Rabbit IgG peroxidase kit (cat. #MP7401; Vector Laboratories, Burlingame, CA) and visualized with 3,3′-diaminobenzidine.

2.3. Identification of partner genes fused to *ALK*

Total RNA was extracted from FFPE tissue scrolls (3-4 per case) using the ExpressArt FFPE Clear RNA Ready kit (Amsbio, Cambridge, MA). RNA quality was assessed using the RNA 6000 Nano Bioanalyzer Kit (Agilent, Mississauga, Ontario, Canada) and quantitated using the Qubit RNA HS Assay Kit (ThermoFisher Scientific, Mississauga, Ontario, Canada). An input of 20-100 ng total RNA and the TruSight RNA Fusion Panel were used to prepare the RNA-seq libraries (Illumina, San Diego, CA), following manufacturer's instructions and as previously described [23]. Sequencing of each sample was performed with 76 base pair paired-end reads on an Illumina MiSeq at 8 samples per flow cell (~3 million reads per sample). The results were then analyzed using the STAR and

Table Results of immunohistochemistry, sequencing, and FISH for Spitz nevi

Case	Age	Sex	ALK IHC	Illumina RNA Fusion Panel	*MLPH* breakapart FISH	*ALK* breakapart FISH
1 [a]	8 y	F	Pos	No *ALK* fusion detected	Neg	Neg
2	6 y	F	Neg		Neg	
3	5 y	F	Neg		Neg	
4	3 y	M	Neg		Neg	
5 [a]	5 y	F	Pos	Failed	Neg	Neg
6	2 y	F	Neg		Neg	
7	5 y	M	Neg		Neg	
8	3 y	M	Neg		Neg	
9	9 mo	F	Neg		Neg	
10	4 y	F	Neg		Neg	
11 [a]	9 y	F	Pos	*MLPH-ALK* fusion	Pos	
12	12 y	M	Neg		Neg	
13	2 y	F	Neg		Neg	
14	6 y	M	Neg		Neg	
15	7 y	M	Neg		Neg	
16 [a]	10 mo	F	Pos	*MLPH-ALK* fusion	Pos	
17	8 y	M	Neg		Neg	
18	5 y	F	Neg		Neg	
19	10 y	M	Neg		Neg	
20	13 y	M	Neg		Neg	
21	1 y	M	Neg		Neg	
22	5 y	M	Neg		Neg	
23	7 y	M	Neg		Neg	
24 [a]	14 y	F	Pos	*MLPH-ALK* fusion	Pos	Pos
25	2 y	M	Neg		Neg	
26	6 y	F	Neg		Neg	
27 [a]	1 y	M	Pos	*MLPH-ALK* fusion	Pos	Pos

[a] Cases that are immunopositive for ALK are highlighted.

BOWTIE2 aligners, and Manta and JAFFA fusion callers, respectively [24,25].

2.4. FISH for *MLPH* and *ALK*

Bacterial artificial chromosome DNA was acquired from the Applied Centre for Genomics, Toronto, Canada (http://www.tcga.ca/), and selected according to the UCSC Genome Bioinformatics Browser (http://genome.uscs.edu/, GRCh37/hg19 Build). For the detection of an *MLPH* translocation, RP11-349L1 labeled with spectrum red and RP11-905D24 labeled with spectrum green were used. RP11-349L1 is located 49 973 base pairs from the 3′ end of *MLPH*, and RP11-905D24 is located 822 base pairs from the 5′ end of *MLPH* (Fig. 1). For the detection of an *ALK* translocation, RP11-701P18 labeled with spectrum red and RP11-100C1 labeled with spectrum green were used. RP11-701P18 is 221 478 base pairs in length and covers intron 4 to intron 13, and RP11-100C1 is 172 880 base pairs long and is located 32 285 base pairs from the 3′ end of *ALK*. The labeled probes were hybridized to normal human lymphocyte metaphases to confirm their chromosomal location.

For FISH on FFPE tissue samples, sections were cut at 5 μm and incubated at 62°C for 45 minutes prior to being

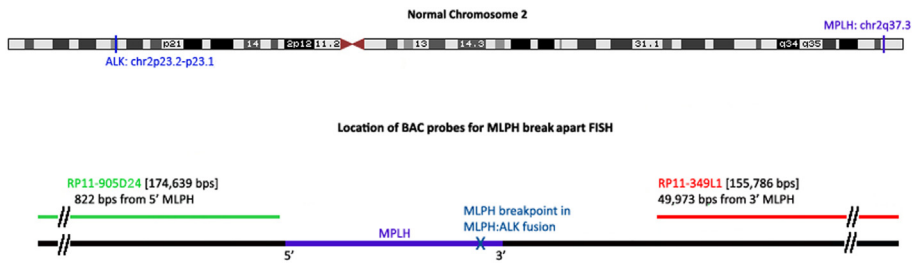

Fig. 1 Breakapart assay for *MLPH*. The upper panel shows a normal chromosome 2 with the locations of the *ALK* gene at 2p23 and the *MLPH* gene at 2q37. The lower panel shows the probes used to detect the *MLPH* break in the *MPLH-ALK* fusion identified by the TruSight RNA Fusion Panel.

processed for FISH. Slides were dewaxed and pretreated with 0. 01 mol/L citrate buffer (pH 6.0) for 10 minutes in a pressure cooker and with pepsin (4 mg/mL in phosphate-buffered saline [pH 1.5]) for 10 minutes at 45°C. Processed slides were then co-denatured with the labeled probes for 10 minutes at 75°C in the ThermoBrite system (Abbot Molecular, Mississauga, Ontario, Canada) and hybridized overnight at 37°C. The slides were washed for 2 minutes at 72°C with 2× SSC/0.3% NP-40 followed by a 1-minute room temperature wash in the same buffer. Slides were stained and mounted with DAPI/Antifade (Vector Laboratories, Burlingame, CA, USA). FISH signals were visualized with an epifluorescence microscope (AxioImager Z1; Carl Zeiss Microimaging, Thornwood, NY).

3. Results

3.1. Identification of *MLPH* as a partner genes for *ALK*

All cases underwent immunostaining for ALK, and 6 cases were scored as positive (Fig. 2 and Table). All positive cases were submitted for RNA extraction and testing on the TruSight RNA Fusion Panel. One case (#5) failed to yield adequate RNA. Of the other 5 cases, 4 showed a fusion of the *ALK* gene to the *MLPH* gene (cases #11, #16, #24, and #27). All 4 cases showed the identical fusion: exon 14 of the *MLPH* gene fused to exon 20 of the *ALK* gene (Fig. 3). This transcript includes the first 479 of 600 amino acids of the melanophilin protein and the terminal 1058 of 1620 amino acids of the ALK protein. For the remaining case (#1), the *ALK* gene was not found to be fused to any other gene.

3.2. FISH for *MLPH* and *ALK*

Because a gene fusion to *MLPH* was a novel finding, we designed a breakapart FISH assay for *MLPH* based on the RNAseq results, with 2 purposes in mind: (1) to confirm the TruSight RNA Fusion Panel results and (2) to screen the remaining 21 cases of Spitz nevi to see if any of them showed a gene fusion involving *MLPH* to a partner gene other than *ALK*. All 4 cases that showed the *MLPH-ALK* fusion on the TruSight RNA Fusion Panel were positive by FISH using the breakapart assay for *MLPH*, with 33%-50% of cells showing the split signal (Fig. 4). Only 1 allele was split in each case; the other allele remained intact. None of the remaining 23 cases of Spitz nevi showed a positive result, including the 2 cases that were positive for ALK by immunohistochemistry but which lacked an ALK fusion using the TruSight RNA Fusion Panel (case #1) or which could not be tested due to poor

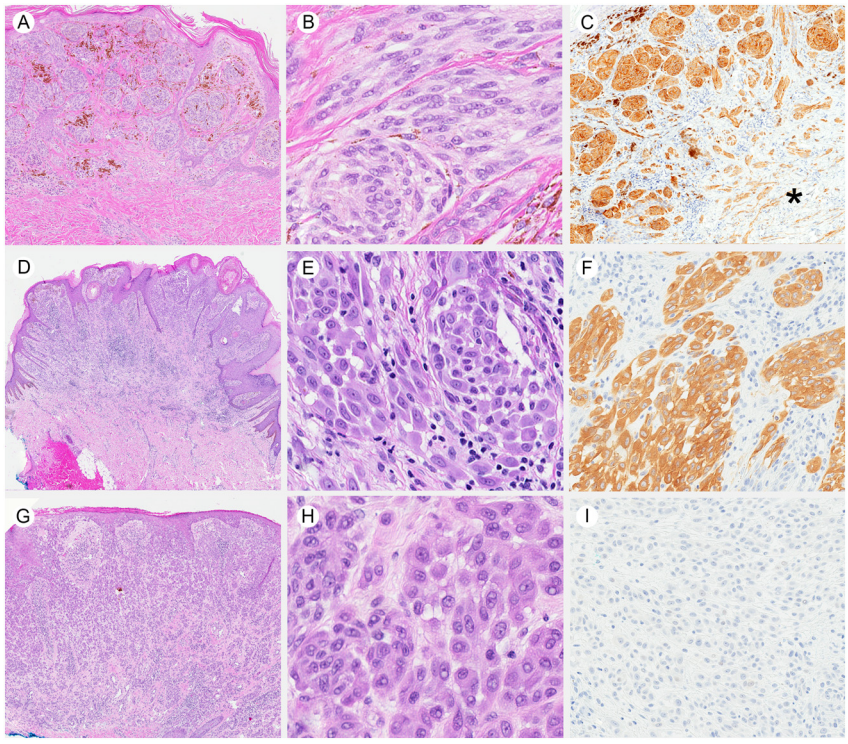

Fig. 2 Histology of the 3 categories of Spitz nevi in the study. A-C, Case with an *MLPH-ALK* fusion (case 24) showing a fascicular growth pattern with lesional cells that range from epithelioid to spindle-shaped and which express ALK by immunohistochemistry (C). The infiltrative deep border is best seen on the ALK stain (asterisk). D-F, Case without an *MLPH-ALK* fusion (case 1) lacking a fascicular growth pattern and showing a more conventional histology for a Spitz nevus, but still expressing ALK by immunohistochemistry (F). G-I, Case lacking an *MLPH-ALK* fusion (case 22) showing conventional Spitz nevus histology and no expression of ALK by immunohistochemistry (I) (A, D, and G: original magnification ×4; B, E, and H: ×40; C, F, and I: ×20.)

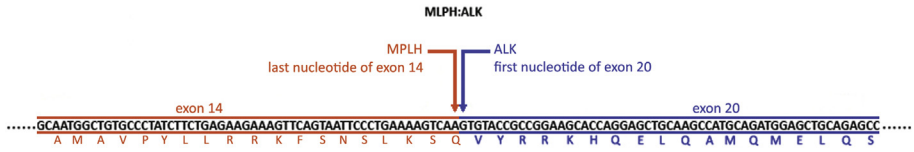

Fig. 3 *MLPH-ALK* fusion detected by the TruSight RNA Fusion Panel. The *MPLH* gene forms the 5′ end and is fused at exon 14 to the 3′ end of the *ALK* gene at exon 20.

RNA quality (case #5). These 2 cases were additionally tested for an *ALK* translocation using a breakapart FISH assay, and both cases were negative (Fig. 4), implying that there was no *ALK* translocation in these 2 cases.

3.3. Histology of Spitz nevi immunopositive for ALK

The 4 cases with the *MLPH-ALK* fusion showed compound (2 cases) or purely dermal involvement (2 cases) and grew in a compact fascicular pattern with an infiltrative border (Fig. 2). The lesional cells ranged from epithelioid to spindle-shaped with occasional multinucleated cells. Rare Kamino bodies were observed in a single case. Three of the cases were amelanotic. The 2 other ALK-positive cases that lacked the *MLPH-ALK* fusion showed a different appearance. Case #1 was a compound nevus with a nested growth pattern, lacking an infiltrative border. The nevus was composed of a mixture of epithelioid and spindle-shaped cells. Kamino bodies and melanin were present, and there was an accompanying lymphocytic infiltrate. Case #5 was a desmoplastic, amelanotic Spitz nevus composed of spindle-shaped cells, dermal in location and lacking an infiltrative border.

4. Discussion

Our study on pediatric Spitz nevi identified the melanophilin gene (*MLPH*) as the fusion partner to *ALK*. Of 27 cases, 6 overexpressed ALK by immunohistochemistry, and 4 of those showed a rearrangement of the *ALK* gene by FISH. All 4 of those cases (100%) showed fusion of the 3′ portion of *ALK* to the 5′ portion of *MLPH* using a breakapart FISH probe for *MLPH* and by sequencing. Genes previously reported as 5′ partners with *ALK* in Spitz tumors include *CLIP1*, *DCTN1, FBXO28, GTF3C2, NPAS2, NPM1*, *PPFIBP1, TPM3,* and *TPR* [1,3,4,14,16,18,19]. Other kinase genes besides *ALK* have been reported in gene fusions in Spitz tumors, including *NTRK1*, *NTRK3*, *ROS1*, *RET*, *MET*, and *BRAF* [3-5,10,15-17]. These fusions involve a variety of 5′ partners including *ARID1B, BAIAP2L1, CEP89, CLIP1, EML4, ERC1, EPS15,*

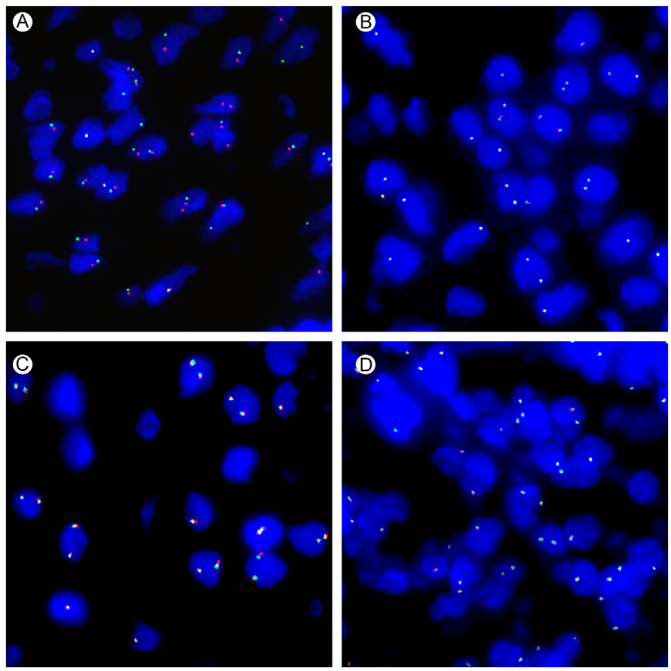

Fig. 4 FISH using breakapart probes for *MLPH* and *ALK.* A, Case 27 showing separation of the green and red signals for *MLPH*, indicating the presence of a translocation. This case was ALK-positive by immunohistochemistry. B, Case 14 showing contiguous green and red signals for *MLPH*, indicating no translocation. This case was ALK negative by immunohistochemistry. C; and D, Case 1, which was ALK positive by immunohistochemistry, showing contiguous green and red signals for *MLPH* (C) and *ALK* (D), indicating no translocation involving either gene.

ETV6, GOLGA5, HLA-A, IL6R, KIAA1598, *KIF5B, LMNA*, *LSM14A*, *LRRFIP1, MYH9, MYO5A, PPFIBP1, PTPRZ1, PWWP2A, TP53, TPM3, TRIM4, ZCCHC8*, and *ZKSCAN1* [3,4,10,15,16,20]. *MLPH* has only been recently reported as a partner gene for *ALK* in a single case of a Spitz nevus in an adult [22]. The finding that *MLPH* was an exclusive partner for *ALK* in our study may relate to the study material because our series was restricted to a small series of pediatric Spitz nevi, whereas other studies included adult patients and atypical Spitz tumor and Spitzoid melanomas [1,3,10,12,14-16,18-21], and the 2 series based on pediatric cases included only a single case with an *ALK* gene fusion (to *TPM3*) [4,17]. None of our 4 cases showed a gain in the fusion protein by FISH, although this has been noted in other studies for other fusion genes in 20%-35% of cases [19,21].

Two other cases were ALK positive by immunohistochemistry but lacked a gene fusion involving *ALK,* as determined by RNA sequencing and FISH. Although not formally tested, it is conceivable that these 2 Spitz nevi express ALK constitutively. This could have the same effect as constitutive expression of the ALK tyrosine kinase domain in a gene fusion, although the role of ALK in the development of Spitz tumors is currently unknown. ALK expression unrelated to a translocation has been documented in 2% of melanomas, related to an alternate transcription form of ALK protein (ALK^{ATI}) [26]. Whether this is true in these 2 Spitz nevi is unknown. Another possibility is that these 2 cases had activating mutations in the tyrosine kinase portion of the *ALK* gene, leading to constitutive kinase activity. This occurs in about 10% of sporadic neuroblastoma cases with hot spot mutations in the *ALK* gene, most commonly R1275 and F1174 [27,28]. Mutation testing for *ALK* was not carried out in our study. There is also a reported case of a Spitz nevus with ALK expression that was felt to be related to *ALK* amplification rather than a translocation [14], but in our 2 cases, *ALK* copy number was normal. This case together with our 2 cases indicate that positive immunohistochemistry for ALK does not always indicate the presence of an *ALK* gene fusion. Whether this situation applies to other kinase proteins requires further study.

In our series, the *ALK* gene was fused at exon 20, which is comparable to previous reports showing the *ALK* gene fused at exon 19, 20, or 21 [3,16,18,19,22]. The *MLPH* portion of the gene fusion included exons 1-14, similar to the one previous report with an *MLPH* fusion [22]. The fusion was in frame, retaining the kinase portion of the *ALK* gene that could be detected by immunohistochemistry. This gene fusion follows the pattern of previously reported kinase fusions, in which the 5′ partner is derived from a gene that is expressed normally in the cell of origin, and the 5′ portion retained in the gene fusion leads to constitutive activation of tyrosine kinase domain of the 3′ partner. Melanophilin is a protein that is normally expressed in melanocytes as part of the transport system for moving melanosomes to the periphery of melanocytes for subsequent transfer to keratinocytes [29-31]. Melanophilin complexes with RAB27A and myosin-5a (myoVa) to move melanosomes along actin tracks in the actin-rich dendritic periphery of melanocytes. Melanophilin is a 65-kDa protein with 3 domains. The N terminal is formed by amino acids 1-147 and binds melanosomes via RAB27a. The central portion is formed by amino acids 147-480 and has 2 binding sites for myoVa. The C terminal is formed by amino acids 480-590 and has the actin binding sites needed for melanosome transport. Melanophilin functions as an adapter protein that links melanosomes to myoVa and to actin, as well as increasing the time melanosomes spend on actin, which enhances the transfer of melanosomes to keratinocytes. The myoVa-melanophilin complex shows preferential binding with actin-Tpm3.1 for most efficient transfer [32]. Tpm3.1 is enriched in the dendritic protrusions of melanocytes, the site where myoVa-melanophilin is involved in melanosome transport. Other Tpm isoforms (eg, Tpm4) slow or block transport of melanosomes.

Because all these proteins involved in melanosome transport are normally expressed in melanocytes, it is reasonable that their respective genes might serve as 5′ partners in gene fusions in Spitz tumors because these tumors are derived from melanocytes. Support for this concept comes from previous studies that have identified both *MYO5A* and *TPM3* as 5′ partners in gene fusions in Spitz tumors, fusing to *ALK*, *MERTK, NTRK1, NTRK3, RET*, or *ROS1* [1,3,4,15-17,20]. Our study adds *MLPH* to this picture. One might also predict that the *RAB27a* gene should participate in these gene fusions, although none have been reported to date. It is noteworthy that no gene fusion with *TPM4* has been reported, in keeping with the fact that the Tpm4 actin isoform is detrimental to melanosome transport and likely not highly expressed in melanocytes or Spitz tumors.

The melanophilin-ALK fusion protein in our study included amino acids 1-479, meaning the actin binding site is lost and replaced by the kinase domain of the ALK protein. Based on what is known about the function of melanophilin, loss of the actin binding site in the fusion protein might be expected to disturb melanocyte trafficking. Defects in any of melanophilin, RAB27A, and myoVa result in a failure of melanosomes to move to the cell periphery, with perinuclear localization [31]. Mutations in any 1 of these 3 proteins results in Griscelli syndrome, with a failure of the transfer of melanosomes to neighboring keratinocytes, causing hypopigmentation. Failure of the melanosome trafficking apparatus might be a contributing factor as to why Spitz nevi are often amelanotic. However, amelanosis varies with the specific gene fusion, and cases with *ALK* involved are more often amelanotic compared to other kinase fusions [1,2,14] (and 75% of our cases were amelanotic), implying that amelanosis is more related to the 3′ partner in the gene fusions. However, this cannot be stated definitively because the feature of amelanosis has never been correlated with the 5′ partner in any previous study. Nor could we do this because *MLPH* was the only 5′ partner identified in our study.

Several studies have shown a correlation between the histology of Spitz tumors and the genetic changes found. For example, Spitz tumors with mutations in *HRAS* and gain of 11p are composed of large epithelioid melanocytes with marked desmoplasia [11], whereas Spitz tumors with *BAP1* loss and *BRAF* mutations are also composed of large epithelioid

melanocytes with enlarged, moderately pleomorphic nuclei and abundant amphophilic cytoplasm [12]. In Spitz tumors, the histology seems to be influenced by the 3′ partner in the gene fusion rather than the 5′, although the latter has not been examined as thoroughly. Spitz tumors with *NTRK1* fusions are typically arranged in small nests with predominantly spindle-shaped cells, occasional rosettes and Kamino bodies [21]. A *BRAF* fusion is associated with large epithelioid cells in a sheet-like growth pattern and high-grade nuclear atypia, and a *RET* fusion is associated with intermediate-sized epithelioid cells with mild nuclear atypia and Kamino bodies [21]. With respect to *ALK* fusions, these lesions have a limited junctional component, with deep dermal expansion by large, spindle-shaped and epithelioid melanocytes arranged in fusiform nests showing a plexiform growth pattern and an infiltrative border [1,2,14,18,19,21,22]. Lesional cells have fibrillary cytoplasm and moderate nuclear atypia, but Kamino bodies are rare. The 4 cases in our study with an *MLPH-ALK* fusion showed similar features to what has been described previously. The 2 cases that were ALK positive by immunohistochemistry but lacked an *ALK* translocation showed different histologic features. One case was a desmoplastic Spitz nevus composed of spindle-shaped cells, with prominent desmoplasia and amelanosis, but a noninfiltrative border. The other case was a compound nevus with a mixture of spindle-shaped and epithelioid cells arranged in nests, with melanin production and Kamino bodies. Thus, although the specific 3′ partner in the gene fusion appears to influence the histology, the typical features seen in *ALK* fusion cases are not seen in our 2 cases expressing full-length ALK. However, our numbers are small, and additional cases are needed to confirm this observation. It is interesting to note that many of the Spitz tumors with kinase fusions are composed of large epithelioid cells. The activated kinases stimulate the PI3K/AKT/mTOR pathway, which regulates cell size, and may account for the increased cell size in these cases [3,16].

The identification of a gene fusion involving *MLPH* has only been reported once in an adult Spitz nevus [22]. The involvement of *MLPH* is a reasonable event in Spitz nevi given the constitutive expression of melanophilin in melanocytes. There are other organs in which MLPH has been shown to play a role. For example, MLPH is expressed in prostate [33,34], and increased expression of melanophilin has been found in favorable risk prostate cancer [33], estrogen receptor–positive breast cancer [35], meningothelial meningiomas [36], and lung cancer [37,38]. Kinase fusions have already been found in secretory breast carcinoma involving *NTRK3* and in lung cancers involving *ALK, ROS1*, and *RET*. It may be of value to screen these tumors for gene fusions involving *MLPH*, which might identify new subgroups of tumors.

References

[1] Busam KJ, Kutzner H, Cerroni L, Wiesner T. Clinical and pathologic findings of Spitz nevi and atypical Spitz tumors with ALK fusions. Am J Surg Pathol 2014;38:925-33.

[2] Kiuru M, Jungbluth A, Kutzner H, Wiesner T, Busam KJ. Spitz tumors: comparison of histological features in relationship to immunohistochemical staining for ALK and NTRK1. Int J Surg Pathol 2016;24:200-6.

[3] Wiesner T, He J, Yelensky R, et al. Kinase fusions are frequent in Spitz tumours and spitzoid melanomas. Nat Commun 2014;5:3116.

[4] Wu G, Barnhill RL, Lee S, et al. The landscape of fusion transcripts in spitzoid melanoma and biologically indeterminate spitzoid tumors by RNA sequencing. Mod Pathol 2016;29:359-69.

[5] Bahrami A, Barnhill RL. Pathology and genomics of pediatric melanoma: a critical reexamination and new insights. Pediatr Blood Cancer 2017;65:1-9.

[6] Gerami P, Scolyer RA, Xu X, et al. Risk assessment for atypical spitzoid melanocytic neoplasms using FISH to identify chromosomal copy number aberrations. Am J Surg Pathol 2013;37:676-84.

[7] Martin V, Banfi S, Bordoni A, Leoni-Parvex S, Mazzucchelli L. Presence of cytogenetic abnormalities in Spitz naevi: a diagnostic challenge for fluorescence in-situ hybridization analysis. Histopathology 2012;60:336-46.

[8] Requena C, Rubio L, Traves V, et al. Fluorescence in situ hybridization for the differential diagnosis between Spitz naevus and spitzoid melanoma. Histopathology 2012;61:899-909.

[9] Yazdan P, Cooper C, Sholl LM, et al. Comparative analysis of atypical spitz tumors with heterozygous versus homozygous 9p21 deletions for clinical outcomes, histomorphology, BRAF mutation, and p16 expression. Am J Surg Pathol 2014;38:638-45.

[10] Yeh I, Botton T, Talevich E, et al. Activating MET kinase rearrangements in melanoma and Spitz tumours. Nat Commun 2015;6:7174.

[11] Bastian BC, LeBoit PE, Pinkel D. Mutations and copy number increase of HRAS in Spitz nevi with distinctive histopathological features. Am J Pathol 2000;157:967-72.

[12] Wiesner T, Murali R, Fried I, et al. A distinct subset of atypical Spitz tumors is characterized by BRAF mutation and loss of BAP1 expression. Am J Surg Pathol 2012;36:818-30.

[13] Lee S, Barnhill RL, Dummer R, et al. TERT promoter mutations are predictive of aggressive clinical behavior in patients with spitzoid melanocytic neoplasms. Sci Rep 2015;5:11200.

[14] Saraggi D, Salmaso R, Zamuner C, et al. Prevalence of ALK gene alterations among the spectrum of plexiform spitzoid lesions. J Am Acad Dermatol 2018;79:728-35.

[15] VandenBoom T, Quan VL, Zhang B, et al. Genomic fusions in pigmented spindle cell nevus of Reed. Am J Surg Pathol 2018;42:1042-51.

[16] Wiesner T, Kutzner H, Cerroni L, Mihm Jr MC, Busam KJ, Murali R. Genomic aberrations in spitzoid melanocytic tumours and their implications for diagnosis, prognosis and therapy. Pathology 2016;48:113-31.

[17] Wang L, Busam KJ, Benayed R, et al. Identification of NTRK3 fusions in childhood melanocytic neoplasms. J Mol Diagn 2017;19:387-96.

[18] Perron E, Pissaloux D, Charon Barra C, et al. Melanocytic myxoid spindle cell tumor with ALK rearrangement (MMySTAR): report of 4 cases of a nevus variant with potential diagnostic challenge. Am J Surg Pathol 2018;42:595-603.

[19] Yeh I, de la Fouchardiere A, Pissaloux D, et al. Clinical, histopathologic, and genomic features of Spitz tumors with ALK fusions. Am J Surg Pathol 2015;39:581-91.

[20] Yeh I, Tee MK, Botton T, et al. NTRK3 kinase fusions in Spitz tumours. J Pathol 2016;240:282-90.

[21] Amin SM, Haugh AM, Lee CY, et al. A comparison of morphologic and molecular features of BRAF, ALK, and NTRK1 fusion spitzoid neoplasms. Am J Surg Pathol 2017;41:491-8.

[22] Fujimoto M, Togashi Y, Matsuzaki I, et al. A case report of atypical Spitz tumor harboring a novel MLPH-ALK gene fusion with discordant ALK immunohistochemistry results. Hum Pathol 2018;80:99-103.

[23] Dickson BC, Sung YS, Rosenblum MK, et al. NUTM1 gene fusions characterize a subset of undifferentiated soft tissue and visceral tumors. Am J Surg Pathol 2018;42:636-45.

[24] Chen X, Schulz-Trieglaff O, Shaw R, et al. Manta: rapid detection of structural variants and indels for germline and cancer sequencing applications. Bioinformatics 2016;32:1220-2.

[25] Liu S, Tsai WH, Ding Y, et al. Comprehensive evaluation of fusion transcript detection algorithms and a meta-caller to combine top performing methods in paired-end RNA-seq data. Nucleic Acids Res 2016;44:e47.
[26] Busam KJ, Vilain RE, Lum T, et al. Primary and metastatic cutaneous melanomas express ALK through alternative transcriptional initiation. Am J Surg Pathol 2016;40:786-95.
[27] Janoueix-Lerosey I, Lequin D, Brugieres L, et al. Somatic and germline activating mutations of the ALK kinase receptor in neuroblastoma. Nature 2008;455:967-70.
[28] Pugh TJ, Morozova O, Attiyeh EF, et al. The genetic landscape of high-risk neuroblastoma. Nat Genet 2013;45:279-84.
[29] Hume AN, Tarafder AK, Ramalho JS, Sviderskaya EV, Seabra MC. A coiled-coil domain of melanophilin is essential for myosin Va recruitment and melanosome transport in melanocytes. Mol Biol Cell 2006; 17:4720-35.
[30] Sckolnick M, Krementsova EB, Warshaw DM, Trybus KM. More than just a cargo adapter, melanophilin prolongs and slows processive runs of myosin Va. J Biol Chem 2013;288:29313-22.
[31] Westbroek W, Klar A, Cullinane AR, et al. Cellular and clinical report of new Griscelli syndrome type III cases. Pigment Cell Melanoma Res 2012;25:47-56.
[32] Sckolnick M, Krementsova EB, Warshaw DM, Trybus KM. Tropomyosin isoforms bias actin track selection by vertebrate myosin Va. Mol Biol Cell 2016;27:2889-97.
[33] Bu H, Narisu N, Schlick B, et al. Putative prostate cancer risk SNP in an androgen receptor-binding site of the melanophilin gene illustrates enrichment of risk SNPs in androgen receptor target sites. Hum Mutat 2015;37:52-64.
[34] Penney KL, Sinnott JA, Tyekucheva S, et al. Association of prostate cancer risk variants with gene expression in normal and tumor tissue. Cancer Epidemiol Biomark Prev 2015;24:255-60.
[35] Thakkar AD, Raj H, Chakrabarti D, et al. Identification of gene expression signature in estrogen receptor positive breast carcinoma. Biomark Cancer 2010;2:1-15.
[36] Fevre-Montange M, Champier J, Durand A, et al. Microarray gene expression profiling in meningiomas: differential expression according to grade or histopathological subtype. Int J Oncol 2009;35:1395-407.
[37] Molina-Pinelo S, Gutierrez G, Pastor MD, et al. MicroRNA-dependent regulation of transcription in non-small cell lung cancer. PLoS One 2014;9:e90524.
[38] Pio R, Blanco D, Pajares MJ, et al. Development of a novel splice array platform and its application in the identification of alternative splice variants in lung cancer. BMC Genomics 2010;11:352-66.