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Identification of *NTRK3* Fusions in Childhood Melanocytic Neoplasms

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Spitzoid neoplasms are a distinct group of melanocytic tumors. Genetically, they lack mutations in common melanoma-associated oncogenes. Recent studies have shown that spitzoid tumors may contain a variety of kinase fusions, including *ROS1*, *NTRK1*, *ALK*, *BRAF*, and *RET* fusions. We report herein the discovery of recurrent *NTRK3* gene rearrangements in childhood melanocytic neoplasms with spitzoid and/or atypical features, based on genome-wide copy number analysis by single-nucleotide polymorphism array, which showed intragenic copy number changes in *NTRK3*. Break-apart fluorescence *in situ* hybridization confirmed the presence of *NTRK3* rearrangement, and a novel *MYO5A-NTRK3* transcript, representing an in-frame fusion of *MYO5A* exon 32 to *NTRK3* exon 12, was identified using a rapid amplification of cDNA ends—based anchored multiplex PCR assay followed by next-generation sequencing. The predicted MYO5A-NTRK3 fusion protein consists of several N-terminal coiled-coil protein dimerization motifs encoded by *MYO5A* and C-terminal tyrosine kinase domain encoded by *NTRK3*, which is consistent with the prototypical structure of TRK oncogenic fusions. Our study also demonstrates how array-based copy number analysis can be useful in discovering gene fusions associated with unbalanced genomic aberrations flanking the fusion points. Our findings add another potentially targetable kinase fusion to the list of oncogenic fusions in melanocytic tumors. *(J Mol Diagn 2017, 19: 387—396; http://dx.doi.org/10.1016/j.jmoldx.2016.11.005)*

Spitzoid melanocytic neoplasms are a distinctive group of melanocytic tumors. In 1948, Sophie Spitz coined the term melanoma of childhood for a group of melanocytic skin tumors composed of spindled or epithelioid melanocytes that developed predominantly in children and adolescents.[1] Most of these neoplasms behaved in an indolent manner, and they were subsequently termed Spitz nevus to indicate their benign nature. Malignant tumors with spitzoid histological features were termed spitzoid melanomas, and these tumors often showed aggressive clinical behavior with widespread metastasis, similar to conventional melanomas. Tumors with ambiguous histological features, overlapping between those of Spitz nevus and melanoma, are termed atypical Spitz tumors.[2,3]

Most spitzoid neoplasms lack mutations in common melanoma-associated oncogenes, such as *NRAS*, *KIT*, *GNAQ*, or *GNA11*.[4,5] Recent studies have uncovered *ROS1*, *NTRK1*, *ALK*, *RET*, *BRAF*, and *MET* fusions in this group of tumors, suggesting that activation of kinase pathways plays an important role in their pathogenesis.[5—7]

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In our clinical practice, we perform genome-wide high-resolution single-nucleotide polymorphism (SNP) array analysis as an adjunct to the histopathological diagnosis for diagnostically challenging melanocytic tumors. Two hundred seven samples have been tested, most of which were diagnosed histologically as atypical Spitz tumors. In a previous study, we reported using array-based DNA copy number analysis to screen for gene fusions associated with unbalanced genomic aberrations flanking the fusion points and successfully identified *KDR-PDGFRA* fusion in a glioblastoma patient sample.[8] This concept has informed our analysis of copy number profiles in the diagnostic setting, and intragenic copy number changes involving common receptor kinase genes are typically further analyzed and, if necessary, studied by alternative methods. Herein, we present the discovery of recurrent *NTRK3* gene rearrangements and a novel *MYO5A-NTRK3* fusion in childhood melanocytic neoplasms.

Materials and Methods

Study Samples

From January 2014 through December 2015, 207 melanocytic neoplasms with atypical, ambiguous, or controversial light microscopic features were submitted by a dermatopathologist (K.J.B.) for DNA copy number array analysis as part of the diagnostic assessment to the Clinical Cytogenetics and Cytogenomics Laboratory of the Department of Pathology. This study was conducted with the approval of the Memorial Sloan Kettering Cancer Center Institutional Review Board.

DNA Copy Number Array Analysis

Tumor-rich areas were macrodissected from sections (10 μm thick) of formalin-fixed, paraffin-embedded (FFPE) tissues. Genomic DNA was extracted using Qiagen DNeasy Tissue and Blood kit (Qiagen, Germantown, MD). Genome-wide DNA copy number alterations and allelic imbalances were analyzed by SNP-array using Affymetrix OncoScan FFPE Assay (Affymetrix, Santa, Clara, CA). We used 80 ng of genomic DNA for each sample. Processing of samples was performed according to the manufacturer's guidelines (Affymetrix). OncoScan SNP-array data were analyzed by the software couple of OncoScan Console version 1.0 (Affymetrix) and Nexus Express version 3.1 (BioDiscovery, El Segundo, CA) using Affymetrix TuScan algorithm. All array data were also manually reviewed for subtle alterations not automatically called by the software.

NTRK3 Break-Apart FISH Assay

FFPE tissue samples were tested for *NTRK3* rearrangements by a break-apart fluorescence *in situ* hybridization (FISH) assay using a commercial *NTRK3* break-apart FISH probe (Empire Genomics, Buffalo, NY). FFPE tissue sections (4 μm thick) generated from FFPE blocks of tumor specimens were pretreated by deparaffinizing in xylene and dehydrating in ethanol. Dual-color FISH assay was conducted according to the protocol for FFPE sections from Vysis/Abbott Molecular with a few modifications. FISH analysis and signal capture were conducted on a fluorescence microscope (Zeiss, Jena, Germany) coupled with ISIS FISH Imaging System (Metasystems, Altlussheim, Germany). We analyzed 100 interphase nuclei from each tumor specimen.

Anchored Multiplex PCR for Targeted RNA-Sequencing

Tumor-rich areas were macrodissected from sections of FFPE tissues, and subjected to RNA extraction using Mineral Oil-Qiagen RNeasy FFPE kit (Qiagen). Random priming was used to synthesize cDNAs from the RNA samples extracted. An anchored multiplex PCR assay based on Rapid Amplification of cDNA Ends (RACE) strategy followed by next-generation sequencing (Archer FusionPlex, ArcherDX) was used to identify specific partners involved in selected gene fusions.[9] We used a custom fusion-screening panel focusing on 35 genes, including *NTRK3*, known to be involved in recurrent gene fusions in several solid tumors. The list of genes in the panel is given in Supplemental Table S1. Within this panel, for *NTRK3* fusions, 5′RACE was conducted using five pairs of *NTRK3*-specific 3′ primers targeting exons 13 to 16, and the multiplex RACE products were sequenced on MiSeq (Illumina), and sequencing data were analyzed by Archer Analysis software version 3.3.0 (ArcherDx).

Results

SNP-Array Study

Based on genome-wide copy number profiling data of 207 melanocytic neoplasms analyzed by high-resolution SNP-array, we identified four tumors with intragenic copy number alteration of *NTRK3* (reference transcript NM_001012338) at 15q25. *NTRK3* encodes TRKC, a transmembrane receptor tyrosine kinase. The copy number plots of *NTRK3* in the first case [709 (Figure 1)] and second case [930 (Figure 2)] showed a dramatic copy number increase of the 3′ portion of *NTRK3*, and no copy number change of the 5′ portion of *NTRK3*. The change point mapped to *NTRK3* intron 13 in sample 709 and to the region between *NTRK3* exons 10 and 12 in sample 930, respectively. An exon-level fine mapping of the breakpoint could not be achieved in 930 because of low probe coverage between exons 10 and 12. The copy number plot of *NTRK3* in the other two cases (3509 and 4583) showed intragenic discontinuity with the change point mapped to *NTRK3* exons 10 to 12 and intron 13, respectively. Case 3509 (Figure 3) is characterized by a copy number loss of the 5′ portion of *NTRK3*, and a copy number gain of 3′ *NTRK3* was the notable finding in case 4583 (Figure 4). A summary of all intragenic copy number change points in *NTRK3* identified by SNP-array analysis in this study is shown in Figure 5.

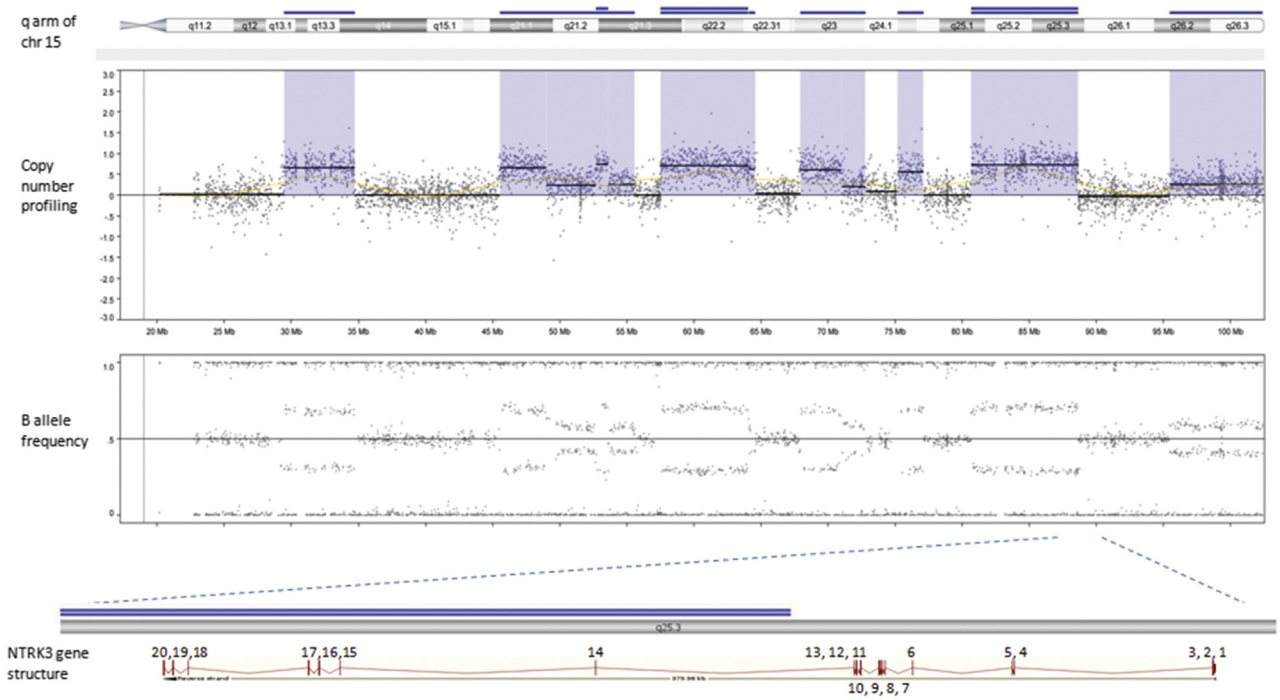

Figure 1 Copy number and B allele frequency alterations across the q arm of chromosome (chr) 15 for case 709 revealed by SNP-array analysis (**violet shadows** highlight gains). A zoom-in view at *NTRK3* gene locus illustrates the intragenic copy number change in *NTRK3*. **Blue line** indicates copy number gain and **double blue line** indicates high-level copy number gain (≥4 copies).

Besides the aberrations leading to intragenic copy number change of *NTRK3*, genome-wide SNP-array analysis also revealed other copy number changes elsewhere in the genome in each sample (Supplemental Figures S1–S4) (ie, multiple segmental gains in 15q and in chromosome 20 in sample 709; multiple segmental gains in 15q, and copy number change at 14q32 in sample 930; gain of 1q in sample 3509; and 6q deletion and complex copy number changes across 11q in sample 4583). The SNP-array data discussed in this publication have been deposited in National Center for Biotechnology

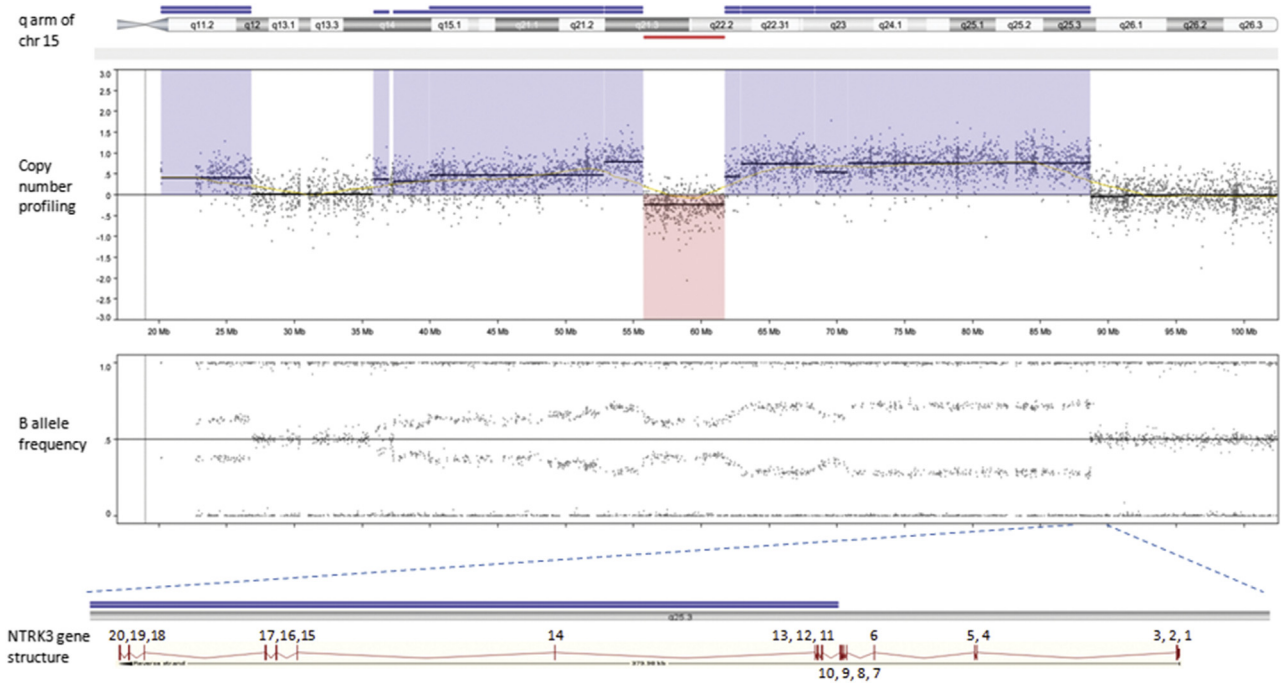

Figure 2 Copy number and B allele frequency alterations across the q arm of chromosome (chr) 15 for case 930 revealed by SNP-array analysis (**violet shadows** and **red shadows** highlight gains and losses, respectively). A zoom-in view at *NTRK3* gene locus illustrates the intragenic copy number change in *NTRK3*. **Red line** indicates copy number loss; **blue line**, copy number gain; and **double blue line**, high-level copy number gain (≥4 copies).

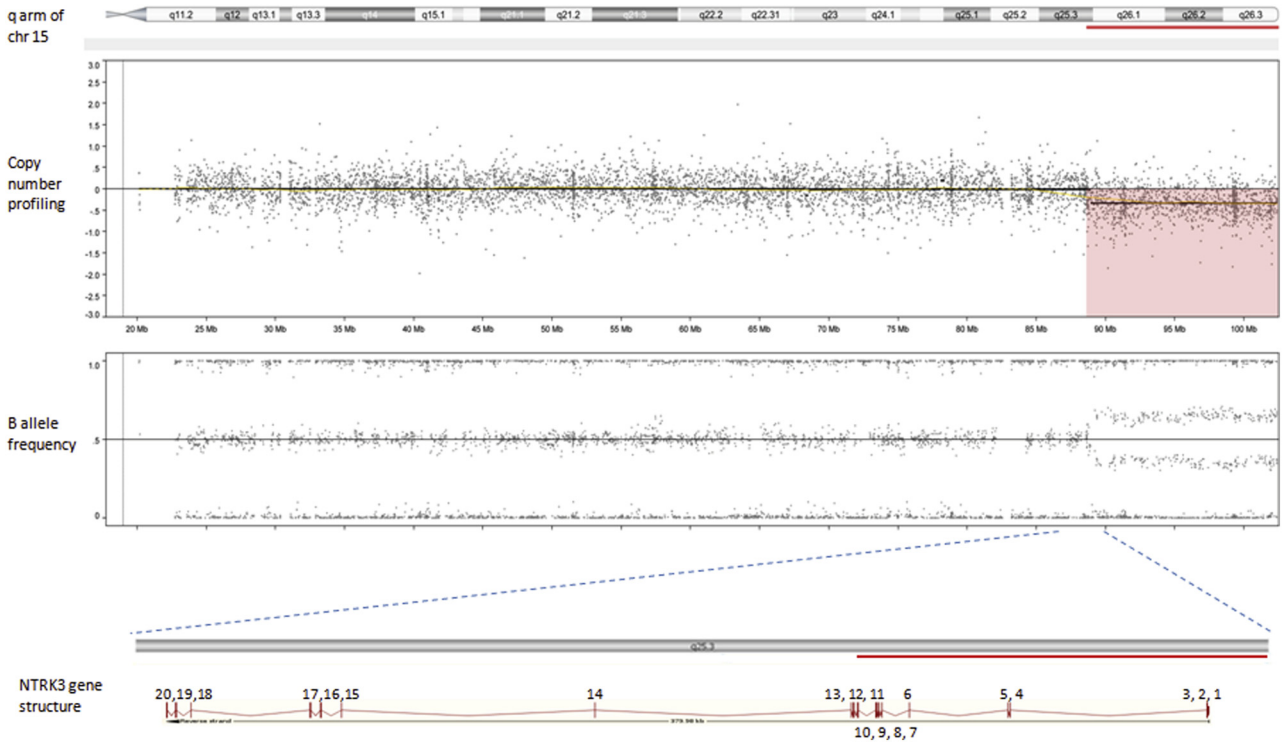

Figure 3 Copy number and B allele frequency alterations across the q arm of chromosome (chr) 15 for case 3509 revealed by SNP-array analysis (the **red shadow** highlights a copy number loss). A zoom-in view at *NTRK3* gene locus illustrates the intragenic copy number change in *NTRK3*. **Red line** indicates copy number loss.

Information's Gene Expression Omnibus (*http://www.ncbi.nlm.nih.gov/geo*; accession number GSE90644).[10]

Verification of NTRK3 Gene Rearrangement by FISH Analysis

NTRK3 break-apart FISH was performed on three tumor samples (930, 3509, and 4583) with *NTRK3* intragenic copy number alterations. Sample 709 has insufficient tumor tissue for FISH study. In sample 930, FISH detected one 3′/5′ fusion signal plus six to eight signals for 3′*NTRK3* and one single signal for 5′*NTRK3*. A similar signal pattern was observed in sample 4583. Because of the lower tumor content in sample 4583 compared to sample 930, SNP-array analysis showed a lesser copy number increase of the 3′ portion of *NTRK3* in 4583 compared to the dramatic copy number increase (>6 copies) of the 3′ portion of *NTRK3* in 930. FISH supported the loss of 5′*NTRK3* in sample 3509 by showing one 3′/5′ fusion signal and one single 3′ *NTRK3* signal. FISH analysis confirmed the SNP-array findings and further supported the presence of *NTRK3* fusions in the three tumor samples. Representative FISH images of all three samples are shown in Figure 6.

Identification of a Novel Fusion Transcript, MYO5A-NTRK3, in a Spitz Nevus

To identify the 5′ fusion partners of *NTRK3*, we used the anchored multiplex PCR platform for targeted RNA-sequencing (seq) (Archer FusionPlex). Using this platform, we successfully identified a novel fusion transcript in sample 930, *MYO5A-NTRK3*. The novel *MYO5A-NTRK3* fusion showed *MYO5A* (NM_001142495) exon 32 fused in-frame to *NTRK3* (NM_001012338) exon 12 (Figure 7), and expressed in high abundance in the tumor sample 930, according to the targeted RNA-seq data. In brief, after the deduplication of high-quality raw sequences, >66,000 on-target RNA-originated sequences were obtained, which represented 4516 unique cDNA molecules (unique start sites). Among these unique reads, approximately 25% are from *NTRK3* transcripts. In contrast, the number of reads representing transcripts from each of the other 34 genes in the panel was much lower (0% to 4% per gene), and no fusion transcript involving the 34 genes was identified according to our data analysis pipeline. In terms of *NTRK3* transcripts, 16% of the reads are bona fide *MYO5A-NTRK3* fusion reads spanning the fusion points. For *X-NTRK3* (*X* represents any 5′ partner) fusion screening, we have five pairs of *NTRK3*-specific 3′ primers, targeting from exon 13 to exon 16, for 5′RACE procedure to uncover 5′ fusion partners of *NTRK3*. Considering the degradation of RNA that occurs because of the formalin fixation process results in RNA species with an average size of approximately 200 nucleotides,[11] only the primers that are close to the fusion point are efficacious in the 5′RACE procedure to pull out the 5′ fusion partner. In fact, all *MYO5A-NTRK3* fusion reads identified in sample 930 were derived from primers targeting *NTRK3* exon 13. In other words, many other

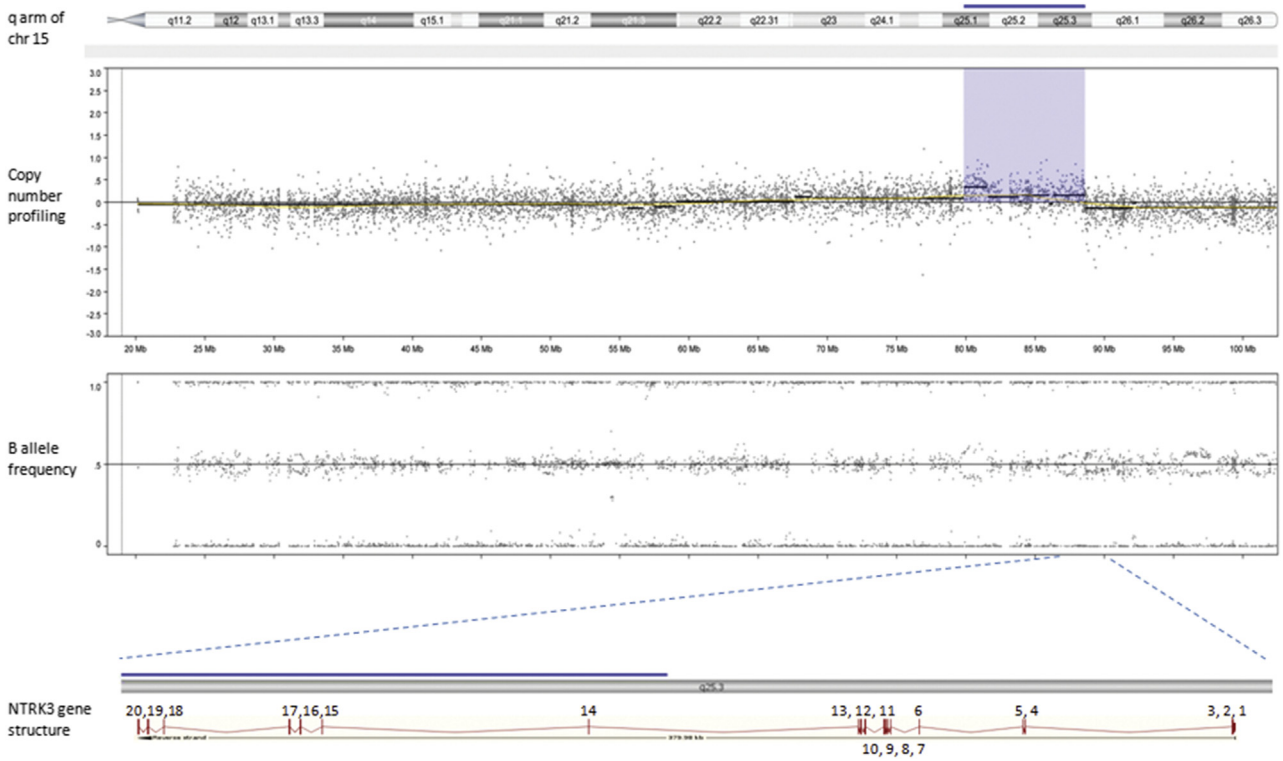

Figure 4 Copy number and B allele frequency alterations across the q arm of chromosome (chr) 15 for case 4583 revealed by SNP-array analysis (the **violet shadow** highlights a copy number gain). A zoom-in view at *NTRK3* gene locus illustrates the intragenic copy number change in *NTRK3*. **Blue line** indicates copy number gain.

NTRK3 transcript reads, if not all, may be from the *MYO5A-NTRK3* fusion product; however, they were not informative in unveiling the novel gene fusion because of the degradation of FFPE RNA. In summary, our targeted RNA-seq data provided strong evidence that *MYO5A-NTRK3* was the sole kinase fusion and expressed in high abundance in case 930.

MYO5A and *NTRK3* are approximately 36 Mb apart on genomic DNA, respectively mapping to 15q21.2 and 15q25.3, and both are on the reverse strand of chromosome 15. In sample 930, complex copy number changes across 15q were detected by SNP-array analysis (Figure 2), including multiple segmental copy number gains (from three to six copies) and a segmental deletion. One copy gain segment overlapped the entire *MYO5A* gene, with no intragenic copy number change observed. Therefore, the *MYO5A-NTRK3* fusion may result from some complex genomic rearrangement in this sample. No evidence of intragenic copy number change of *MYO5A* gene was observed in the other cases. Whether *MYO5A* is the recurrent fusion partner could not be verified because of the unavailability of material of cases 709, 3509, and 4583 for RNA extraction and the targeted RNA-seq study.

The predicted MYO5A-NTRK3 fusion protein consist of several N-terminal coiled-coil protein dimerization motifs from MYO5A and C-terminal tyrosine kinase domain provided by NTRK3, which is consistent with the fusion structure typically demonstrated in *NTRK* (*NTRK1*, *NTRK2*, and *NTRK3*) oncogenic fusions.[12] Figure 7 shows the schematic diagram of the predicted MYO5A-NTRK3 chimeric protein.

Clinical and Histopathological Features of NTRK3-Rearranged Melanocytic Tumors

The four tumor samples with evidence of *NTRK3* fusion were from four children, including three girls (1, 7, and 15 years old) and one 8-year-old boy. Two lesions (3509 and 4583) were from the cheek, and two (709 and 930) were from the foot. Three lesions had spitzoid cytologic features

Figure 5 Schematic diagram of *NTRK3* gene structure (reference sequence: NM_001012338) with **green diamond arrows** indicating the intragenic copy number change point in each sample. From left to right: 930, 3509, 709, and 4583. NTRK3 kinase domain coding exons are 15 to 20.

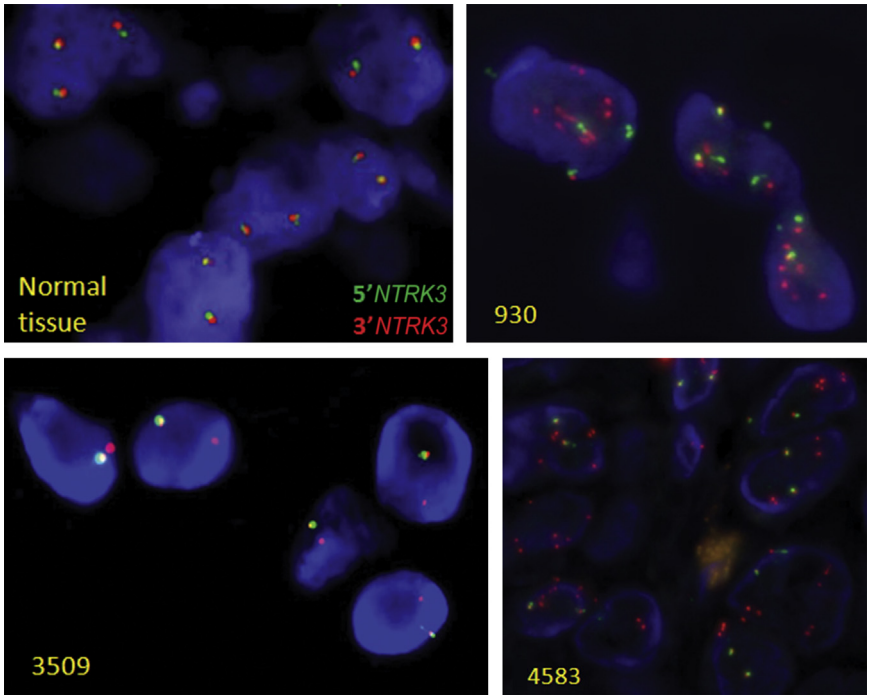

Figure 6 Interphase fluorescent *in situ* hybridization detection of *NTRK3* gene rearrangement. Green signals represent 5′*NTRK3*; and red signals, 3′*NTRK3*. In comparison to the 5′/3′-fusion signals seen in normal cells, multiple signals for 3′*NTRK3* and one separated signal for 5′*NTRK3* were observed in samples 930 and 4583; and a single 3′*NTRK3* signal pattern (loss of the signal for 5′*NTRK3*) was observed in sample 3509. Original magnification, ×63.

(ie, 930 and 3509 were interpreted as Spitz nevi, whereas 4583 was designated as spitzoid melanoma of childhood because of nuclear pleomorphism and additional cytogenetic aberrations, including loss of the q arm of chromosome 6 and segmental gains and losses across 11q). The remaining one lesion (709) was an acral nevus in a one-year-old child with unusual features. The tumor displayed a congenital nevus pattern and contained a subpopulation of heavily pigmented epithelioid melanocytes. Accordingly, the histopathological findings of the four tumors were variable. Two lesions were entirely composed of amelanotic large epithelioid cells (Figure 8, B and D); the other two lesions contained a mixed population of pigmented large melanocytes superficially and amelanotic epithelioid melanocytes in the deeper portion of the lesion (Figure 8, A and C).

Discussion

We report herein the evidence for recurrent *NTRK3* gene fusions in four childhood melanocytic tumors based on genome-wide copy number analysis by SNP-array. In addition, in one case of which sufficient material was available for RNA extraction, a novel *MYO5A-NTRK3* fusion was identified by targeted RNA-seq. The approach used in the current study builds on our previous studies,[8] which had shown that high-resolution copy number array analysis can be used to screen for gene fusions associated with unbalanced genomic aberrations flanking the fusion points without prior knowledge of the genetics of a given case. In addition to the identification of *NTRK3* fusions, we also detected samples with *ALK*, *BRAF*, and *NTRK1* gene rearrangements that have known to be associated with recurrent gene fusions in spitzoid tumors. FISH assays were performed on each case, when tumor material was available, and verified the prediction based on the SNP-array findings (data not shown).

In a recent study, Wiesner et al[6] used a high-throughput next-generation sequencing approach to analyze 140 spitzoid tumors, and demonstrated that recurrent kinase fusions involving *ROS1*, *NTRK1*, *ALK*, *BRAF*, and *RET* are common genetic aberrations in spitzoid neoplasms, and these aberrations present in a mutually exclusive pattern. And, more recently, Yeh et al[7] reported *MET* fusions in a rare subtype of spitzoid tumor. All these kinase genes, except *MET*, are included in our custom fusion-screening panel designed for anchored multiplex PCR for targeted RNA-seq. This fusion-screening strategy enables the identification of both known and novel fusions involving genes in the panel. Using the screening platform, we discovered a *MYO5A-NTRK3* fusion in sample 930. Besides the strong evidence from the target RNA-seq showing the bona fide *MYO5A-NTRK3* transcript, *MYO5A-NTRK3* is the only fusion transcript identified in this sample, which suggests that *NTRK3* fusion is mutually exclusive with other kinase fusions in spitzoid tumors. Given that *MET* was not included in our fusion-screening panel, we could not obtain data from the targeted RNA-seq to verify if *NTRK3* and *MET* fusions are mutually exclusive. All *MET*-fusion cases reported by Yeh et al[7] were associated with unbalanced structural abnormalities at or near *MET* gene locus by array analysis. However, this phenomenon was not observed in any of our *NTRK3*-rearranged cases by high-resolution copy number analysis. Therefore, it is likely that *NTRK3* and *MET* fusions in melanocytic tumors follow the mutually exclusive pattern as well.

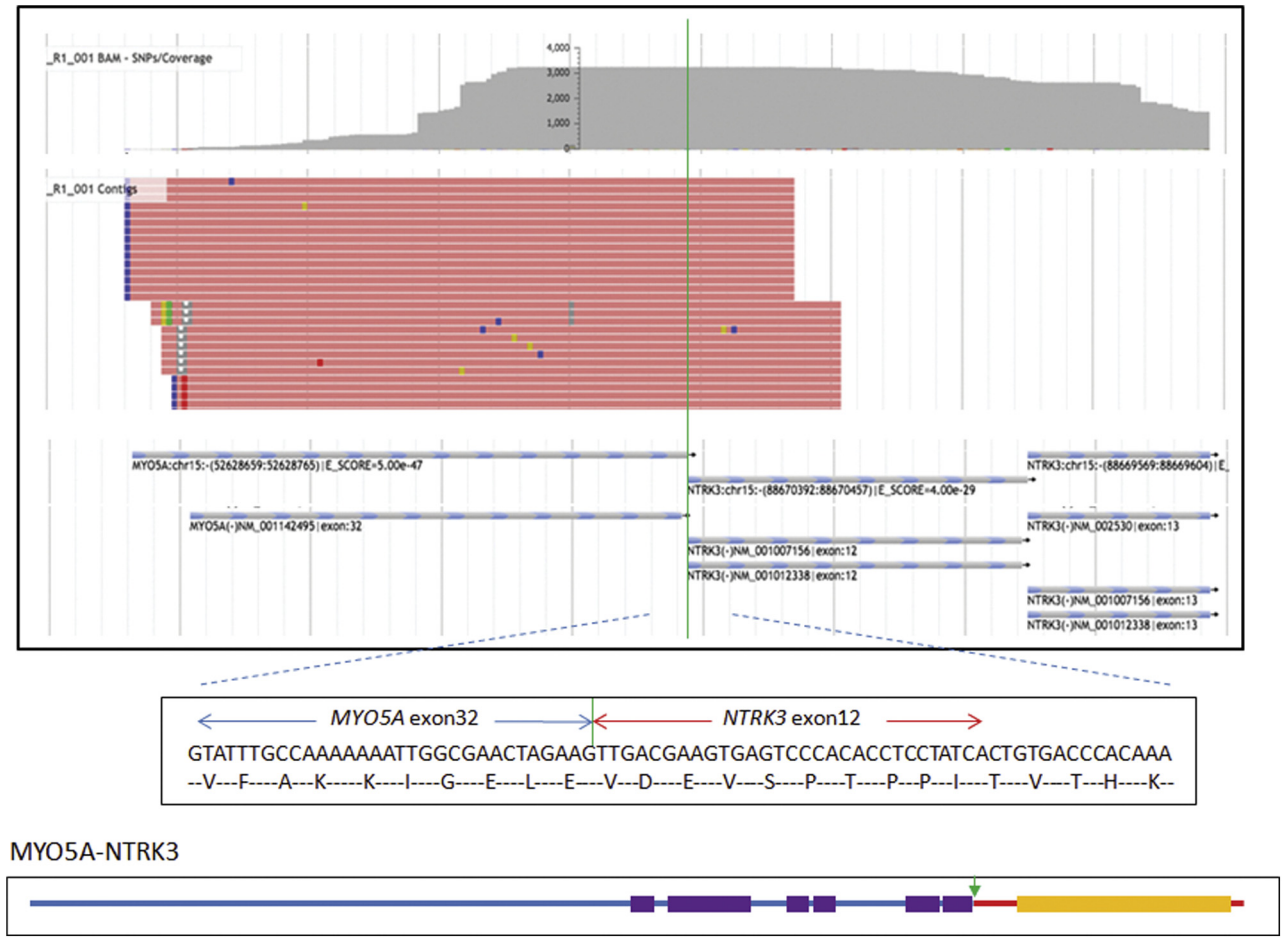

Figure 7 Identification of the novel *MYO5A-NTRK3* fusion in the spitzoid tumor sample 930. **Top panel:** A representative image of *MYO5A-NTRK3* fusion visualization by Archer JBrowse software version 3.3.0. **Middle panel:** Partial sequence of the *MYO5A-NTRK3* fusion transcript along with predicted amino acid sequence. The transcript is an in-frame fusion of *MYO5A* exon 32 to *NTRK3* exon 12. **Bottom panel:** Schematic diagram of the predicted MYO5A-NTRK3 chimeric protein. **Blue line** indicates N-terminal of MYO5A; **red line**, C-terminal of NTRK3; **purple bars**, coiled-coil dimerization domains of MYO5A; **orange bar**, the kinase domain of NTRK3; **vertical green lines** in **top** and **middle panels** and the **green arrow** in **bottom panel**, the fusion point.

The *NTRK3* gene encodes TRKC, a transmembrane receptor tyrosine kinase, which is primarily involved in neuronal cellular processes.[13] TRKC is a member of the tropomyosin-receptor kinase (TRK) family that also includes TRKA (encoded by *NTRK1*) and TRKB (encoded by *NTRK2*). *NTRK1/2/3* oncogenic gene fusions have been identified in several tumor types to date.[14–28] The first *NTRK3* gene fusion, *ETV6-NTRK3*, was described in congenital fibrosarcoma in 1998.[19] Since then, *ETV6-NTRK3* rearrangements have been identified in several other tumor types, including congenital mesoblastic nephroma,[20] secretory breast carcinoma,[21] acute myeloid leukemia,[22] mammary analog secretory carcinoma of the salivary gland,[23] radiation-associated thyroid cancer,[24] and colorectal cancer.[26] In contrast to *NTRK1* and *NTRK2* fusions in which a variety of 5′ fusion partners have been identified, almost all *NTRK3* gene fusions reported so far are *ETV6-NTRK3* regardless of the tumor type, and only a few other fusion partners have been identified in individual cases.

In most of the *NTRK* fusions identified so far, the 5′ fusion partner provides a portion encoding one or more dimerization domains and 3′ portion of *NTRK* encoding the kinase domain is always preserved in the fusion gene. Several functional studies have shown that chimeric TRK proteins are constitutively active tyrosine kinases and oncogenic. For instance, Wai et al[29] expressed a serial of *ETV6-NTRK3* constructions in NIH3T3 cells and demonstrated that the protein product of *ETV6-NTRK3* functions as a chimeric protein tyrosine kinase that is autophosphorylated on tyrosine residues and has transforming activity. Both an intact dimerization domain and a functional protein tyrosine kinase domain are required for its transforming activity. Further on, Tognon et al[30] illustrated that the expression of *ETV6-NTRK3* in NIH3T3 cells leads to constitutive activation of two effector pathways of wild-type *NTRK3* (ie, the mitogen-activated protein kinase signaling pathway and phosphatidylinositol 3-kinase−AKT pathway), and mitogen-activated protein kinase and phosphatidylinositol 3-kinase−AKT activations act synergistically to mediate *ETV6-NTRK3* transforming activity. As *ETV6-NTRK3* fusion is fairly dominant in secretory breast cancer, Tognon et al[21] also demonstrated the transforming activity of

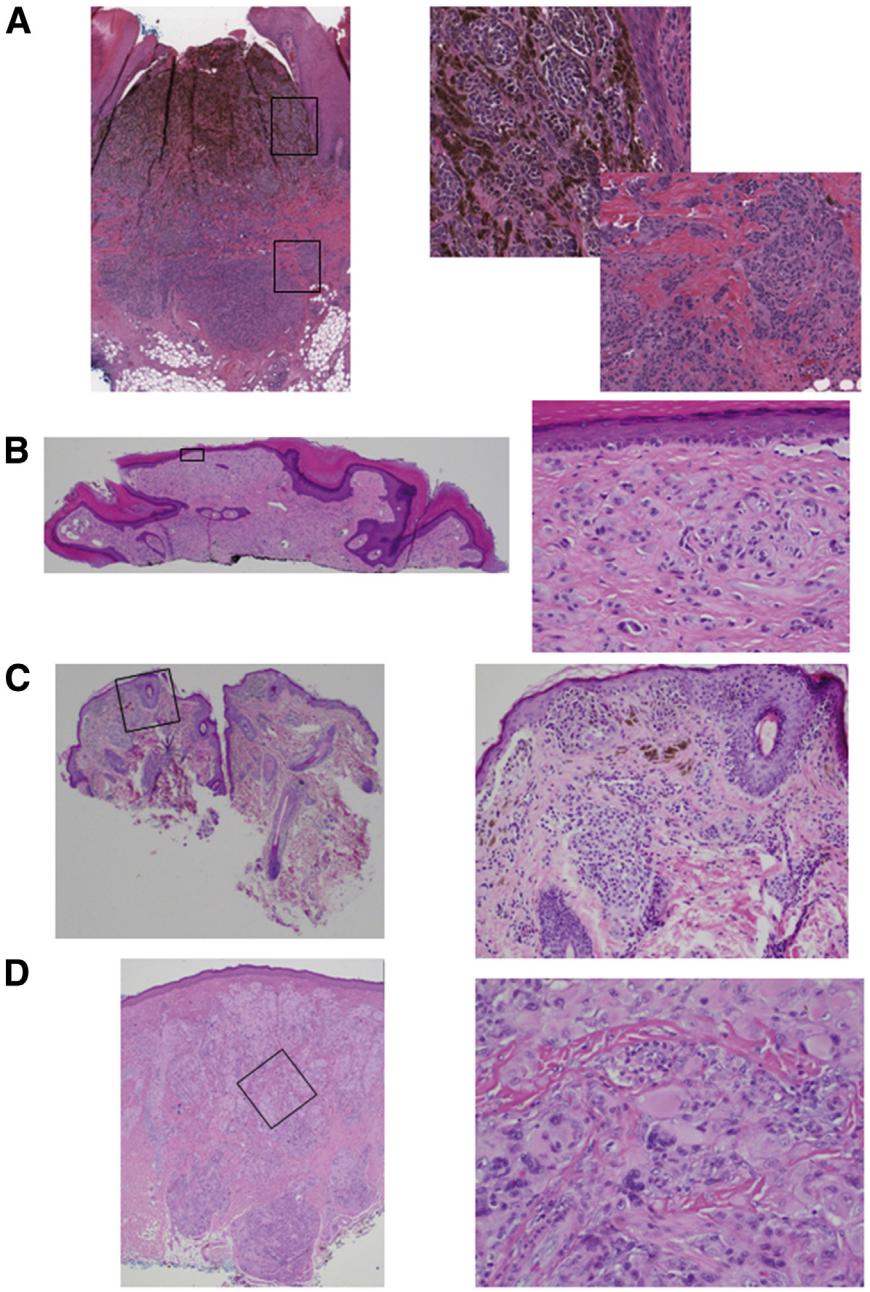

Figure 8 Light microscopic findings of four *NTRK3*-rearrangement melanocytic tumors. Sample 709 (**A**), 930 (**B**), 3509 (**C**), and 4583 (**D**). **A–D: Left panels**: Low-magnification images. **Right panels**: High-magnification images showing **boxed areas** of detail. **A:** Pandermal acral epithelioid melanocytic tumor with heavy pigmentation in its superficial portion. **Top right panel:** Superficial pigmented epithelioid cell component. **Bottom right panel:** Deep dermal amelanotic spindle and epithelioid cell proliferation. **B:** Polypoid silhouette of a predominantly intradermal amelanotic pauci-cellular melanocytic nevus; the intradermal melanocytes are predominantly epithelioid in appearance. **C:** Compound melanocytic nevus, the tumor cells are epithelioid in appearance; a few melanophages are present. **D:** Spitzoid melanoma of childhood, large atypical compound melanocytic tumor with extension into subcutis, the tumor cells are amelanotic, epithelioid in appearance, and display nuclear pleomorphism. Original magnifications: ×2 (**A–D**, **left panels**); ×20 (**A–D**, **right panels**).

ETV6-NTRK3 in mammary epithelial cells. Similarly, several studies reported oncogenic activity of *NTRK1* fusions with different 5′ fusion partners.[16,17] Given that chimeric TRK proteins are constitutively active tyrosine kinases and oncogenic, clinical trials to evaluate the benefit of pan-TRK inhibitors in patients with *NTRK* fusions are ongoing.[31,32] We recently reported the first clinical response to TRKC inhibition in a patient with an *NTRK3*-rearranged malignancy, underlining the role of *NTRK3* fusions as targetable drivers of oncogenesis.[33]

In our study, the predicted MYO5A-NTRK3 fusion protein consists of several N-terminal coiled-coil protein dimerization motifs encoded by *MYO5A* and C-terminal tyrosine kinase domain encoded by *NTRK3*, which perfectly fits the TRK oncogenic fusion paradigm and suggests it may function as a chimeric protein tyrosine kinase with potent transforming activity (Figure 7). Although *MYO5A* is a novel fusion partner for *NTRK3*, a *MYO5A-ROS1* fusion was identified in the Wiesner study of spitzoid tumors.[6] *MYO5A* is one of three myosin V heavy-chain genes, belonging to the myosin gene superfamily. Myosin V is a class of actin-based motor proteins involved in cytoplasmic vesicle transport and anchorage, spindle-pole alignment, and mRNA translocation. The expression of *MYO5A* is abundant in melanocytes and

nerve cells.[34] Hence, we hypothesize that *MYO5A*, as a recurrent fusion partner involved in kinase gene fusions in spitzoid tumor, may facilitate the oncogenic activity of the fusion products not only by providing the coiled-coil dimerization domain but also by its strong promoter to up-regulate the expression of the fusion transcript. As shown by the targeted RNA-seq, the *MYO5A-NTRK3* fusion transcripts expressed in high abundance in tumor sample 930, which is consistent with our hypothesis. Unfortunately, the expression of NTRK3 chimeric protein could not be evaluated by immunohistochemical staining on the FFPE tumor tissues in the present study because of the unavailability of a reliable antibody. Future studies will continue to characterize *NTRK3* fusion(s) in childhood melanocytic neoplasms and determine whether *NTRK3* fusions contribute to the initiation and/or maintenance of the fusion-positive childhood melanocytic neoplasms.

In summary, we report herein the identification of a novel *NTRK3* gene fusion in childhood melanocytic tumors based on genome-wide copy number analysis by SNP-array. Because the prevalence and pattern of DNA copy number changes differ dramatically between benign and malignant melanocytic tumors, DNA copy number analysis by array-based studies has been used as an important ancillary method for diagnostic evaluation of melanocytic proliferations with ambiguous histopathological findings.[35–37] As such, we suggest that intragenic copy number changes, in particular when genes affected are known to be involved in recurrent gene fusions in human tumors, should be closely reviewed and further evaluated (if needed) to confirm potential gene fusions that may be rational drug targets.

Our findings add to the list of kinase fusions that have so far been found in melanocytic tumors. *NTRK3* fusion may define an additional subset of melanocytic tumors with a potentially targetable driver oncogene. Among the four examined lesions, three lesions had spitzoid cytologic features, whereas one was not believed to belong into the group of Spitz tumors. Thus, our findings suggest that, although kinase activation by receptor-kinase–gene fusions is a common mechanism that drives tumorigenesis in spitzoid neoplasms, kinase fusions could be found in childhood melanocytic neoplasms outside of the spectrum of Spitz tumors. With regard to the significance of *NTRK3* fusions in Spitz tumors, as previously documented with other kinase fusions,[5] kinase fusions can be found in the full spectrum of spitzoid neoplasms ranging from lesions classified as nevi to lesions designated as spitzoid melanoma of childhood. The clinical significance of *NTRK3* fusions in childhood melanocytic tumors will be determined on following up on the clinical outcomes of patients bearing the fusions.

Supplemental Data

Supplemental material for this article can be found at *http://dx.doi.org/10.1016/j.jmoldx.2016.11.005*.

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