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CASE REPORT

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A pediatric case of pigmented epithelioid melanocytoma with chromosomal copy number alterations in 15q and 17q and a novel *NTRK3-SCAPER* gene fusion

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Abstract

Pigmented epithelioid melanocytoma (PEM) represents a group of rare, heavily pigmented melanocytic tumors encompassing lesions previously designated as "animal-type melanomas" and "epithelioid blue nevi." Despite the association of multiple such tumors in the setting of Carney complex, most cases of PEM occur spontaneously as solitary neoplasms in otherwise healthy patients. PEM may arise in both children and adults, and has a known propensity to spread to the regional lymph nodes. Despite this latter finding, recurrence at the biopsy site or spread beyond the lymph node basin is exceptionally uncommon. Although the molecular basis for PEM continues to be characterized, findings to date suggest that this category of melanocytic neoplasia has genetic alterations distinct from those seen in common nevi, dysplastic nevi, Spitz nevi, and melanoma. Herein, we present an in-depth clinical, histopathologic, and molecular analysis of a case of PEM occurring on the scalp of a young African American girl found to have a novel *NTRK3-SCAPER* gene fusion.

KEYWORDS

melanocytic neoplasms, melanocytic lesions, dermatopathology, pigmented epithelioid melanocytoma, NTRK3 fusion

1 | BACKGROUND

Pigmented epithelioid melanocytoma (PEM) represents a group of rare, heavily pigmented melanocytic tumors encompassing lesions previously designated as "animal-type melanomas" and "epithelioid blue nevi."[1,2] Despite the association of multiple such tumors in the setting of Carney complex, most cases of PEM occur spontaneously as solitary neoplasms in otherwise healthy patients. The typical clinical presentation for PEM is a slow-growing deep-seated blue or gray nodule, which may clinically resemble cellular blue nevi, pigmented cystic, adnexal or other vascular neoplasms, and occasionally melanoma.[3] PEM may arise in both children and adults, and has a known propensity to spread to the regional lymph nodes.[2,4] Despite this latter finding in up to 46% of patients, recurrence at the biopsy site or spread beyond the lymph node basin is exceptionally uncommon.[2,5] Although the molecular basis for PEM continues to be characterized, findings to date suggest that this category of melanocytic lesions has genetic alterations distinct from those seen in common nevi, dysplastic nevi, Spitz nevi, and melanoma.[5,6] Herein, we present an in depth clinical, histopathologic, and molecular analysis of a case of PEM occurring on the scalp of a young African-American girl found to have a novel *NTRK3-SCAPER* gene fusion.

2 | CASE REPORT

A 4-year-old African American female was referred for a 2-month history of an enlarging lesion on her right parietal scalp that was reportedly tender with brushing of her hair. On physical examination there was a firm but mobile, largely skin-colored subcutaneous nodule measuring 1.5 x 1.5 cm. A small focus of dark pigmentation was observed at the center (Figure 1). A 4-mm punch biopsy was obtained from the nodule given the unusual clinical presentation.

Histopathologic analysis revealed deep dermal and subcutaneous nodular aggregations of heavily pigmented epithelioid melanocytes, interspersed with melanophages and occasional lymphocytes (Figure 2). The melanocytes were fairly uniform in size and shape and showed vesicular nuclei with thin nuclear membranes and conspicuous nucleoli. In some foci, mild-to-moderate nuclear pleomorphism and rare mitotic figures were observed. The neoplastic cells were highlighted by a Melan-A immunohistochemical stain and showed a proliferation index (Ki-67) of less than 5%. Immunohistochemistry for PRKAR1A showed retained cytoplasmic expression in the lesional melanocytes. No pre-existing congenital-pattern nevus or junctional component was observed.

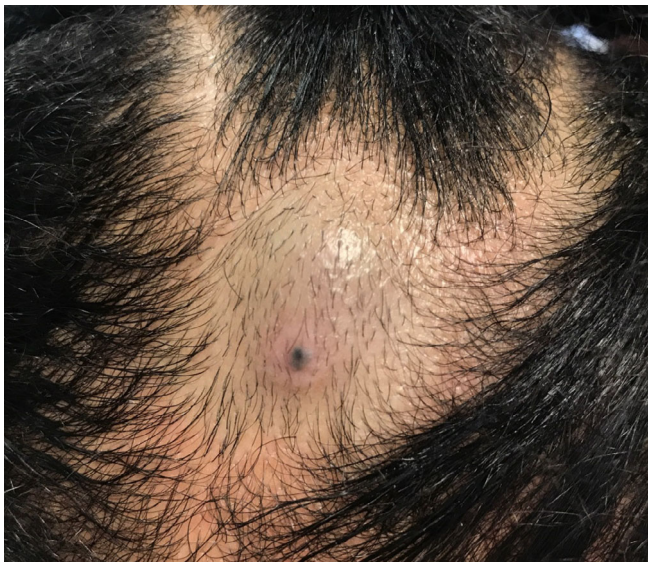

FIGURE 1 Clinical. Deep-seated 1.5 × 1.5 cm nodule with eccentric focus of pigmentation on the right parietal scalp

Single nucleotide polymorphism and comparative genomic hybridization (SNP/CGH) microarray analysis revealed two distinct segmental abnormalities consisting of chromothripsis involving chromosomes 15q (23 copy number gains and three losses) and 17q (13 gains). Prior studies and experience have showed that the vast majority of benign melanocytic nevi typically do not harbor any copy number changes (rarely, n = 1 or 2); on the other hand melanoma often shows three or more segmental amplifications or deletions in reproducible loci.[7,8] The two abnormalities detected in this sample have not classically been seen in melanoma, and therefore this result was interpreted as "borderline" with a low risk of progression (Figure 3).

Within the amplified regions a breakpoint was identified within the *NTRK3* gene with amplification of 5′ end and loss of 3′ end, indicating rearrangement and potentially forming a gene fusion at this locus. Subsequent analysis by fluorescence in-situ hybridization (FISH) using break apart probes and target capture gene fusion analysis by next generation sequencing (NGS) confirmed amplification and deletion of the 5′ and 3′ regions, respectively, of *NTRK3* and detection of an in-frame fusion of exon 7 with exon 13 of *SCAPER* gene at its 3′ region. Subsequent FISH analysis of the *SCAPER* gene also confirmed the array CGH finding of amplification and deletion of the 3′ and 5′ regions, respectively (Figures 4 and 5). The remainder of the tumor was excised with narrow margins, revealing identical histopathology (Figure 2). Follow-up history and thorough physical evaluation of the patient showed no evidence to suggest underlying Carney complex.

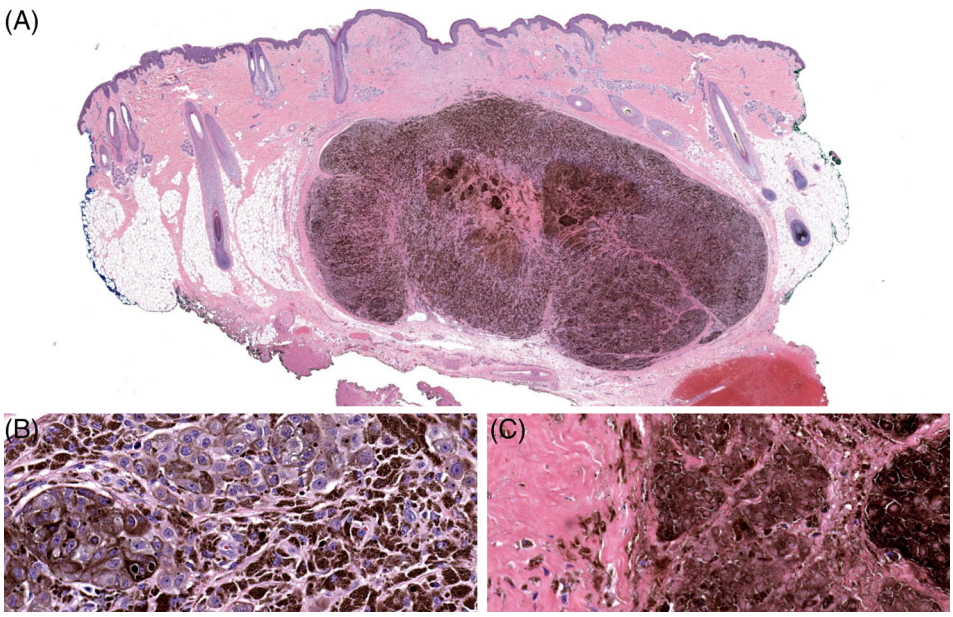

FIGURE 2 Histopathology. A, Scanning view showing a circumscribed, pigmented nodular aggregation of neoplastic cells in the deep dermis and subcutis (original magnification, ×20). B, Nests of heavily melanized epithelioid melanocytes containing vesicular nuclei with centrally placed nucleoli (original magnification, ×200). C, Sclerosis enveloping nests of melanocytes with dense cytoplasmic melanin accumulation obscuring nuclear detail (original magnification, ×200)

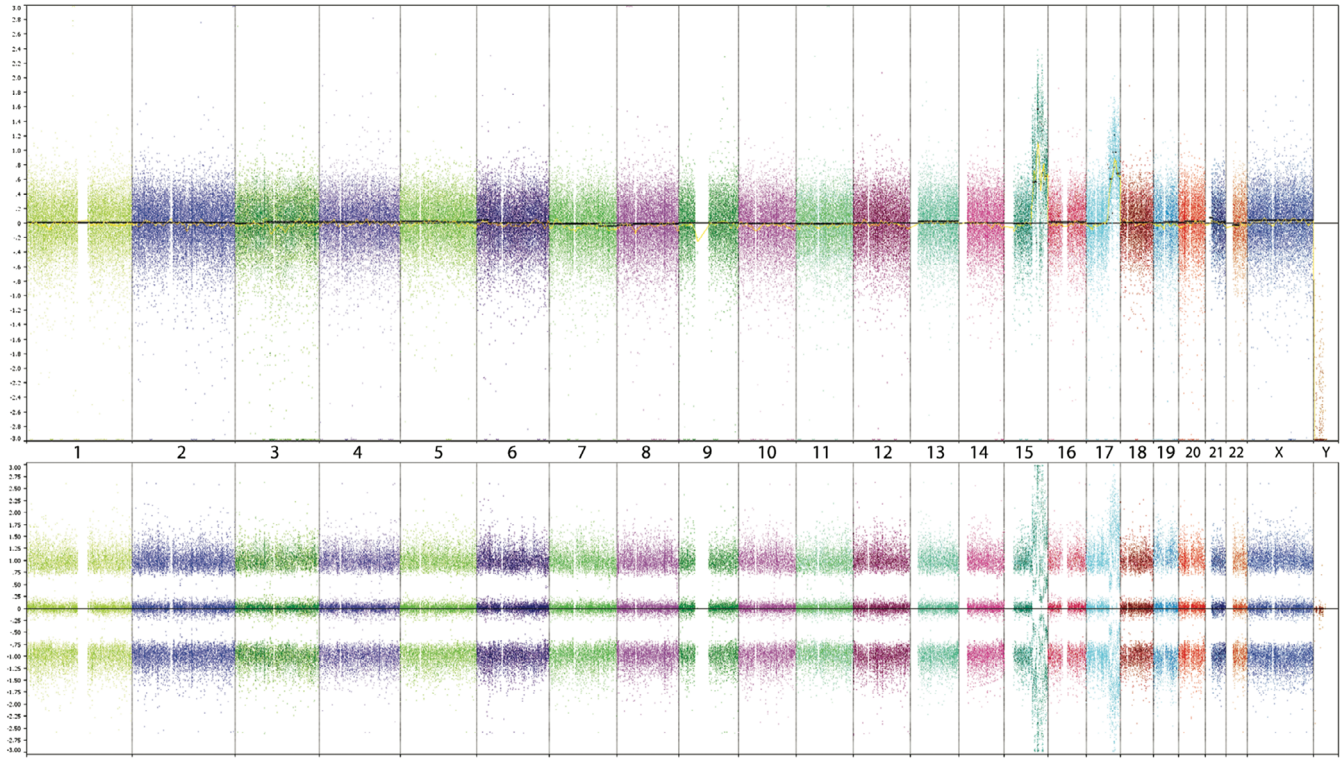

FIGURE 3 Chromosomal SNP array (upper panel: copy number data, lower panel: allelic ratio plot) showing gains and losses in chromosomes 15q and gains in chromosome 17q

3 | DISCUSSION

Limited published molecular data to date provide support for the notion that sporadic PEM may be a distinct category of melanocyte neoplasia separate from dysplastic (or Clark's), congenital, Spitz, and blue nevi. In one of the larger studies exploring this issue,[5] expression of *PRKAR1A* was found to be lost in 6/11 cases of sporadic PEM, with variable alterations in the underlying gene (locus 17q24.2) detected in three of those cases (missense, splice cite, and deletion). Of note, these latter three cases were "combined PEM" with a component of ordinary compound nevus and unsurprisingly also had the more common *BRAF* p.V600E hotspot mutations.[5] Two of the remaining three cases showed loss of *PRKAR1A* expression (but no genetic alterations were detected at this focus) in addition to *MAP2K1* in frame deletions (locus 15q22). In the five cases with preserved *PRKAR1A* expression, two showed *PRKCA* fusions (locus 17q24.2) and three showed no genetic aberrations.[5] A second group recently found similar alterations in cases of sporadic PEM as they detected *PRKCA* fusions in 5/16 of their analyzed cases.[6] One of their cases also showed both a *MYO5A- NTRK3* fusion along with a point mutation in *PRKAR1A*.[6]

In this report, we describe a case of sporadic PEM occurring in a pediatric patient, with the diagnosis being supported by characteristic clinical and histopathological features. Moreover, the absence of characteristic copy number alterations as is usually seen in melanoma on SNP array helped better exclude a malignant phenotype.[7,8] Interestingly, a small number of copy number alterations restricted to chromosomes 15q and 17q were detected. This latter finding may provide additional support for the notion that at least some cases of PEM are intermediate/borderline melanocytic tumors. Very limited data exist on copy number status in the context of PEM, but it is noteworthy that Cohen et al (2018)[5] failed to detect significant copy number alterations in any of their 11 cases. Interestingly, many of the genetic loci previously implicated in PEM as described above (*PRKAR1A*, *MAP2K1*, and *PRKCA*) are found on either chromosomes 15q or 17q within the areas found to have chromothripsis in this case. This raises the possibility that one or more of these or related genes could have been affected in this case, potentially driving the copy number changes detected on our SNP array analysis. Interestingly, PRKAR1A staining was preserved on immunohistochemistry, which argues against a deleterious mutation or gene silencing at this locus.

A novel finding in this case is the detection of an in-frame *NTRK3-SCAPER* fusion. NTRK3 fusions (most often with a 5′ partner such as *MYO5A* or *ETV6*) have recently been reported to occur in the context of some pediatric Spitz nevi (especially pigmented nevus of reed variant) and atypical spitzoid tumors,[9-11] To our knowledge, this particular fusion combination has not been reported previously in PEM or any other melanocytic neoplasm. The functional significance of this inframe fusion, which is producing a truncated SCAPER protein is unclear and may be worthy of future study. We can only speculate as to why the *NTRK3-SCAPER* fusion in this case was associated with PEM-like morphology as opposed to pigmented Spitz nevus-like morphology that has been described in prior series.[9-11] In the case of PEM with a *MYO5A-NTRK3* fusion reported by Isales et al (2018), it is likely that the concomitant *PRKAR1A* mutation influenced the ultimate

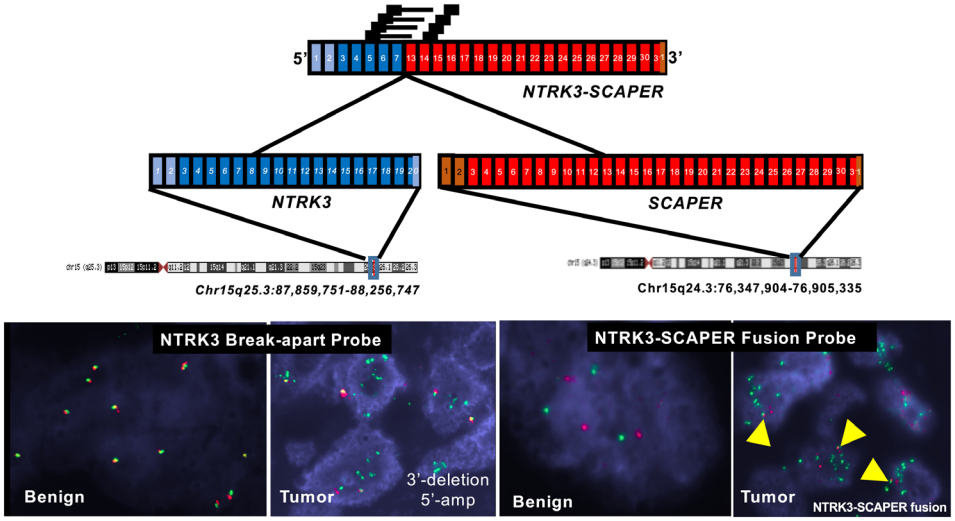

FIGURE 4 Fluorescence in situ hybridization confirming amplification of the 5′ portion of *NKTR3* and the presence of a fusion with *SCAPER* at its 3′ portion

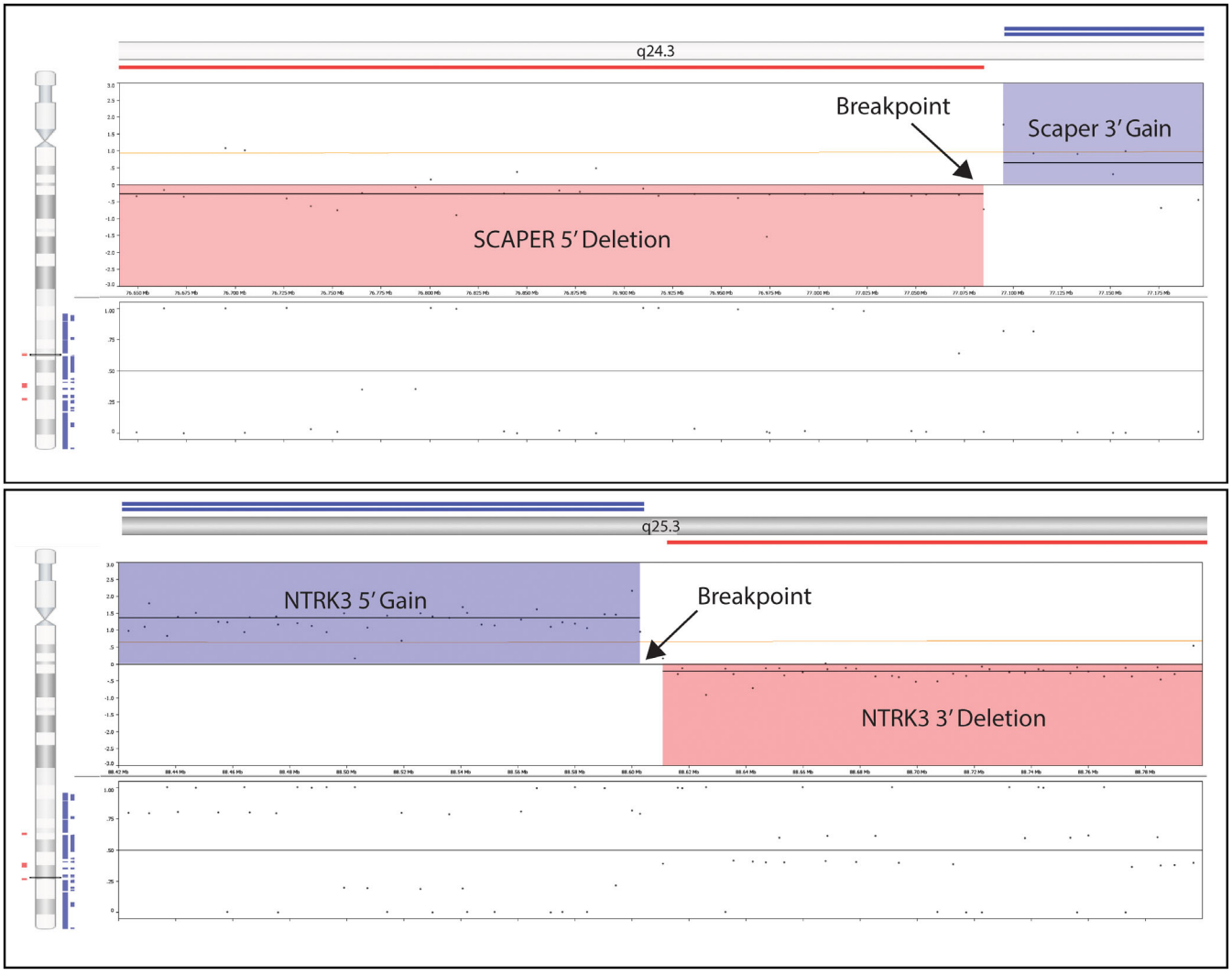

FIGURE 5 Array CGH analysis shows breakpoint in *NTRK3* and *SCAPER* genes

morphology.[6] By deduction, one could hypothesize that abnormalities in NTRK3 may predispose to dense melanization, but that a second alteration in the cAMP signaling pathway (present, but not discovered in this case) is needed to induce the more medium-sized epithelioid cell type seen in PEM. An additional possibility is that the cell of origin could possibly have represented a follicular or matrical melanocyte in

this case (given the deep dermal and subcutaneous position of the tumor), while classical pigmented Spitz nevi more clearly originate from the dermo-epidermal junction and have a different milieu.

4 | MATERIALS AND METHODS

4.1 | Next generation sequencing

A multiplex RNA fusion panel (Archer FusionPlex Solid Tumor Kit) was run on RNA extracted from representative FFPE tumor block. Briefly, this assay is a targeted sequencing assay that uses anchored multiplex polymerase chain reaction (AMP) to prepare target-enriched cDNA libraries from RNA to detect fusions and other mutations in over 50 genes linked to carcinomas, other solid tumors, known sarcoma and hematological malignancy-associated fusions using NGS. This technology allows the detection of not only known recurrent fusions but also previously unidentified fusions at key breakpoints in target genes. AMP creates target enriched libraries by using a combination of unidirectional gene-specific primers and universal adapters to enrich for both known and unknown mutations. Adapters that contain both molecular barcode adapters (MBCs) and sample indices permit quantitative multiplex data analysis, read deduplication and accurate mutation calling. MBCs help in error correction, sample identification, de duplication, and duplicate read binning for confident mutation detection.

4.2 | Array comparative genomic hybridization

Thick sections (10 μM, n = 10) were cut from a representative formalin fixed and paraffin embedded (FFPE) tissue block and tumor was macrodissected using a hematoxylin and eosin-stained slide as a guide. DNA was extracted and purified using the QIAmp DNA FFPE Tissue Kit (Qiagen, Dusseldorf, Germany) according to the manufacturer's protocols. Extracted DNA was quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA) following the manufacturer's instructions, and copy number alteration studies were performed using the OncoScan FFPE Express 3.0 gene chip probe SNP microarray (Affymetrix, Santa Clara, CA), as described previously.[12] Briefly, DNA from each sample (80 ng) was probed with more than 335 000 SNP-containing molecular inversion probes targeting interrogation sites of 40 base pairs. Probe fluorescence was compared with a reference human genome, yielding information on both copy number changes (gains and losses) and allelic frequency/zygosity. Copy number alteration data from each sample were analyzed using Nexus Copy Number software version 9.0 (BioDiscovery, El Segundo, CA). Diploid correction was performed, when necessary, according to the manufacturer's instructions. Clinical quality control threshold of MAPD <0.3 was applied.

4.3 | Fluorescence in situ hybridization

Interphase FISH was carried out as described previously.[13] Bacterial artificial chromosomes (BACs) were obtained from the BACPAC Resource Center (Oakland, CA), and probes were prepared as described.[13] For detection of gene rearrangement, deletion or amplification, the following probes were used: for *NTRK3*, RP11-241D10, 5′end and RP11-93B23, 3′end, and for *SCAPER*, 3′end, RP11-289H7. The integrity and correct localization of all probes were verified by hybridization to metaphase spreads of normal peripheral lymphocytes. Slides were examined using an ImagingZ1 microscope (Carl Zeiss, Oberkochen, Germany). FISH signals were scored manually (X100 oil immersion) in morphologically intact and non-overlapping nuclei by study author NP, and a minimum of 100 cancer cells from both cancer and benign regions were scored.

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