Friedman Ben (Orcid ID: 0000-0002-0075-836X)
Harms Paul (Orcid ID: 0000-0002-0802-2883)
Palanisamy Nallasivam (Orcid ID: 0000-0002-0633-9772)

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

Ben J. Friedman, MD[1,2], Simon Hernandez, BS[3], Chelsea Fidai, MD[1], Angela Jiang, MD[1], Tor A. Shwayder, MD[1], Shannon Carskadon[4], Aleodor A. Andea, MD, MBA[5,6], Paul W. Harms, MD, PhD[5,6], Dhananjay Chitale MD[2], Nallasivam Palanisamy, PhD[4].

Author Affiliations:

1) Department of Dermatology, Henry Ford Health System, Detroit, MI 48202

2) Department of Pathology and Laboratory Medicine, Henry Ford Health System, Detroit, MI 48202

3) College of Medicine, State University of New York Upstate Medical University, 766 Irving Ave, Syracuse, NY 13210

4) Department of Urology, Vattikuti Urology Institute, Henry Ford Health System, Detroit, MI 48202

5) Department of Pathology, Michigan Medicine, University of Michigan, Ann Arbor MI 48109

6) Department of Dermatology, Michigan Medicine, University of Michigan, Ann Arbor MI 48109

Corresponding Author:

Ben J. Friedman

3031 West Grand Blvd

Suite 800

Detroit, MI 48202

P#- 313-916-2151

Author Manuscript

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/cup.13566

This article is protected by copyright. All rights reserved.

Author Manuscript

F#-888-959-0927

Email: bfriedm1@hfhs.org

Figure #: 5

Word Count: 1750

Acknowledgement: The authors would like to thank Dr. Phil Leboit for performing the PRKAR1A immunohistochemistry in this case.

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 grey 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

This article is protected by copyright. All rights reserved.

case of PEM occurring on the scalp of a young African American girl found to have a novel *NTRK3-SCAPER* gene fusion.

Author Manuscript

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 demonstrated vesicular nuclei with thin nuclear membranes and conspicuous nucleoli. In some foci, mild-to-moderate nuclear pleomorphism and rare mitoses were observed. The neoplastic cells were highlighted by a Melan-A immunohistochemical stain and demonstrated a proliferation index (Ki-67) of less than 5%. Immunohistochemistry for *PRKAR1A* demonstrated retained cytoplasmic expression in the lesional melanocytes. No pre-existing congenital pattern nevus or junctional component was observed.

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 3 losses) and 17q (13 gains). Prior studies and experience

This article is protected by copyright. All rights reserved.

Author Manuscript

have demonstrated that the vast majority of benign melanocytic nevi typically do not harbor any copy number changes (rarely, n=1 or 2), whereas melanoma often demonstrates 3 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 relatively 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 (**Figure 4 & 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 demonstrated no evidence to suggest underlying Carney Complex.

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)

This article is protected by copyright. All rights reserved.

detected in 3 of those cases (missense, splice cite, and deletion). Of note, these latter 3 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 demonstrated loss of *PRKAR1A* expression (but no genetic alterations were detected at this focus) in addition to *MAP2K1* in frame deletions (locus 15q22). In the 5 cases with preserved *PRKAR1A* expression, 2 demonstrated *PRKCA* fusions (locus 17q24.2) and 3 demonstrated 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 demonstrated both a *MYO5A- NTRK3* fusion along with a point mutation in PrkaR1α.(*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 exists 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 chromotrypsis in this case. This raises the possibility that one or more of these or related genes could have been affected

This article is protected by copyright. All rights reserved.

Author Manuscript

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*)'(*10*)'(*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 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), whereas classical pigmented spitz nevi more clearly originate from the dermo-epidermal junction and have a different milieu.

This article is protected by copyright. All rights reserved.

Author Manuscript

Materials and Methods

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 next generation sequencing. 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.

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 (H&E)-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

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript

the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) 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, USA). Diploid correction was performed, when necessary, according to the manufacturer's instructions. Clinical quality control threshold of MAPD < 0.3 was applied.

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, USA), 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

This article is protected by copyright. All rights reserved.

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.

Figure Legends:

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

Figure 2. Histopathology. A) Scanning view demonstrating a circumscribed, pigmented nodular aggregation of neoplastic cells in the deep dermis and subcutis [Original Magnification, 20x]. B) Nests of heavily-melanized epithelioid melanocytes containing vesicular nuclei with centrally placed nucleoli. [Original Magnification, 200x] C) Sclerosis enveloping nests of melanocytes with dense cytoplasmic melanin accumulation obscuring nuclear detail [Original Magnification, 200x].

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.

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.

References:

Author Manuscript

This article is protected by copyright. All rights reserved.

1. Gavriilidis P, Michalopoulou I, Chatzikakidou K, Nikolaidou A. Pigmented epithelioid melanocytoma: a new concept encompassing animal-type melanoma and epithelioid blue nevus. *BMJ Case Rep*. 2013;2013:bcr-2013-008865. Published 2013 Mar 22.
2. Zembowicz A, Carney JA, Mihm MC. Pigmented epithelioid melanocytoma: a low grade melanocytic tumor with metastatic potential indistinguishable from animal-type melanoma and epithelioid blue nevus. *Am J Surg Pathol* 2004; **28**:31–40.
3. Moscarella E, Ricci R, Argenziano G, Lallas A, Longo C, Lombardi M, et al. Pigmented epithelioid melanocytoma: clinical, dermoscopic and histopathological features. *Br J Dermatol*. 2016 May;174(5):1115–7
4. Lim C., Murali R., McCarthy S.W., Krivanek J., and Scolyer R.A.: Pigmented epithelioid melanocytoma: a recently described melanocytic tumor of low malignant potential. *Pathology* 2010; 42: pp. 284-286
5. Cohen JN, Joseph NM, North JP, et al. Genomic analysis of pigmented epithelioid melanocytomas reveals recurrent alterations in PRKAR1A, and PRKCA genes. *Am J Surg Pathol.* 2017;41:1333–1346.
6. Isales MC, Mohan LS, Quan VL, et al.Distinct Genomic Patterns in Pigmented Epithelioid Melanocytoma. *The American Journal of Surgical Pathology.* 2019;43(4):480–488.
7. Bastian BC, Olshen AB, LeBoit PE, Pinkel D. Classifying melanocytic tumors based on DNA copy number changes. *Am J Pathol*. 2003;163(5):1765–1770. doi:10.1016/S0002-9440(10)63536-5
8. Bastian BC, Leboit PE, Hamm H, et al: Chromosomal gains and losses in primary cutaneous melanomas detected by comparative genomic hybridization. *Cancer Res.* 1998. 58(10); 2170 - 2175.
9. VandenBoom T, Quan VL, Zhang B, et al.Genomic Fusions in Pigmented Spindle Cell Nevus of Reed. *The American Journal of Surgical Pathology.* 2018;42(8):1042–1051
10. Yeh I, Tee MK, Botton T, et al. *NTRK3 kinase fusions in Spitz tumours. J Pathol*. 2016;240(3):282–290.
11. Wang L, Busam KJ, Benayed R, et al. Identification of NTRK3 Fusions in Childhood Melanocytic Neoplasms. *J Mol Diagn*. 2017;19(3):387–396. doi:10.1016/j.jmoldx.2016.11.005
12. Carter MD, Durham AB, Miedema JR, et al.Molecular testing of borderline cutaneous melanocytic lesions: SNP Array is more sensitive and specific than FISH. *Hum Pathol*. 2019 Apr;86:115-123
13. Han B, Mehra R, Dhanasekaran SM, et al. A fluorescence in situ hybridization screen for E26 transformation-specific aberrations: identification of DDX5-ETV4 fusion protein in prostate cancer. *Cancer Res*. 2008;68(18):7629–7637.

Author Manuscript

This article is protected by copyright. All rights reserved.

Department of Dermatology
Henry Ford Hospital

Ben J. Friedman, M.D.
Senior Staff Physician
Department of Dermatology

3031 West Grand Boulevard
Suite 800
Detroit, MI 48202
(313) 829-8312 Office
1-888-959-0927 Fax
Email: bfriedm1@hfhs.org

Author Manuscript

05/21/2019

Dear Dr. Shea,

We are submitting a unique case study regarding a pigmented epithelioid melanocytoma arising on the scalp of a pediatric patient. The molecular findings in this case build upon and expand the spectrum of genomic alterations that can be seen in this unique category of melanocyte neoplasia, (recently published in your journal by Leboit & Gerami). We believe that this information will greatly appeal to your authorship.

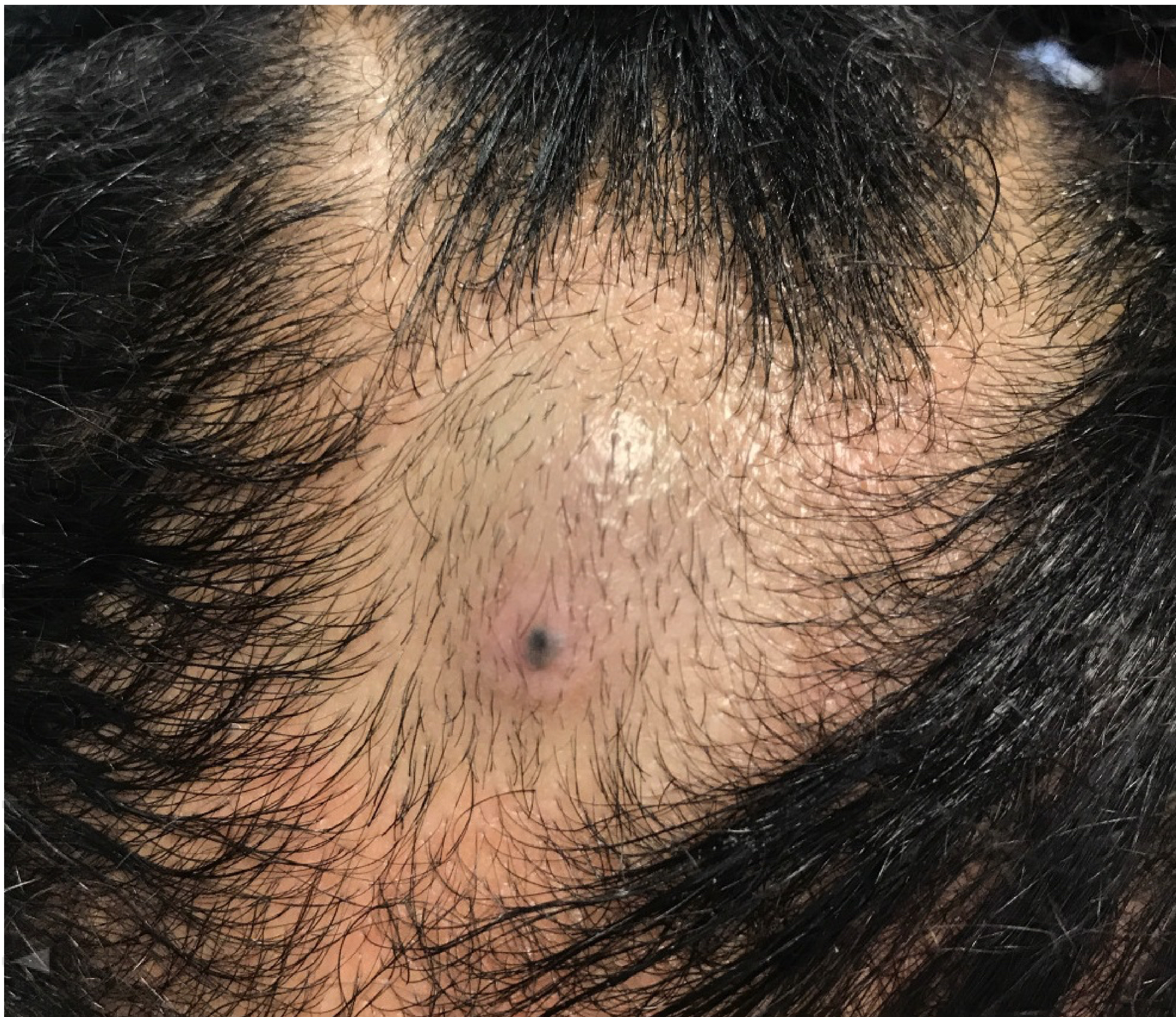
Thank you kindly for your consideration of our manuscript and please do not hesitate to contact me with any questions or clarifications.

Sincerely,

Ben J. Friedman, M.D.

Senior Staff Physician
Henry Ford Hospital
Department of Dermatology
Department of Pathology and Laboratory Medicine

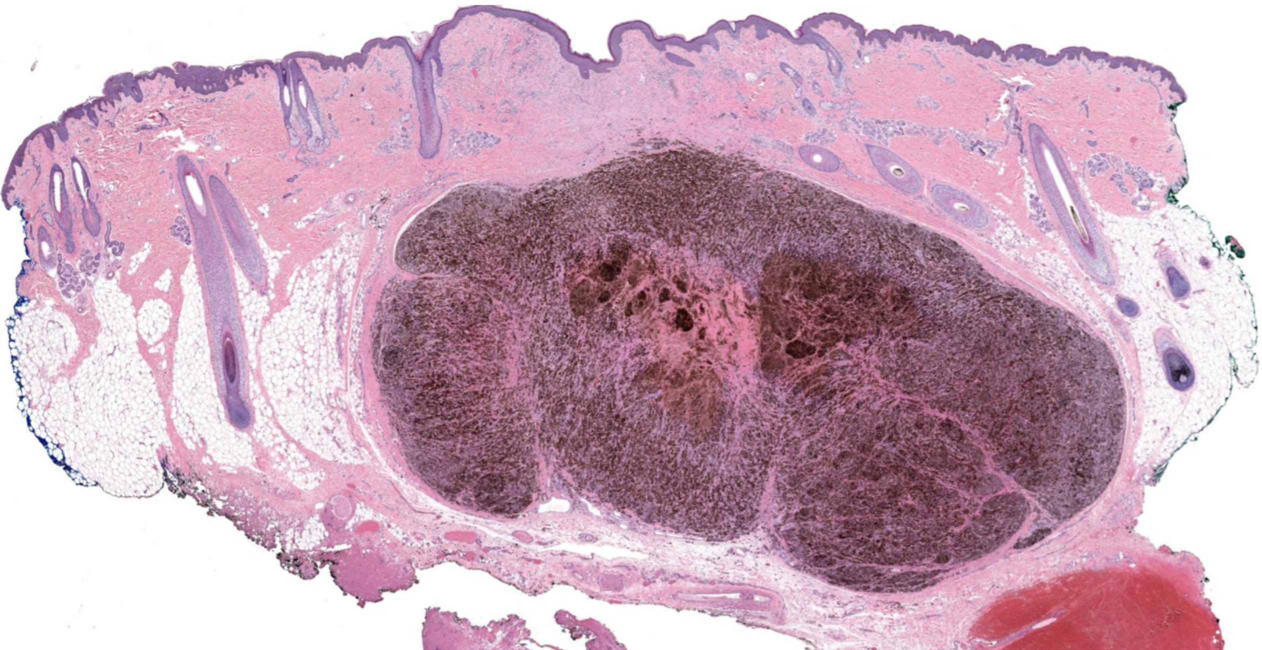
This article is protected by copyright. All rights reserved.

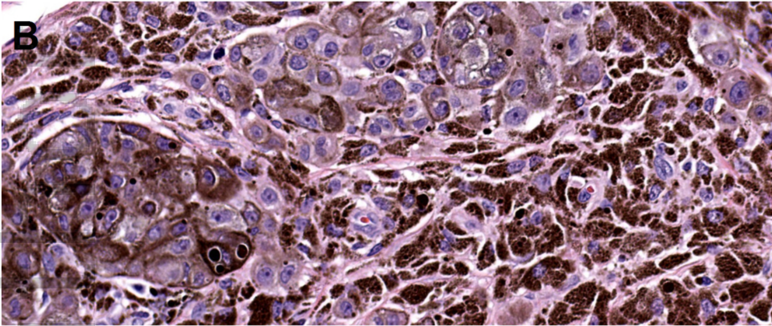


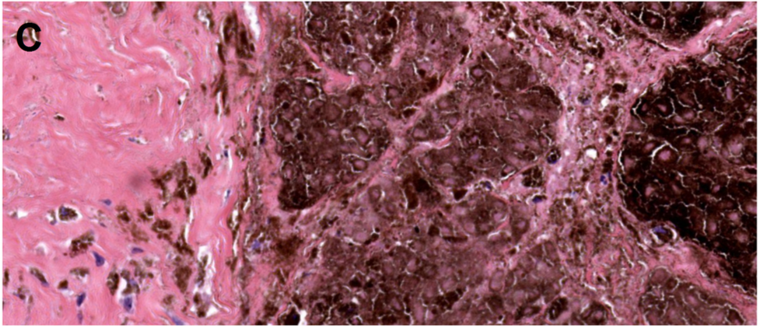
CUP_13566_Figure 1.jpg

This article is protected by copyright. All rights reserved.

Author Manuscript



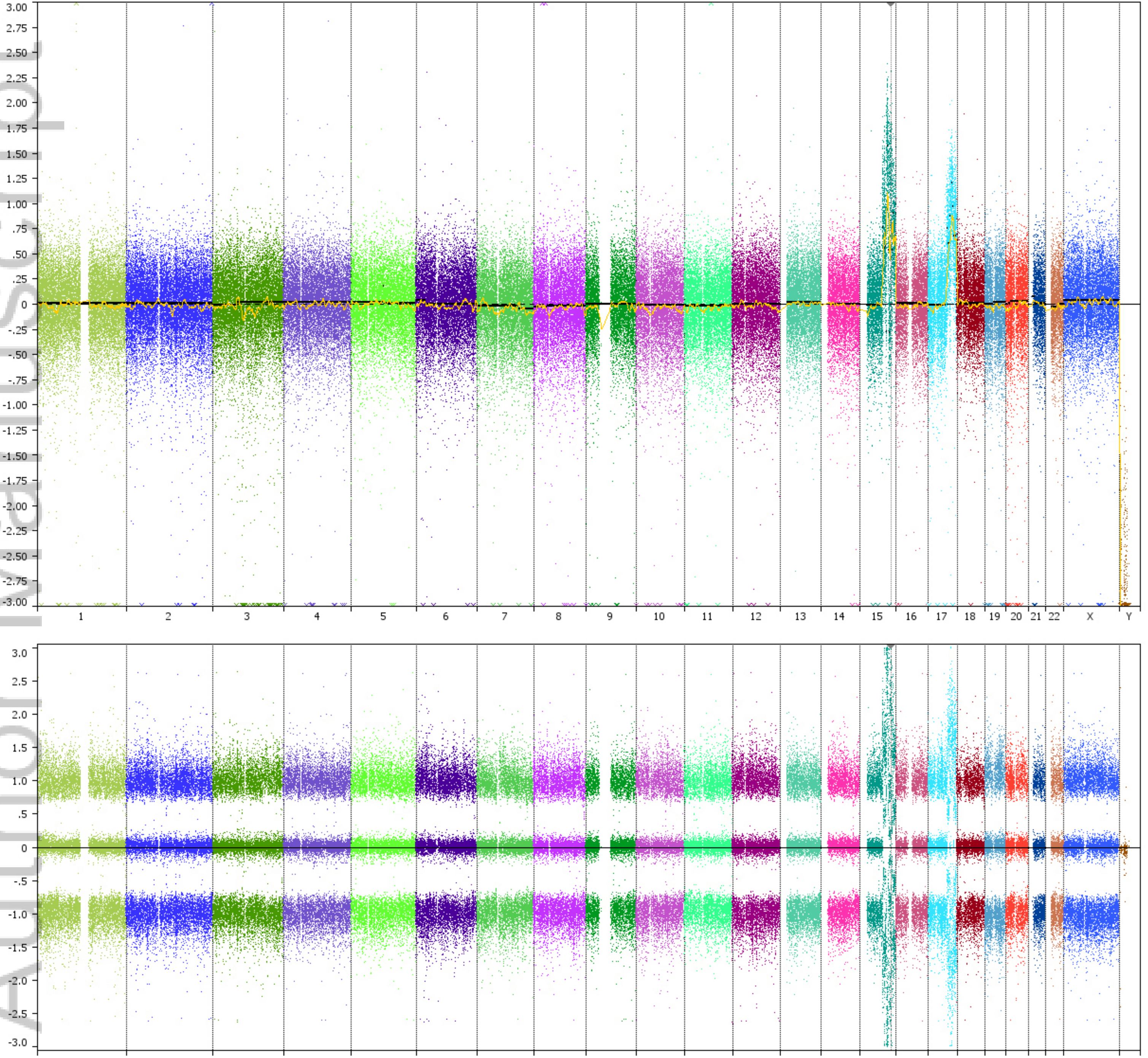




CUP_13566_Figure 2.jpg

This article is protected by copyright. All rights reserved.

Author Manuscript



CUP_13566_Figure 3.jpg

This article is protected by copyright. All rights reserved.

5' 1 2 3 4 5 6 7 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 3'

NTRK3-SCAPER

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

NTRK3

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31

SCAPER

Chr15q25.3:87,859,751-88,256,747

Chr15q24.3:76,347,904-76,905,335

NTRK3 Break-apart Probe

Benign

Tumor

3'-deletion
5'-amp

NTRK3-SCAPER Fusion Probe

Benign

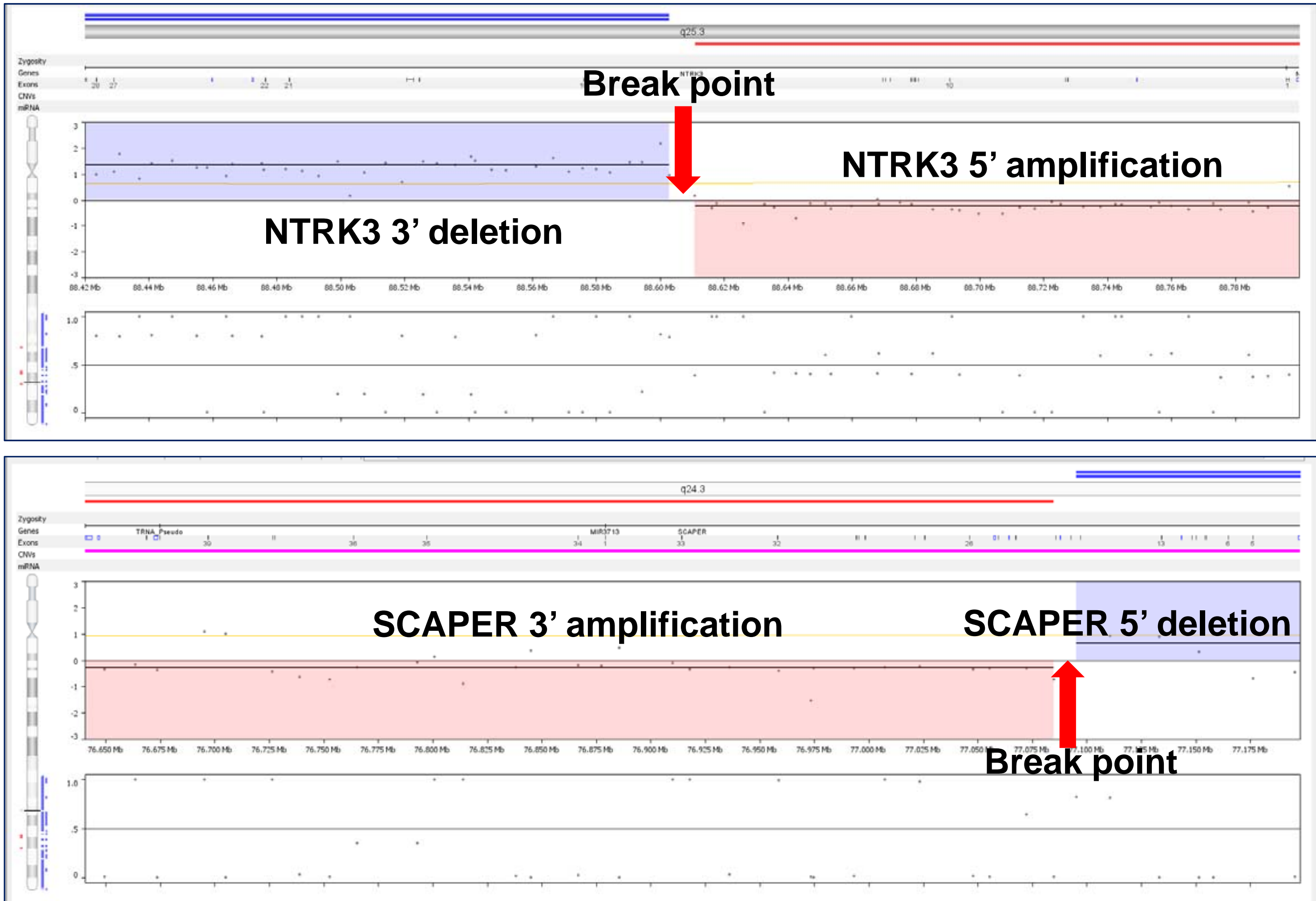
Tumor

NTRK3-SCAPER fusion

Author Manuscript

This article is protected by copyright. All rights reserved.

Author Manuscript



This article is protected by copyright. All rights reserved.

Author Manuscript

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

Ben J. Friedman, MD[1,2], Simon Hernandez, BS[3], Chelsea Fidai, MD[1], Angela Jiang, MD[1], Tor A. Shwayder, MD[1], Shannon Carskadon[4], Aleodor A. Andea, MD, MBA[5,6], Paul W. Harms, MD, PhD[5,6], Dhananjay Chitale MD[2], Nallasivam Palanisamy, PhD[4].

Author Affiliations:

1) Department of Dermatology, Henry Ford Health System, Detroit, MI 48202

2) Department of Pathology and Laboratory Medicine, Henry Ford Health System, Detroit, MI 48202

3) College of Medicine, State University of New York Upstate Medical University, 766 Irving Ave, Syracuse, NY 13210

4) Department of Urology, Vattikuti Urology Institute, Henry Ford Health System, Detroit, MI 48202

5) Department of Pathology, Michigan Medicine, University of Michigan, Ann Arbor MI 48109

6) Department of Dermatology, Michigan Medicine, University of Michigan, Ann Arbor MI 48109

Corresponding Author:

Ben J. Friedman

3031 West Grand Blvd

Suite 800

Detroit, MI 48202

P#- 313-916-2151

This article is protected by copyright. All rights reserved.

F#-888-959-0927

Email: bfriedm1@hfhs.org

Figure #: 5

Word Count: 1750

Acknowledgement: The authors would like to thank Dr. Phil Leboit for performing the PRKAR1A immunohistochemistry in this case.

Author Manuscript

This article is protected by copyright. All rights reserved.