Melanocytic neoplasms with prominent pigment synthesis mimicking equine melanoma and with histologic features overlapping those of epithelioid blue nevus (EBN) represent a rare variant of biologically indeterminate or low-grade malignant melanocytic tumors in which the molecular profile and exact histologic classification are not established. Tumors with these characteristics rarely occur as congenital lesions. We performed genomic analysis of a congenital pigment synthesizing melanocytic neoplasm with indeterminate biological potential.

CONCLUSIONS AND RELEVANCE To our knowledge, this is the first report of PRKCA fusions in melanocytic neoplasms. Future studies need to determine the frequency of PRKCA fusions in pigment-synthesizing melanocytic neoplasms.

There is considerable overlap between the histologic features of pigment-synthesizing melanocytic neoplasms and EBN, a rare variant of blue nevus that commonly occurs in the setting of the Carney complex, a familial lentigiosis and multiple endocrine, multiorgan low-grade neoplasia syndrome. Most cases of the Carney complex are caused by inactivating germline mutations in the protein kinase, cAMP-dependent, regulatory, type I, alpha (PRKAR1A) gene (NM_002737). Because of the histologic similarities and commonly indolent behavior, a unifying concept for EBN and pigment-synthesizing (animal-type) melanoma under the term pigmented epithelioid melanocytoma has been proposed, but whether the 2 entities are related at the genomic level has not been established.

Recently, kinase activation by gene fusions has been identified as a common oncogenic mechanism in spitzoid melanocytic tumors. For most EBNs of both Carney complex-associated and sporadic forms, however, loss of expression of the tumor suppressor gene PRKARIA is postulated to be involved in pathogenesis.

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We performed targeted mutational analysis and whole-transcriptome sequencing in a congenital heavily pigmented melanocytic tumor. We identified a novel protein kinase C-alpha (PRKCA) fusion that has not been previously reported in association with melanocytic neoplasms.

Report of a Case

A 5-month-old girl presented with a large scalp mass (Figure 1A). She had been delivered by cesarean section because of failure of descent due to the head mass. Over a period of 5 months, the mass grew rapidly and doubled in size. The family history was negative for melanoma, multiple neoplasia, or early-onset cancers. The mother was healthy and had no suspicious melanocytic lesions. Physical examination revealed a nontender protuberant mass on the vertex of the scalp. A computed tomographic scan of the head revealed a $5.3 \times 5.8 \times 3.6$-cm$^3$ hyperdense, heterogeneous mass with invasion of the underlying calvarium (Figure 1B). The patient underwent complete surgical excision of the mass (Figure 1C). Gross examination revealed a $6.0 \times 5.5 \times 3.8$-cm$^3$ mass with a dark-brown cut surface (Figure 1D).

Microscopic examination identified a heavily pigmented dermal infiltration of epithelioid melanocytes arranged in confluent nests and sheets abutting the epidermis (Figure 2A). The melanocytes percolated into the subcutaneous tissue without maturation. Abundant fine and dark-brown coarse deposits of melanin were present. The melanin-bleached preparations revealed the tumor cells with round nuclei, conspicuous nucleoli, and focally mild nuclear pleomorphism (Figure 2B). There was focal necrosis en masse of tumor cells. The mitotic rate was 1 to 2 per mm$^2$. There was no evidence of a preexisting melanocytic nevus. The histologic diagnosis was a severely atypical pigmented epithelioid cell melanocytic proliferation of indeterminate malignant potential. There was no clinical or radiographic evidence of distant metastases. Sentinel lymph node sampling was withheld. Close clinical follow-up revealed no evidence of recurrence 1 year after the diagnosis.

Array comparative genomic hybridization (aCGH) analysis was performed on the formalin-fixed paraffin-embedded (FFPE) tissue (see the eMethods in the Supplement). aCGH analysis showed losses in chromosomes 1p36.33-p35.3, 1q32.1-q44, and 17q11.1-24.2 (see the eFigure in the Supplement).

For mutational analysis, genomic DNA was extracted from FFPE tumor tissue according to the manufacturer’s protocol.
by using the Maxwell 16 FFPE Plus LEV DNA Purification Kit (Promega). Tumor DNA was screened for mutational hot spots for BRAF (exon 15), NRAS (exons 1 and 2), GNAQ (exons 4 and 5), GNA11 (exons 4 and 5), PRKARIA (exons 1A, 1B, 3, 4A, 4B, 5, 6, 8, 9, and 10), and the TERT promoter (HG19 coordinates, chr5: 1295151–1295347), as previously described.10 The tumor was negative for a hotspot mutation in BRAF, NRAS, GNAQ, GNA11, or the TERT promoter. Sequencing of PRKARIA showed the wild-type gene.

For RNA sequencing (RNA-seq) analysis, RNA from FFPE tumor tissue was extracted with the Maxwell 16 LEV RNA FFPE Kit (Promega) and quantitated by fluorescence dye by using the Quant-IT RNA assay (Life Technology). The RNA quality was evaluated by a 2100 Bioanalyzer (Agilent Technologies) with a Nano RNA 6000 Chip. RNA-seq libraries enriched for coding regions were prepared by using the Truseq RNA Access Library Prep Kit (Illumina) following the manufacturers protocol. Sequencing was performed on a HiSeq2000 to generate 100 base-paired end reads. A novel assembly-based method (CICERO) was used to detect structural variations. Details of the RNA-seq analysis are provided in the Methods in the Supplement. RNA-seq analysis revealed a single fusion transcript that was predicted to encode the intact catalytic domain of the serine/threonine PRKCA fused in frame with the N-terminal of ATP2B4 (Figure 3). When compared with samples of pediatric melanocytic tumors for which RNA-sequencing data were available (data not shown), this tumor had the highest PRKCA expression.
For reverse transcriptase–polymerase chain reaction (RT-PCR), total RNA was isolated from FFPE sections of the tumor and reverse-transcribed using the SuperScript VILO cDNA Synthesis Kit (Invitrogen) as per manufacturer’s protocol. The RT-PCR analysis was performed by using GoTaq Long PCR Master Mix (Promega) to amplify the ATP2B4-PRKCA fusion by using the following primers for 35 cycles: (forward: 5′-AGGAGATCACCAAGGATGCC-3′, reverse: 5′-TCCAAAACCT-CCTTTGCC-3′). The RT-PCR products were successfully amplified and processed for Sanger sequencing. RT-PCR and direct sequencing detected the fusion product (Figure 3 and Figure 4B).

For fluorescence in situ hybridization (FISH), bacterial artificial chromosome clones (BAC PAC Resources) were used to develop break-apart probes for PRKCA (CH17-384F08 and CH17-209D17) and ATP2B4 (RP11-813P3 and CH17-72A07). Dual-color FISH was performed as previously described. In addition, PRKCA FISH was performed on 8 atypical spitzoid melanocytic tumors, known from previous analysis to be negative for the known recurrent kinase fusions in spitzoid neoplasms.

Break-apart FISH showed PRKCA rearrangement with loss of one 5′ green signal, which suggested an unbalanced translocation. In addition, a subset of cells had 1 or 2 extra 3′ red signals (Figure 4A). Break-apart FISH for ATP2B4 was not successful even after multiple attempts because of background interference from heavy melanin pigmentation. FISH was negative for PRKCA rearrangement in the 8 kinase fusion-negative spitzoid tumors tested, suggesting that PRKCA fusions are not likely to occur frequently in spitzoid tumors.

To investigate whether the lesion was related to those EBNs described as being deficient for R1a (the protein product of PRKAR1A), we analyzed PRKAR1A expression in the patient sample. PRKAR1A was expressed at levels similar to those in control melanoma samples. Also, immunohistochemical analysis revealed that R1o expression was retained in tumor cells (see the Supplement).

Discussion

In this study, we show a novel PRKCA fusion with a potential pathogenic role in a pigment-producing melanocytic neoplasm. The fusion event likely leads to constitutive activation of the kinase domain of PRKCA by removing its autoinhibitory domain or by the promoter activity of a ubiquitously expressed gene, such as ATPase, Ca2+ transporting, plasma membrane 4 (ATP2B4). PRKCA, a serine-threonine kinase, is an isofrom of protein kinase C that is implicated in various cellular functions such as proliferation, apoptosis, differentiation, cell transformation, and adhesion. Note that the conserved PRKCA kinase domain induces phosphorylation of RAF-1, which in turn activates the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK/MAPK) cascade, a key signaling pathway in melanocyte growth and survival. Furthermore, PRKCA fusions have been identified as putative pathogenic events in other tumors, such as papillary glioneuronal tumor and lung squamous cell carcinoma.

In immunohistochemical studies, Zembowicz et al showed that most sporadic and Carney complex–associated EBNs lose expression of the PRKAR1A-encoded R1a protein. Tumors (including EBNs) in patients with the Carney complex commonly show loss of heterozygosity at the gene locus on chromosome 17q24.2, suggesting a double-hit model of tumorigenesis.

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investigated the status of PRKARIA and its protein in the patient’s sample. PRKARIA was expressed at normal levels, and immunohistochemical analysis revealed that its expression was retained in tumor cells. In addition, the mutational analysis showed no evidence of PRKARIA mutations. Therefore, our case does not seem to be associated with the R1α (PRKARIA)-deficient EBN type. Given the proximity of PRKARIA and PRKCA, it might be useful to analyze PRKCA as potential candidate for families with the Carney complex who are negative for inactivating germline PRKARIA mutations.

**Conclusions**

We show the presence of a PRKCA fusion in a congenital melanocytic neoplasm with indeterminate biological potential with prominent pigment synthesis. The frequency of this genetic alteration in heavily pigmented melanocytic neoplasms or other melanocytic tumors needs to be determined in future studies. The association of PRKCA fusion-positive melanocytic neoplasms with EBNs of both Carney complex-associated and sporadic types also remains to be determined.