COLD SPRING HARBOR
Molecular Case Studies

RESEARCH REPORT

Molecular insights into malignant progression of atypical choroid plexus papilloma

Maxim Yankelevich,[1,2,9] Jonathan L. Finlay,[3] Hamza Gorsi,[2] William Kupsky,[4] Daniel R. Boue,[5] Carl J. Koschmann,[6,7] Chandan Kumar-Sinha,[7,8] and Rajen Mody[6,7]

[1]Pediatric Hematology/Oncology Program, Rutgers Cancer Institute of New Jersey, New Brunswick, New Jersey 08901, USA; [2]Division of Pediatric Hematology/Oncology, Children's Hospital of Michigan and Wayne State University School of Medicine, Detroit, Michigan 48201, USA; [3]Division of Hematology, Oncology, and BMT, Nationwide Children's Hospital and The Ohio State University, College of Medicine, Columbus, Ohio 43205, USA; [4]Department of Pathology, Wayne State University School of Medicine, Detroit, Michigan 48201, USA; [5]Department of Pathology, Nationwide Children's Hospital and The Ohio State University, College of Medicine, Columbus, Ohio 43205, USA; [6]Department of Pediatrics, Michigan Medicine, [7]Rogel Cancer Center, [8]Michigan Center for Translational Pathology, Michigan Medicine, University of Michigan, Ann Arbor, Michigan 48109, USA

Abstract Choroid plexus tumors are rare pediatric neoplasms ranging from low-grade papillomas to overtly malignant carcinomas. They are commonly associated with Li–Fraumeni syndrome and germline *TP53* mutations. Choroid plexus carcinomas associated with Li–Fraumeni syndrome are less responsive to chemotherapy, and there is a need to avoid radiation therapy leading to poorer outcomes and survival. Malignant progression from choroid plexus papillomas to carcinomas is exceedingly rare with only a handful of cases reported, and the molecular mechanisms of this progression remain elusive. We report a case of malignant transformation of choroid plexus papilloma to carcinoma in a 7-yr-old male with a germline *TP53* mutation in which we present an analysis of molecular changes that might have led to the progression based on the next-generation genetic sequencing of both the original choroid plexus papilloma and the subsequent choroid plexus carcinoma. Chromosomal aneuploidy was significant in both lesions with mostly gains present in the papilloma and additional significant losses in the carcinoma. The chromosomal loss that occurred, in particular loss of Chromosome 13, resulted in the losses of two critical tumor suppressor genes, *RB1* and *BRCA2*, which might play a possible role in the observed malignant transformation.

Corresponding author:
my414@cinj.rutgers.edu

Ontology terms: choroid plexus papilloma; neoplasm of the nervous system

Published by Cold Spring Harbor Laboratory Press

doi:10.1101/mcs.a005272

INTRODUCTION

Choroid plexus tumors are rare central nervous system (CNS) neoplasms with varying malignant potential ranging from low-grade papillomas to overtly malignant carcinomas. Atypical choroid plexus papilloma (CPP) is an intermediate lesion characterized by increased mitotic rate but still clearly distinguishable from carcinoma (Safaee et al. 2013). The Li–Fraumeni syndrome, associated with germline mutations of the *TP53* tumor suppressor gene, is known to increase the risk of choroid plexus tumors (Tabori et al. 2010; Orr et al. 2020). Malignant

[9]Present address: Pediatric and Young Adult Hematology/Oncology, Beaumont Health, Royal Oak, Michigan, USA

transformation of choroid plexus papillomas is extremely rare with less than a handful of cases reported (Chow et al. 1999; Jeibmann et al. 2007; Ruggeri et al. 2018). The most comprehensive retrospective report on transformation of papilloma to carcinoma followed 124 children with CPP, 12 of whom developed recurrences with two of 124 (1.6%) cases of confirmed transformation (Jeibmann et al. 2007). Because of the uncommon nature of these cases, the molecular mechanisms that trigger this malignant progression remain elusive. Here we report a child with histologically confirmed progression of papilloma into carcinoma 7 yr after initial presentation for whom we were able to perform comprehensive molecular sequencing analyses of both tumor samples from initial diagnosis of papilloma and ultimate transformation to carcinoma.

RESULTS

Clinical Presentation

The patient is a Caucasian male, 9 yr old at the time of this report. He had a history of poor weight gain, fussiness, and macrocephaly since birth and magnetic resonance imaging (MRI) at 3 wk of age demonstrated a large lobulated and hypervascular mass in the left cerebral hemisphere causing mass effect and midline shift. The patient underwent staged gross total resection of the mass that required three separate procedures. Pathology was diagnostic of atypical choroid plexus papilloma based on the increased mitotic activity. Despite surgical removal of a significant part of the left hemisphere, the patient had only mild right-sided hemiparesis and was developing well, showing no developmental delays and good school performance.

During his scheduled interval brain MRI evaluation 7 yr and 2 mo after the original surgery the patient was found to have a 2-cm nodular recurrent mass at the resection cavity (Fig. 1) and underwent an uncomplicated gross total resection with pathology demonstrating clear progression to a higher grade choroid plexus carcinoma (Fig. 2), with pathology showing highly cellular tumor invading brain parenchyma with a high nuclear to cytoplasmic ratio, marked nuclear pleomorphism, and high mitotic activity (up to eight mitoses per high power field compared to less than one in the original sample). Staining with Ki-67 labeled at least

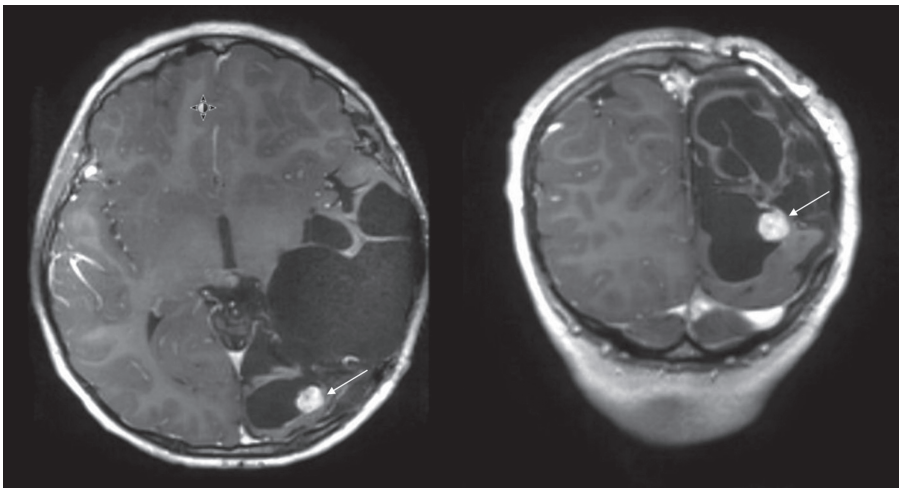

Figure 1. Recurrent mass in the resection cavity found on a routine surveillance MRI 7 yr and 2 mo after initial surgery.

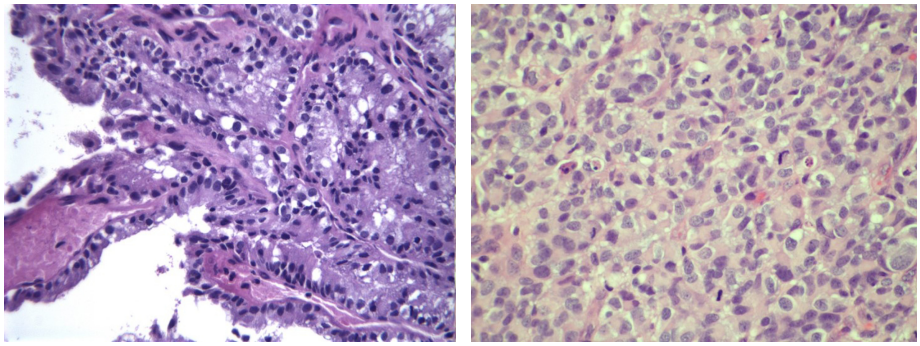

Figure 2. (*Left*) Atypical CPP, 2009, hematoxylin and eosin (H&E), 400×, tumor cells in a papillary configuration; (*right*) CPC, 2017, H&E, 400×, patternless sheets of densely packed cells with atypia and high mitotic activity.

40%–50% of tumor cell nuclei compared to 20%–30% in the original lower grade tumor. The papillary architecture noted in the original papilloma specimen was not evident at this time. The p53 immunostain was positive in nearly 100% of the tumor cells. The original and recurrent pathology specimens were reviewed at both Children's Hospital of Michigan and Nationwide Children's Hospital, Columbus and the progression from atypical CPP into choroid plexus carcinoma (CPC) was confirmed by two independent pathologists.

The patient's initial germline *TP53* gene polymerase chain reaction (PCR)-based sequencing at an outside genetics laboratory that was done during initial diagnosis and treatment planning for the recurrent tumor was reported to be negative for *TP53* mutations; however, the subsequent repeat test by the same laboratory and at the University of Michigan did demonstrate *TP53* germline mutation. The patient received chemotherapy according to a modified "HeadStart II" protocol including three cycles of induction with high-dose methotrexate, vincristine, cyclophosphamide, etoposide, and cisplatin, followed by three cycles of tandem high-dose thiotepa and carboplatin with autologous hematopoietic cell support (Chi et al. 2004; Cohen et al. 2015).

Molecular Studies

During his recurrence treatment, the patient was enrolled on a clinical integrative sequencing study consisting of targeted 1711 gene panel and tumor whole transcriptome (RNA-seq) along with matched germline DNA sequencing, performed on the patient's original papilloma, recurrent carcinoma and the patient's blood DNA samples using the PEDS-MI-Oncoseq clinical sequencing protocol (Mody et al. 2015). Chromosomal copy-number alterations were evaluated as well by analyzing the distribution of the sequencing reads from the patient's sample aligned to the reference genome. The sequencing analysis revealed a pathogenic germline variant of *TP53 p.R248W* with loss of heterozygosity (LOH) by uniparental disomy (UPD) in the tumor in both the papilloma and the carcinoma samples. As noted above, the *TP53* variant was not detected in the initial test; interestingly the results at the University of Michigan showed a variant allele frequency of 39% in the germline sample, suggesting a possible mosaicism state. No notable gene expression outliers were detected by RNA-seq. Analysis of copy-number alterations in the two samples identified extensive aneuploidy and similar pattern of gains in Chromosomes 7, 8, 12, 20, 21, and X, and uniparental disomy of Chromosomes 2, 3, 4, 6, 9, 10, 11, 14, 16, 17, and 18 in both the samples. Striking copy-number aberrations newly acquired in the carcinoma included copy gain of Chromosomes 5p, 12, 15q, and 20, and copy loss of Chromosomes 5q, 13, and 22 that were not present in the papilloma sample (Fig. 3). In terms of somatic mutations, the original

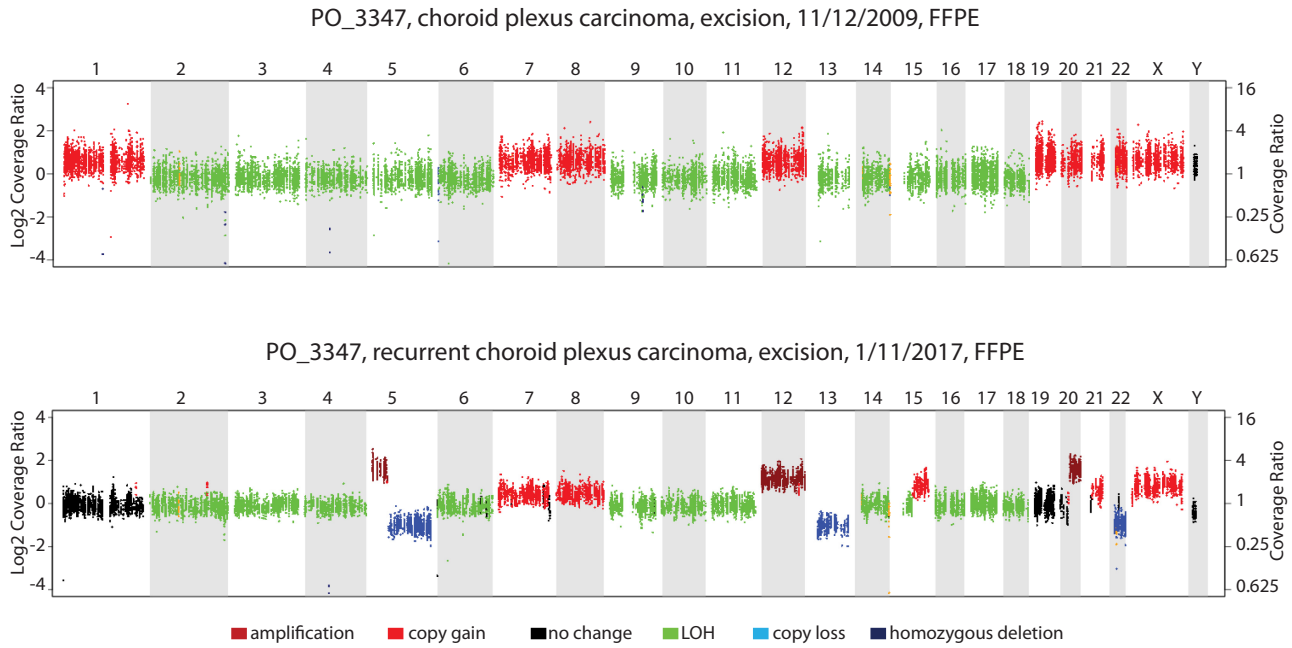

Figure 3. Copy-number profile of the choroid plexus carcinoma sample, primary excision on 11/12/2009 (*top*) and that of the recurrent CPC sample excised on 1/11/2017 (*bottom*). The color code corresponding to different aberrations is indicated below. (FFPE) Formalin-fixed, paraffin-embedded, (LOH) loss of heterozygosity.

papilloma was found to harbor three missense mutations with low allelic fractions (4%–21%), including *HDAC9 p.R248Q*, and two different *NCOR1* mutations, *p.G5V* and *p.Y20S*; notably, these were not detected in the subsequent carcinoma, which was found to harbor a subclonal somatic indel *NCOA3 p.Q1263*dup insertion with an allelic fraction of 6% (Table 1).

Table 1. List of mutations in atypical choroid plexus papilloma (aCPP) and choroid plexus carcinoma (CPC) samples

Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect	dbSNP/ dbVar ID	Genotype	Tumor allelic fraction
aCPP, 2009 sample								
NCOR1	17, somatic	NM_006311.3: c.14G > T	NP_006302.2: p. Gly5Val	Single nucleotide	Missense	rs76145228	LOH by UPD	21%
NCOR1	17, somatic	NM_006311.3: c.59A > C	NP_006302.2: p. Tyr20Ser	Single nucleotide	Missense	rs73281920	LOH by UPD	14%
HDAC9	7, somatic	NM_178425.3: c.743G > A	NP_848512.1: p. Arg248Gln	Single nucleotide	Missense	rs759089852	Heterozygous	4%
TP53	17, germline	NM_000546.6: c.742C > T	NP_000537.3: p. Arg248Trp	Single nucleotide	Missense	rs121912651	LOH by UPD	
CPC, 2017 sample								
NCOA3	20, somatic	NM_181659.2: c.3788_3789ins ACA	NP_858045.1: p.Gln1263	Indel	Frameshift	rs753491875	Heterozygous	6%
TP53	17, germline	NM_000546.6: c.742C > T	NP_000537.3: p. Arg248Trp	Single nucleotide	Missense	rs121912651	LOH by UPD	

(LOH) Loss of heterozygosity; (UPD) uniparental disomy.

Treatment Outcome

The patient was in uneventful remission for two and a half years off therapy, but at 2 yr and 8 mo off therapy he presented with worsening cytopenias and was found to have acute myeloid leukemia (AML). His AML cytogenetics revealed trisomy 21, deletion 5q, monosomy 7, deletion 9q, gain 5p consistent with therapy-related myelodysplastic syndrome/AML.

DISCUSSION

The common molecular defect in the patient's papilloma and carcinoma tumors was *R248W* mutation of the *TP53* gene. This particular mutation is one of the most common *TP53* alterations in choroid plexus tumors and is associated with the loss of an apoptotic role of *TP53* as well as a gain of tumorigenic function (Willis et al. 2004; Song et al. 2007; Thompson and Compton 2010).

Although some of the other genes found to be altered by somatic mutations in both the patient's papilloma and subsequent carcinoma samples are known to play a role in epigenetic regulation of gene expression (Gil et al. 2016), most of the specific mutations detected here represent nonrecurrent mutations of unknown significance, and their allelic fractions were low and unlikely responsible for the observed malignant transformation (Table 1).

More pertinently, chromosomal aneuploidy was significant in both lesions with mostly gains present in the papilloma and additional significant losses in the carcinoma. It has been shown by Thomas et al. (2016) that for supratentorial choroid plexus tumors in young patients, mainly losses in DNA copy-number alterations were prevalent and thus potentially relevant to CPC oncogenesis in this group of patients (Thomas et al. 2016). Aneuploidy is typically a result of chromosomal instability phenotype (CIN) (Sansregret et al. 2018). A functional *TP53* pathway has been shown to prevent CIN and aneuploidy via accumulation of nuclear *TP53* during chromosome missegregation and subsequent elimination of aneuploid cells (Thompson and Compton 2010). The chromosomal loss that occurred—in particular loss of Chromosome 13—resulted in the losses of two critical tumor suppressor genes, *RB1* and *BRCA2*, which might play a possible role in the observed malignant transformation in our case (Dick and Rubin 2013; Zámborszky et al. 2017).

Although we cannot entirely rule out a possibility that the CPC did not arise from the CPP and these represent two distinct tumors, development of two distinct rare tumors in the same location is very unlikely. Also, the pattern of chromosomal number alterations has striking similarities (Fig. 3) and is suggestive of the common origin.

Consistent with the observations in this case, Li–Fraumeni syndrome (LFS)-associated choroid plexus tumors (LFS-CPC) have been noted to harbor a significantly higher burden of chromosomal structural variations as well as significant risk of progression (Tabori et al. 2010). Furthermore, LFS-CPC patients were found to display marginally detrimental response to radiation therapy as compared to those with wild-type *TP53* (Bahar et al. 2015). Consistent with this observation, a case report of a 3-yr-old girl with LFS-CPC described a complete remission upon treatment with surgery and chemotherapy (without radiation therapy) (McEvoy et al. 2017). Similarly, the "Head Start" Consortium experience suggested that with intensive myeloablative-chemotherapy containing regimens, the prognosis of TP53 mutant CPC may be improved in the absence of radiation therapy (Zaky et al. 2015). It is worth noting that both OncoSeq and repeat *TP53* testing results that confirmed *TP53* mutation were received when the patient was undergoing therapy and did not influence our decisions over therapy choices. Our patient was treated using an etoposide-containing regimen, and it has been shown that *BRCA*-deficient cancers are sensitive to topoisomerase II inhibitors (Treszezamsky et al. 2007); however, the risk of secondary malignancies after etoposide should not be underestimated in patients with germline *TP53* mutation as demonstrated

by our patient's case. Chemotherapy agents used to treat CPCs including etoposide, alkylating, and platinum-based drugs have an increased risk of secondary cancers and their use versus avoidance should always be carefully considered in children with LFS-associated CPCs who carry predisposition to multiple primary cancers and increased sensitivity to radiation and chemotherapy induced carcinogenesis.

METHOD

The patient was consented and enrolled on a prospective, institutional review board–approved (HUM00056496), integrative clinical sequencing trial (PEDS-MI-OncoSeq), in which patient samples undergo paired tumor/normal DNA sequencing, tumor RNA sequencing, and bioinformatics analyses, details of which have been previously described (Mody et al. 2015).

ADDITIONAL INFORMATION

Database and Deposition

Coding variants identified in the PEDS-MI-OncoSeq analysis are reported in Table 1. The patient did not provide consent for public deposition of all raw sequencing data for all genes included in the assay. The variants were submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession number VCV000012347.13.

Ethics Statement

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The patient's parents provided informed consent to participate in PEDS-MI-OncoSeq and received mandatory preenrollment genetic counseling in which no family history of cancer was noted. In addition, written informed consent was obtained from the patient for publication of this case report and any accompanying images.

Acknowledgments

We acknowledge the patient and his family.

Author Contributions

M.Y., J.L.F., and H.G. were responsible for patient care and clinical decision-making. M.Y., J.L.F., C.J.K., and R.M. were responsible for the concept and design. W.K. and D.R.B. provided pathologic diagnosis and figures. C.J.K., C.K.-S., M.Y., and R.M. obtained, analyzed, and interpreted molecular data. M.Y. wrote the manuscript. All authors performed a final review of the manuscript.

Funding

We acknowledge the support by the National Institutes of Health (NIH) Clinical Sequencing Exploratory Research (CSER) Award NIH 1UMIH006508. We acknowledge the Om Foundation for the support of this publication (M.Y.). R.M. is a Hyundai Hope on Wheels Scholar. None of the sponsors played a role in the design; data collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

Competing Interest Statement

The authors have declared no competing interest.

Received February 12, 2020; accepted in revised form November 16, 2020.

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