Molecular Characterization of Choroid Plexus Tumors Reveals Novel Clinically Relevant Subgroups

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Abstract

Purpose: To investigate molecular alterations in choroid plexus tumors (CPT) using a genome-wide high-throughput approach to identify diagnostic and prognostic signatures that will refine tumor stratification and guide therapeutic options.

Experimental Design: One hundred CPTs were obtained from a multi-institutional tissue and clinical database. Copy-number (CN), DNA methylation, and gene expression signatures were assessed for 74, 36, and 40 samples, respectively. Molecular subgroups were correlated with clinical parameters and outcomes.

Results: Unique molecular signatures distinguished choroid plexus carcinomas (CPC) from choroid plexus papillomas (CPP) and atypical choroid plexus papillomas (aCPP); however, no significantly distinct molecular alterations between CPPs and aCPPs were observed. Allele-specific CN analysis of CPCs revealed two novel subgroups according to DNA content: hypodiploid and hyperdiploid CPCs. Hyperdiploid CPCs exhibited recurrent acquired uniparental disomy events. Somatic mutations in TP53 were observed in 60% of CPCs. Investigating the number of mutated copies of p53 per sample revealed a high-risk group of patients with CPC carrying two copies of mutant p53, who exhibited poor 5-year event-free (EFS) and overall survival (OS) compared with patients with CPC carrying one copy of mutant p53 (OS: 14.3%, 95% confidence interval, 0.71%–46.5% vs. 66.7%, 28.2%–87.8%, respectively, P = 0.04; EFS: 0% vs. 44.4%, 13.6%–71.9%, respectively, P = 0.03). CPPs and aCPPs exhibited favorable survival.

Discussion: Our data demonstrate that differences in CN, gene expression, and DNA methylation signatures distinguish CPCs from CPPs and aCPPs; however, molecular similarities among the papillomas suggest that these two histologic subgroups are indeed a single molecular entity. A greater number of copies of mutated TP53 were significantly associated to increased tumor aggressiveness and a worse survival outcome in CPCs. Collectively, these findings will facilitate stratified approaches to the clinical management of CPTs. Clin Cancer Res; 21(1); 184–192. ©2014 AACR.
Genomic Profiling of Choroid Plexus Tumors

Translational Relevance
This report is the first to dissect the aberrant complexity in copy number, methylation, and gene expression of one of the largest cohorts of pediatric choroid plexus tumors (CPT). Our findings revealed molecular homogeneity among choroid plexus papillomas (CPP) and atypical choroid plexus papillomas (aCPP), reflecting the favorable survival of these patients and suggesting these histologically distinct subgroups are single-tumor entities. Choroid plexus carcinomas (CPC) were significantly different from CPPs and aCPPs. Moreover, CPCs exhibited molecular heterogeneity, and patient outcomes varied widely. We identified novel CPC subgroups with significantly distinct copy-number signatures, suggesting different mechanisms drive CPC development. We identified that patient overall and event-free survival significantly decreased with an increasing number of mutated copies of TP53. By defining the molecular landscape of CPTs, this study has provided a comprehensive molecular background on which to explore mechanisms of tumorigenesis and develop stratified approaches to the clinical management of CPTs.

Cytogenetic studies of central nervous system (CNS) tumors have revealed high chromosomal instability in more than 90% of CPTs analyzed (Supplementary Table S1). Defining the molecular landscape of CPTs and identifying actionable molecular aberrations have been challenging due in part to the limited number of patients and high-quality samples available for genome-wide studies. Here, we use an integrative molecular approach to characterize the genomic, transcriptomic, and epigenomic landscape of the largest cohort of CPTs to date. The information derived from these analyses creates a molecular foundation on which to develop approaches to improve the clinical management of this devastating disease.

Materials and Methods

Patients and sample preparation
CPT samples and/or clinical data were collected from institutions in Canada, the United States of America, Brazil, Israel, and Germany (see Supplementary Appendix) in accordance with each institution’s Research Ethics Board. Informed consent was obtained from the parents/legal guardians of all patients. We studied 100 unique tumor samples (58 CPP, 30 CPP, and 12 aCPP) from 91 pediatric patients (ages 0.03–16.50 years old) for which TP53 sequence data were available (Supplementary Table S2). Pathologic review of CPTs was conducted by C. Hawkins, D. W. Ellison, and B.T. Harris when samples were available. In all other institutions, expert neuropathologists critically examined each case. In 15 CPC cases, immunohistochemical analysis of hHFS/INI1 was conducted and revealed immunopositivity excluding the diagnosis of atypical teratoid/rhabdoid tumors.

Nucleic acids were derived from fresh-frozen (n = 75), optimal cutting temperature compound (n = 9), and formalin-fixed paraffin-embedded (FFPE; n = 12) samples. We received isolated tumor DNA from 4 samples. Twenty-two nucleic acid samples exhibited suboptimal quality and/or quantity, leaving 78 high-quality samples from 73 patients for analysis (Supplementary Fig. S1). Detailed clinical data were obtained for 68 patients. Tumor DNA was extracted using standard phenol-chloroform extraction from fresh-frozen samples, and the RecoverAll Total Nucleic Acid Isolation Kit for FFPE (Ambion) from FFPE samples. Total RNA was isolated from fresh-frozen samples using the TRIzol method (Invitrogen) according to the manufacturer’s instructions.

TP53 sequencing
Sequencing of the coding region of TP53 (exons 2–11) was performed in the molecular diagnostic laboratory at The Hospital for Sick Children (Toronto) by direct Sanger sequencing of whole genome DNA as previously described (5).

Microarray processing and bioinformatics analysis
Fifty RNA samples were hybridized to GeneChip Human Exon 1.0ST gene expression microarrays (Affymetrix), and 36 DNA samples were hybridized to Illumina 450 K Infinium methylation bead arrays (Illumina) as per manufacturer’s instructions. An initial set of 55 tumor DNA samples was hybridized to Genome-Wide Human SNP Array 6.0 (Affymetrix), whereas an independent set of 20 tumor DNA samples was hybridized to Affymetrix OncoScan FFPE Express 2.0 arrays. One technical replicate was included in both genotyping platforms and analyzed for copy-number call consistency. One FFPE-derived sample was removed from further copy number analysis due to poor microarray quality.

Partek Genomics Suite 6.5 (PGS; Partek Inc.) and BioDiscovery Nexus Copy Number software (Discovery Edition 7.0; BioDiscovery) were used for copy-number analysis as previously described (5; see Supplementary Appendix). Copy-number changes encompassing more than 75% of the chromosome were called whole-chromosome aberrations. Allele-specific copy number analysis of tumors (ASCAT) was performed in R as previously described (10) and verified by Nexus software. Tumor ploidy, where hypodiploid <1.90 and hyperdiploid >2.10, heterogeneity, and allelic imbalances were inferred from the output. ASCAT failed to resolve the ploidy of two samples with very low aberrant fraction, so these were excluded from further analysis.

Clustering of gene expression and methylation data was investigated in R (version 3.0.1) by unsupervised hierarchical clustering (uHCL), non-negative matrix factorization (NMF), and PCLLIST algorithms. Differential gene expression analysis was conducted with PGS 6.5 (see Supplementary Appendix). Gene set enrichment analysis (GSEA) was performed as previously described (11), and its visualization was obtained by Cytoscape and Enrichment Map using an in-house–curated database containing freely available NCI, Kyoto Encyclopedia of Genes and Genomes (KEGG), Protein families database (PFPAM), Biocarta, and GO databases as described in Witt and colleagues (12). Differences in DNA methylation status were analyzed with the Illumina GenomeStudio software (see Supplementary Appendix).

All probe sets were annotated according to the human genome build hg19 (GRCh37). Microarray data can be accessed from GEO GSE60886.

Statistical analysis

Statistical analyses of copy number and gene expression were performed in PGS 6.5, whereas methylation was analyzed in Genome Studio. Patient survival was calculated in StataSE (version 12), whereas other statistical analyses were conducted in R (version 3.0.1; see Supplementary Appendix). Survival estimates for tumor subgroups, and for CPCs by TP53 and ploidy status were generated using the Kaplan–Meier method, and curves were compared using a log-rank test. Overall survival (OS) measured time from initial diagnosis to death from any cause or last follow-up.
up as of December 1, 2013. Event-free survival (EFS) measured time from initial diagnosis to tumor progression, recurrence, or death from any cause.

**Results**

Genomic, transcriptomic, and DNA methylation profiling of CPTs reveals significant segregation of CPCs from CPPs and aCPPs

Unsupervised clustering analyses performed with gene expression and methylation data revealed clear segregation of CPCs from CPPs and aCPPs (Fig. 1). NMF analysis of gene expression (Fig. 1A) and methylation (Fig. 1B) data demonstrated greatest from CPPs and aCPPs (Fig. 1). NMF analysis of gene expression and methylation data revealed clear segregation of CPCs from CPPs and aCPPs. This significant molecular stratification was also observed using a smaller number of probe sets for gene expression and methylation differences analyzed by PVCLUST (Supplementary Fig. S2). The concordance between tumors stratified by gene expression and methylation was significant (Rand index = 0.73, \( P < 1.0 \times 10^{-4} \)) and revealed consistent molecular segregation of CPTs into unique molecular subgroups.

Although copy-number analysis revealed widespread chromosomal instability in all tumor subgroups (Fig. 2), a distinct signature characterized by increased frequency of chromosome-wide gains and losses was observed in CPCs (average 5.31 chromosomes gained and 6.17 lost per CPC), compared with very few losses in CPPs and aCPPs (average 0.32 lost per CPP, and 0.64 vs. 0.09 per acPP; Fig. 2). The frequency of chromosome-wide losses in CPCs was significantly greater than in CPPs and aCPPs (Mann-Whitney test, \( P < 1.00 \times 10^{-4} \)). Allele-specific copy-number analysis allowed us to investigate the allelic ratios in our samples. This technique revealed a striking pattern of copy-number–neutral LOH. This phenomenon is commonly observed in cancer cells and may also be referred to as acquired uniparental disomy (aUPD), wherein a chromosome pair is homozygous, thus having two copies of the same allele (13). CPCs exhibited frequent aUPD events with an average of 2.31 aUPD events per sample, whereas the phenomenon occurred less frequently in CPPs and aCPPs (average 0.32 and 1 events per sample, respectively).

Analysis of clinical variables between the three histologic subgroups revealed no significant difference of age at diagnosis (Kruskal–Wallis test, \( P = 0.30 \) or ratio of males to females (two-way ANOVA, \( P = 0.26 \); Supplementary Table S2). Survival outcomes for CPCs were significantly worse than for CPPs and aCPPs. Five-year OS for CPCs was 56.3% [95% confidence interval (CI), 36.5%–76.0%], compared with 92.9% (59.1%–99.0%) and 100% for CPPs and aCPPs, respectively (\( P = 0.03 \); Fig. 3B). Only one patient with CPP died due to complications from a concurrent diagnosis of ependymoma. Five-year EFS for CPCs was 39.7% (95% CI, 22.8%–56.2%), compared with 87.4% (58.1%–91.9%) and 70.0% (22.5%–91.8%) for CPPs and aCPPs, respectively (\( P = 4.90 \times 10^{-7} \); Fig. 3C).

CPCs and aCPPs share similar molecular signatures, which correlate with favorable survival outcomes

Analyzing CPPs and aCPPs independently from CPCs revealed a striking molecular similarity between the papilloma subgroups (Fig. 4). Unsupervised clustering analysis demonstrated that CPPs and aCPP did not segregate according to differences in gene

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**Figure 1.** Unsupervised clustering of (A) gene expression normalized intensities and (B) methylation beta values by NMF demonstrates significant segregation of CPCs (red) from CPPs (yellow) and aCPPs (light blue). No segregation was observed between CPPs and aCPPs. NMF was conducted using 5,000 probe sets with the largest median absolute deviation. This clustering algorithm identified the most significant measures of similarity (cophenetic coefficient) when the data were at \( k = 2 \) (clusters). In the matrix, red represents the highest measure of similarity (0), whereas blue/purple represents the lowest measure of similarity (0). Any other colors within the matrix represent a spectrum of changing measures of similarity, from red to blue/purple. Colors: TP53 mutation status: black, TP53 mutant; white, TP53 wild type.
expression or methylation (Fig. 4A and B). Supervised analysis using the Wilcoxon rank-sum test between CPPs and aCPPs revealed no significant differences in gene expression or methylation (Fig. 4C and D). In addition, signatures of chromosomal instability characterized by recurrent chromosome-wide gains and very few losses were observed in both CPPs and aCPPs; no significant alterations per subgroup and the chromosome-wide copy-number changes were calculated using the frequency of chromosome-wide gains and losses were observed (Mann-Whitney test, P = 0.32 and P = 0.49, respectively; Fig. 4E). There were no differences in age at diagnosis (Mann-Whitney test, P = 0.45) or ratio of males to females (Fisher exact test, P = 0.31) between CPPs and aCPPs. Moreover, survival outcomes for patients with CPP and aCPP were not significantly different (log-rank test, OS P = 0.51, EFS P = 0.30).

Ploidy analysis reveals novel CPC subgroups with unique molecular alterations

Ploidy analysis revealed the presence of aneuploidy in 89% of tumors (Supplementary Fig. S3). CPPs and aCPPs exhibited ploidy greater than 2 (hyperdiploidy); however, CPPs exhibited a wide distribution of ploidy values, with two significantly distinct subgroups observed: hyperdiploid CPPs (average ploidy 2.76; range, 2.21–3.34) and hypodiploid CPPs (average ploidy 1.45; range, 1.25–1.71; Mann–Whitney test, P < 1.00 × 10⁻⁴). Only three of 35 CPPs were diploid. Hypodiploid CPPs exhibited recurrent chromosome-wide losses and very few gains with an average of 12.71 chromosomes lost and 0.06 gained per tumor. Chromosome 3 was lost in all hypodiploid CPPs, with loss of chromosomes 6, 22, 11, and 16 observed in more than 80% of tumors (Fig. 3A). Hyperdiploid CPPs exhibited a high frequency of chromosome-wide gains and almost no losses (average 11.33 and 0.33 chromosomes, respectively). Chromosomes 12, 7, 20, and 1 were gained in more than 80% of hyperdiploid CPPs (Fig. 3A). In addition to a high frequency of chromosomal gains, hyperdiploid CPPs also exhibited aUPD more frequently than hypodiploid CPPs (average of 4.93 affected chromosomes per tumor compared with 0.35 chromosomes in hypodiploid CPPs; Fisher exact test, P < 0.0001; Fig. 3A). Moreover, significant enrichment in aUPD was observed in TP53 mutant hyperdiploid CPPs compared with hyperdiploid CPPs with wild-type TP53 (Fisher exact test, P < 0.0001). aUPD was most frequently observed in chromosome 17, affecting 29% (10/35) of CPPs.

We conducted GSEA to identify biologic pathways and processes that are differentially expressed between hypodiploid and hyperdiploid CPPs. GSEA revealed enrichment in RNA processing, DNA replication and repair, and chromosome segregation in hyperdiploid CPPs. Hypodiploid CPPs exhibited enrichment in cellular metabolism, signaling, and cell migration pathways, as well as leukocyte activation and proliferation (5% FDR, P < 0.05; Supplementary Fig. S4). The patterns of enrichment observed suggest hypodiploids are more proliferative than hypodiploid CPPs, and that the latter tumors are undergoing a significant immune response. A greater understanding of these distinct enrichment patterns will elucidate the mechanisms underlying the progression of these molecularly distinct CPC subgroups (Supplementary Fig. S4). There were no significant differences in DNA methylation between subgroups, although this may be due to the low number of samples in the comparison groups (hypodiploid, n = 4; hyperdiploid, n = 9).

Increased number of copies of mutant TP53 is associated with tumor aggressiveness and unfavorable survival outcomes

Mutations in TP53 were assessed in our cohort by Sanger sequencing. Sixty percent of CPPs (35/58) were mutant for TP53. Fifteen (15/58, 26%) of these samples belonged to 12 patients with LFS carrying a germline mutation in TP53. Mutations in TP53 were observed in both hypodiploid and hyperdiploid CPPs; however, in our cohort, the frequency of TP53 mutations was significantly greater in hypodiploid CPPs (15/17, 88%) than hyperdiploid CPPs (7/15, 47%; Fisher exact test, P = 0.02). Diploid CPPs (n = 3) were TP53 wild type (Supplementary Table S3). No significant enrichment for patients with LFS was observed in either hypo- or hyperdiploid subgroups.
Unsupervised clustering using gene expression and methylation data segregated CPCs into two significantly distinct clusters (P = 0.05; Fig. 5A and B). Although CPCs did not segregate according to ploidy status, we observed two clusters, which were significantly distinct according to TP53 status using DNA methylation data (Fisher exact test, P = 0.007), but did not reach significance using gene expression data (Fisher exact test, P = 0.089). LFS CPCs did not segregate from the spontaneous CPCs, suggesting no unique aberrations were present in tumors arising from patients with an inherited TP53 mutation. An in-depth analysis of the type of TP53 mutations revealed that a few samples, which appeared to be misclassified by unsupervised clustering, had an uncharacterized intronic alteration (c.28-28G>A/wt) and mutations outside the DNA-binding domain (i.e., c.290T>A in the SH3-like/Proline-rich domain), which may account for differences in the transcriptomic and epigenomic signature of these samples (Fig. 5).

Combining TP53 sequencing results with allele-specific copy-number status of chromosome 17 in 35 CPCs, we estimated the number of mutated copies of TP53. We found that 34.3% of CPCs (12/35) had 2 copies of mutant p53, 28.6% (10/35) had 1 copy of mutant p53, and 37.1% (13/35) had zero copies of mutant p53 (wild type). CPCs with 2 mutant copies of p53 exhibited a homozygous TP53 mutation status in all but one tumor sample with a low aberrant cell fraction (46%), suggesting this sample was largely contaminated with normal cells. Seventy-five percent of samples with 2 copies of mutated p53 (9/12) exhibited aUPD in chromosome 17. Eighty-three percent of CPCs with 2 copies of mutated p53 (10/12) had missense mutations in the DNA-binding domain, whereas 1 sample had a missense mutation in the SH3-like/Proline-rich domain and the other sample, a splicing mutation. CPCs with 1 copy of mutated p53 had missense mutations in the DNA binding (9/10) and tetramerization (1/10) domains, and carried a single copy of chromosome 17, exhibiting LOH of the entire chromosome. Three samples exhibited a heterozygous TP53 mutation status by sequencing, which may be a result of normal cell contamination. Gene expression and methylation analyses revealed no significant differences among CPCs carrying 1 or 2 mutated copies of p53 because of the limited sample sizes (gene expression: 3 and 6 samples, respectively; methylation: 1 and 6 samples, respectively).

Examining clinical variables among CPCs revealed no differences in the age of diagnosis (Mann–Whitney t test, P = 0.80 and P = 0.59) or the ratio of males to females (Fisher exact test, P = 1.0 and P = 1.0) according to ploidy nor p53 status, respectively (Supplementary Table S2). No significant differences were seen in the OS or EFS estimates of patients with CPT by diagnosis. Statistical values were obtained with the log-rank (Mantel–Cox) test.
differences in OS or EFS estimates were observed between patients with CPC exhibiting a hyper- or hypodiploid tumor genome \((P = 0.82, P = 0.94, \text{respectively}; \text{Supplementary Fig. S5A and S5B})\). TP53 status had a significant effect on the OS of our CPC cohort (log-rank test, \(P = 3.8 \times 10^{-5}\)), however, EFS was not significantly different between TP53 mutant and wild-type CPCs \((P = 0.07; \text{Fig. 5C and D})\). Investigating survival differences according to the number of mutant copies of TP53 in CPCs revealed a significant reduction in OS (log-rank test 2 copies vs. 1 copy, \(P = 0.04\); 2 copies vs. 0 copies, \(P < 1.0 \times 10^{-4}\)), and EFS (log-rank test 2 copies vs. 1 copy \(P = 0.03\); 2 copies vs. 0 copies \(P = 0.003\)) in patients harboring a greater number of mutant TP53 copies. The estimated OS of patients with CPCs harboring wild-type TP53 (zero copies) was 88.9\% (95\% CI, 43.3\%–98.4\%) and EFS was 66.5\% (32.9\%–86.1\%). Patients with CPCs harboring a single copy of mutant TP53 exhibited an estimated OS of 66.7\% (28.2\%–87.8\%) and EFS of 44.4\% (13.6\%–71.9\%), whereas patients with CPCs harboring two copies of mutant TP53 showed an OS of 14.3\% (0.71\%–46.5\%) and EFS of 0\% (Fig. 6).

Discussion

Our study is the first and largest comprehensive investigation of the molecular alterations found in CPTs, demonstrating that the molecular profile of CPCs is significantly distinct from that of...
CPCs and aCPPs, and that the papillomas are not significantly distinct from each other. In addition, using an innovative allele-specific approach in combination with TP53 sequencing, we identified a particularly poor prognostic subgroup in patients with TP53 mutant CPC exhibiting aUPD in chromosome 17, and who as a result had an elevated number of mutated copies of p53. This study provides evidence for the crucial role of molecular stratification as a tool to improve the clinical management of patients with CPT.

Atypical CPCs are currently distinguished from CPPs by histopathology, where aCPPs exhibit increased mitotic activity (14); yet, survival outcomes for both CPPs and aCPPs are comparably favorable. Standard of care for these tumors consists of surgical resection with very few aCPP cases requiring adjuvant chemotherapy. In our cohort, all patients with aCPP for which we had clinical history (6/11) were treated with surgical resection alone, yet demonstrated favorable survival comparable with CPPs. We suggest that the benign phenotype of aCPPs may re

...variation in clinical outcome has been observed among patients with CPC despite the use of similar treatment protocols (2, 16, 17). Our findings demonstrate that the molecular heterogeneity of CPCs may be driving this clinical variability.

Extensive chromosomal alterations were recurrent in CPCs. An allele-specific copy-number approach allowed us to identify aneuploidy in 89% of CPCs, and distinguish between hypo- and hyperdiploid CPCs, exhibiting numerous chromosomal losses, and hyperdiploid CPCs, exhibiting numerous chromosomal gains and concurrent aUPD. Our findings uncovered that chromosomal instability is a common mechanism involved in CPC development; however, further examination of the molecular differences driving hypo- and hyperdiploid development will identify distinct mechanisms responsible for tumor progression. Ploidy was not significantly associated with age at diagnosis or patient survival; nonetheless, we identified that hyperdiploid CPCs were significantly enriched in chromosomes exhibiting aUPD. A recent study reported similar subgroupings in CPCs, where a higher frequency of chromosomal losses were observed in younger children and chromosomal gains in older children, and loss of 12q was associated with shorter survival (18). In our cohort, we found no significant correlations between patient age and CPC subgroups or TP53 status. Moreover, survival differences were identified only when TP53 copy number and mutation status were examined concomitantly.

A rise from somatic recombination errors during mitosis, aUPD is an important mechanism leading to LOH with an...
unaffected copy number, and is therefore associated with the enrichment of chromosomes or regions harboring preexisting mutations, specific promoter methylation patterns, and focal deletion of genes (13). In our study, we observed that aUPD affects entire chromosomes in all tumor subgroups; however, aUPD was most frequent in CPCs harboring TP53 mutations. We identified chromosome 17 to be the most frequent site affected by aUPD, and that 90% of CPCs exhibiting aUPD of chromosome 17 harbored a mutation in TP53, increasing the number of mutant p53 copies to 2 in these tumors. Our findings suggest aUPD is a mechanism by which CPCs accumulate deleterious aberrations, such as TP53 mutations, while retaining the normal function of other genes due to an unaffected chromosome copy number.

Focusing on the known association between p53 mutations and CPCs, we identified that the number of mutated copies of p53 was significantly associated with patient survival. Our findings support the concept that in addition to the loss of tumor-suppressive activity of p53, mutant TP53 also acquires oncogenic activities that promote CPC development. The gain of function (GOF) properties of mutant p53 include cellular invasion, proliferation, genomic instability, and polyploidy, among others (reviewed in ref. 19). Because of increased GOF activity, in addition to a complete loss of the tumor suppressor functions of p53, an elevated number of mutant p53 copies could result in an aggressive phenotype associated with decreased survival as we observed in our high-risk CPC patient cohort. Because we did not assess the mutation status of other cancer genes in chromosome 17, we cannot infer that the number of mutant copies of p53 is the only aberration on this chromosome-wide gains and losses. However, we have demonstrated that TP53 mutations alone do not drive chromosomal instability in CPCs as TP53 wild-type tumors also exhibit high levels of chromosome-wide gains and losses. However, we have demonstrated that TP53 mutations are associated with changes in gene expression and methylation patterns that may result in increased tumor aggressiveness and may elucidate the different clinical outcomes observed. Alterations affecting the p53 pathway, either upstream or downstream of p53, may generate the molecular background necessary for CPC development, and should be investigated further.

Recurrent lesions, such as the chromosome-wide gains of chromosome 1, which was not only recurrently gained but also the least frequently lost in CPCs, chromosome 12, and chromosome-wide loss of chromosome 3, may also be contributing to CPC’s unique genotype and would need to be further investigated to identify unique targets for effective therapies.

Our study demonstrates that investigating the molecular characteristics of CPTs is crucial to further refine the molecular stratification of patients to improve patient care. We suggest that the prognostic significance of TP53 mutation and copy-number status in CPCs be validated prospectively in future cooperative clinical trials. Validation of these data in future prospective studies will inform risk stratification of patients with CPC, and set the framework for future treatment intensification for high-risk patients.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Authors’ Contributions**

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