**Myc and Loss of p53 Cooperate to Drive Formation of Choroid Plexus Carcinoma**


**Abstract**

Choroid plexus carcinoma (CPC) is a rare brain tumor that occurs most commonly in very young children and has a dismal prognosis despite intensive therapy. Improved outcomes for patients with CPC depend on a deeper understanding of the mechanisms underlying the disease. Here we developed transgenic models of CPCs by activating the Myc oncogene and deleting the Trp53 tumor suppressor gene in murine neural stem cells or progenitors. Murine CPC resembled their human counterparts at a histologic level, and like the hypodiploid subset of human CPC, exhibited multiple whole-chromosome losses, particularly of chromosomes 8, 12, and 19. Analysis of murine and human CPC gene expression profiles and copy number changes revealed altered expression of genes involved in cell cycle, DNA damage response, and cilium function. High-throughput drug screening identified small molecule inhibitors that decreased the viability of CPC. These models will be valuable tools for understanding the biology of choroid plexus tumors and for testing novel approaches to therapy.

**Significance:** This study describes new mouse models of choroid plexus carcinoma and uses them to investigate the biology and therapeutic responsiveness of this highly malignant pediatric brain tumor.

**Introduction**

Choroid plexus tumors are rare pediatric neoplasms that arise around the ventricles of the brain, and account for up to 20% of brain tumors in children under 1 year of age. These tumors can be divided into three subgroups based on histology: choroid plexus papillomas (CPP; WHO grade I), atypical CPPs (aCPP; WHO grade II), and choroid plexus carcinomas (CPC; WHO grade III). CPPs have a favorable prognosis after surgical resection and rarely require additional treatment. CPCs, in contrast, usually require surgical removal followed by radiation and chemotherapy. Despite aggressive treatments, the 5-year overall survival rate is less than 60% (1, 2) and the median progression-free survival (PFS) is only 13 months (3). Patients who do survive often suffer devastating side effects from the therapy, including neurocognitive deficits, endocrine disorders, and secondary cancers. Effective treatments for CPC are lacking due to poor understanding of CPC biology and the paucity of patient specimens and animal models for studying the disease.

The pathogenesis of choroid plexus tumors is not well understood. Mutations in the TP53 tumor suppressor gene are present in 60–90% of CPCs and have been associated with poor prognosis (4). However, whole genome sequencing of CPC patient specimens has not identified other recurrent single-nucleotide variants, insertions/deletions, or focal copy number alterations (5). Rather, CPCs exhibit frequent chromosomal imbalances, with some
tumors exhibiting multiple large chromosomal gains (hyperdiploid) and others showing predominantly large chromosomal losses (hypodiploid, refs. 6–8). These studies suggest that copy number alterations might be oncogenic drivers of CPC.

To understand the pathogenesis of CPC, we have created mouse models of hypodiploid CPC by activating the Myc oncogene and inactivating the Trp53 tumor suppressor in neural stem cells or progenitors. The resulting models are useful for understanding the biology of CPC and for testing novel therapies.

Materials and Methods

Animals

Atoh1-Cre [B6.Cg-Tg(Atoh1-cre)1Bri/J, stock number 011104] and p53Lox1 [B6.129P2-Trp53tm1Bnr/J, stock number 008462] mice, Nestin-Cre [B6.129S7-creNek10/J, stock number 003771], and hGFAP-Cre [FVB.Tg(GFAP-cre)25Mes/J, stock number 004600] were purchased from JAX. B6p-Cre mice were purchased from NCI mouse repository [B6;CB-Tg(Fabp7-creLacZ)3Gtm/Nci, strain number: 01XM9]. LSL-MycT58A mice were kindly provided by Rosalie Sears at Oregon Health and Science University. All animals were bred in parafilm and protected in 30% sucrose, frozen in Tissue Tek-OCT (Sakura Finetek), and cut into 12 μm sagittal sections. Sections were blocked and permeabilized for 1 hour with PBS containing 0.1% Triton X-100 and 10% normal donkey serum, stained with primary antibodies (anti-Otx2: Millipore AB9566; anti-Aqp1: Santa Cruz SC-20810; and 10% normal goat serum) after SDS-PAGE electrophoresis and blocked in non-fat dry milk for 30 minutes. Membrane was incubated with primary antibodies (anti-MYC: Cell Signaling Technology 5605S; anti-GAPDH: Cell Signaling Technology 5174S; anti-phospho-RB: Cell Signaling Technology 8516T; anti-RNAPII: Cell Signaling Technology 2629) overnight and washed three times in Tris-buffered saline + 0.1% Tween-20 (TBST). Membrane was then incubated with secondary antibody for 1 hour at room temperature and washed three times in TBST. Membrane was incubated with Bio-Rad Clarity Western substrate before being imaged on a Bio-Rad ChemiDoc MP imaging system.

Array CGH

Array CGH data analysis was performed at St. Jude Children's Research Hospital on Agilent SurePrint G3 Mouse Genome CGH microarrays using genomic DNA samples prepared from normal mouse choroid plexus, CPPs and CPCs. DNA copy number changes were visualized in Integrative Genomics Viewer. Raw array CGH data have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) and are accessible through the GEO Series accession number GSE126408.

Gene expression profiling and pathway enrichment analysis

Gene expression profiling was done using Affymetrix GeneChip Mouse Gene ST 2.0 microarrays. RMA normalization was applied and differentially expressed genes were generated using R/Limma package (cut off: fold change >2 and P value < 0.05). Pathway enrichment analyses were determined using Metascape (Metascape.org). Chromosome enrichment analysis was done in DAVID Bioinformatics 6.8 (https://david.ncifcrf.gov/). Raw microarray data have been deposited in the National Center for Biotechnology Information GEO and are accessible through the GEO Series accession number GSE126327.

Sanger sequencing of TP53

Exons 2 to 11 of TP53 were PCR amplified from the genomic DNA of CHLA-CPC02 and CHLA-CPC03 patient specimen. PCR products were column purified and Sanger sequencing was performed to determine the mutational status of TP53. Mutation calls were made with Mutation Surveyor software.

Cell culture

CPC tumor tissue was isolated from the mouse ventricle and dissociated with papain for 30 minutes at 37°C. Dissociated CPC cells were plated for subsequent experiments in Neurocult Basal Medium (STEMCELL Technologies) with proliferation supplement (STEMCELL Technologies) and Penicillin-Streptomycin.

High-throughput drug screening

A total of 7,902 compounds from drug libraries including StemSelect (EMD), Spectrum (Microsources), LOPAC (Sigma), FDA/IN drugs (Microsources), Prestwick chem library

Viability assay

Cells were plated in 384 well plates prior to drug treatment. Forty-eight hours after treatment, cell viability was analyzed by CellTiter Glo assay (Promega) and results were collected on a Perkin Elmer Envision plate reader.

qRT-PCR

RNA was isolated using Qiagen RNeasy Mini Kit. Reverse transcription was done using iScript cDNA Synthesis Kit (Bio-Rad). Primers for qPCR are listed below. Myc (forward: 5’-ATGCCCTCTAAGCTGACTCT-3’; reverse: 5’-GGCGACATATCGTGAGGACA-3’); Trp53 (forward: 5’-CACAGCGTGTGGTACCCTA-3’; reverse: 5’-GTGATTGACGCTGGCTTCT-3’); Hspa1b (forward: 5’-GAGATGCACACTCTGTTGGAG-3’; reverse: 5’-GCCCTGTGAAAGAGCCTG-3’).

Western blot analysis

Protein lysates were prepared in RIPA buffer (Millipore) and protein concentration was determined by BCA assay (Bio-Rad). Proteins were transferred to nitrocellulose membrane (Invitrogen) after SDS-PAGE electrophoresis and blocked in non-fat dry milk for 30 minutes. Membrane was incubated with primary antibodies (anti-MYC: Cell Signaling Technology 5605S; anti-GAPDH: Cell Signaling Technology 5174S; anti-phospho-RB: Cell Signaling Technology 8516T; anti-RNAPII: Cell Signaling Technology 2629) overnight and washed three times in Tris-buffered saline + 0.1% Tween-20 (TBST). Membrane was then incubated with secondary antibody for 1 hour at room temperature and washed three times in TBST. Membrane was incubated with Bio-Rad Clarity Western substrate before being imaged on a Bio-Rad ChemiDoc MP imaging system.
Compounds were loaded into 384 well plates at a concentration of 1 μmol/L and 5,000 CPC cells were seeded into each well. Celltiter Glo assay was used to determine the effect of drugs on cell viability 48 hours later. More than 50% reduction in viability compared with vehicle control (DMSO) treated cells was used as a cut off to select candidate compounds.

Small molecule compounds

Dinaciclib, Flavopiridol, and Abemaciclib were purchased from SelleckChem. Triptolide was purchased from Cayman Chemical. 10058-F4 was purchased from Sigma. CD-532, Meriolin 3, and Cdk/Crk inhibitor were obtained from the Con­rad Prebys Center for Chemical Genomics at SBP.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software and Limma R package. Monte Carlo simulation was performed by randomly generating 1,712 human genes and 113 mouse genes respectively from the 19,676 possible human-mouse orthologous genes and the overlap was calculated across 1,000,000 simulations.

Drug synergy analysis

Drug synergy was tested using CompuSyn software (www.combosyn.com). CI < 0.5 is highly synergistic, 0.5 < CI < 0.9 is synergistic, 0.9 < CI < 1.1 is additive, CI > 1.1 is antagonistic.

Results

Ectopic expression of Myc and loss of Trp53 in neural progenitor cells leads to CPC

We previously generated mouse models of MYC-driven medulloblastoma by using retroviruses to overexpress Myc and inactivate Trp53 in purified cerebellar stem cells or granule neuron precursors and transplanting those cells into the cerebellum of naïve mice (9, 10). In an effort to create a transgenic version of these models, we crossed mice carrying a Cre-inducible allele of Myc (Rosa-LoxP-Stop-LoxP-MycT58A, hereafter called LSL-Myc; ref. 11) and a Cre-deletable allele of Trp53 (LoxP-Trp53-LoxP, or Trp53-flox) with animals that express Cre recombinase in neural stem or progenitor cells, including Atoh1-Cre (12), Blbp-Cre (13), and hGFAP-Cre (Fig. 1A; Supplementary Fig. S1A; ref. 14). Within 13 to 28 weeks, mice developed signs of increased intracranial pressure and neurologic symptoms and had to be sacri­ficed (Fig. 1B; Supplementary Fig. S1B and S1C). Unexpectedly, fewer than 5% of mice had cerebellar tumors that resembled medulloblastoma; the remaining mice had tumors in the choroid plexus, and these exhibited histologic characteristics of CPP or CPC.

Animals in which Myc was activated but Trp53 expression was retained [Atoh1-Cre; LSL-Myc (hereafter referred to as AM); Figure 1. Expression of Myc and loss of p53 induce CPC. A, Mice used in this study. Crossing Atoh1-Cre mice to lox-STOP-lox (LSL) Myc<sup>T58A</sup> mice results in progeny that develop CPP; further crossing of these animals to p53-flox mice results in progeny that develop CPC. B, Kaplan–Meier survival curve shows median survival times: Atoh1-Cre;Myc/Myc;p53<sup>fl</sup>/+ (CPP), 400 days. Mice that have p53 loss without MycT58A expression or mice that do not express Atoh1-Cre do not develop tumors. C and D, Hematoxylin and eosin (H&E) staining shows histology of murine CPP and CPC. E and F, Ki67 staining shows proliferation in murine CPP and CPC. G–I, Aqp1 and Otx2 immunostaining on brain sections of murine CPP and CPC. J–L, p-H2A.X and Ki67 immunostaining shows DNA damage and proliferation status in murine CPC, CPP, or normal choroid plexus. Scale bars in C–F, 100 μm; G–J, 200 μm; K–P, 50 μm.
hGFAP-Cre; LSL-Myc (GM) and Blbp-Cre; LSL-Myc (BM)] developed tumors that showed increased numbers of cells but maintained architecture of normal choroid plexus, with a single layer of epithelial cells surrounding a fibrovascular core; histologically, these tumors resembled CPPs (Fig. 1C). In contrast, tumors induced by activation of Myc and deletion of both copies of Trp53 [Atoh1-Cre; LSL-Myc; Trp53-flox (AMP), hGFAP-Cre; LSL-Myc; Trp53-flox (AMP) and Blbp-Cre; LSL-Myc; Trp53-flox (AMP)] exhibited frequent nuclear pleomorphism and loss of papillary structure, and resembled CPCs (Fig. 1D). CPCs had increased numbers of mitotic figures and much higher proliferative indices than CPPs as shown by Ki67 staining (Fig. 1E and F). Mice that developed CPPs survived much longer (median survival = 400 days for AMP) than those that developed CPCs (median survival = 131 days for AMP; Fig. 1B). Interestingly, tumors induced by Atoh1-Cre and hGFAP-Cre developed in the fourth ventricle whereas Blbp-Cre induced tumors were found in all four ventricles.

Normal choroid plexus epithelial cells express aquaporin 1 (Aqp1) and orthodenticle homeobox 2 (Otx2; refs. 15, 16). Although murine CPPs retain expression of these markers (Fig. 1G and H), murine CPCs have reduced expression of Aqp1 and Otx2 (Fig. 1I and J), suggesting that they are relatively undifferentiated. Furthermore, leptomeningeal metastasis was observed in a subset of mouse CPCs (Supplementary Fig. S2A–S2D). Because inactivation of p53 often results in genomic instability (17), we examined DNA double-stranded breaks by staining with antibodies against Phospho-Histone H2A.X (pH2A.X). In animals as young as 4 weeks old, we observed pH2A.X staining in CPCs (Fig. 1K) but not in CPPs (Fig. 1I) or normal choroid plexus (Fig. 1M). We also observed higher mitotic indices in CPCs (Fig. 1N) than in CPPs (Fig. 1O) and normal choroid plexus (Fig. 1P). These studies demonstrate that activation of Myc and loss of Trp53 can cooperate to generate aggressive choroid plexus tumors in mice.

Atoh1-expressing neural progenitor cells contribute to the choroid plexus epithelial lineage

Most previous studies have suggested that Atoh1 is expressed in granule neuron precursors of the cerebellum and in progenitor cells destined to give rise to the deep cerebellar nuclei (18). The induction of choroid plexus tumors by Atoh1-Cre suggested that Atoh1-expressing progenitors might also contribute to the choroid plexus. To test whether this was the case, we performed lineage tracing by crossing Atoh1-Cre mice with Cre-inducible fluorescent reporter mice (Rosa-CAG-LoxP-stop-LoxP-tdTomato), in which tdTomato expression is permanently induced upon excision of the stop sequence (Fig. 2A). Brains were collected from progeny of these crosses at embryonic day 15.5 and postnatal day 7. In Atoh1-Cre; LSL-tdTomato mice, tdTomato expression was detected in a subset of choroid plexus epithelial cells in the fourth ventricle as early as E15.5. These tdTomato-expressing cells also expressed the choroid plexus epithelial cell markers Aqp1 and Otx2 (Fig. 2B). To determine whether tumor cells in AMP mice are derived from Atoh1+ CP progenitors, we crossed these animals with tdTomato reporter mice to generate tumor-bearing mice in which Atoh1+ cells and their progeny are labeled with tdTomato (AMP-tdTomato). These mice developed CPCs and most of the tumor cells expressed tdtomato, indicating that CPCs are derived from Atoh1+ progenitors (Fig. 2C). Similar studies performed using Blbp-Cre mice showed that Blbp-expressing progenitor cells give rise to choroid plexus epithelial cells in both the forebrain and the hindbrain (Supplementary Fig. S3). These studies revealed that CPCs can arise from Atoh1-expressing or Blbp-expressing progenitors in the choroid plexus.

Choroid plexus tumors are dependent on Myc

Although previous studies have shown that Trp53 mutations are common in CPC, the role of Myc in CPC pathogenesis has not been studied in detail. To confirm the overexpression of Myc and loss of p53 in murine tumors, we performed quantitative RT-PCR. As expected, Myc was highly expressed in murine CPPs and CPCs (Fig. 3A), whereas loss of Trp53 expression was only observed in CPCs (Fig. 3B). We also observed high MYC expression in primary human CPPs and CPCs compared with normal fetal and adult brain tissue (Fig. 3C and Supplementary Fig. S4A–S4D). These results suggest that MYC is highly expressed in murine and human choroid plexus tumors.

To determine whether Myc is required for tumor cell survival, we treated mouse CPCs with compounds that have been shown to inhibit Myc function. KJ-Pyr-9 (19) and 10058-F4, both of which disrupt Myc/Max dimerization, decreased CPC cell viability in a dose-dependent manner (Fig. 3D and E). CD-532, a novel compound that destabilizes Myc by preventing its interactions with Aurora kinase (20), also potently reduced mouse...
choroid plexus tumor cell viability (Fig. 3F). To determine whether human CPC cells are sensitive to MYC inhibition, we obtained primary tumor cells from patients with CPC. These samples (CHLA-CPC02 and CHLA-CPC03) both contained polymorphisms in the TP53 locus [P72R (rs1042522) and intron 3 duplication (rs17878362), respectively] that are associated with increased cancer risk. Consistent with the results from murine tumors, the viability of human CPC cells was decreased when treated with compounds that disrupt MYC function (Fig. 3G). These studies suggest that MYC is required for survival of murine and human CPCs.

Mouse CPCs are characterized by frequent losses of chromosomes 8, 12, and 19

Human choroid plexus tumors frequently exhibit large chromosomal gains (hyperdiploid tumors) or losses (hypodiploid tumors). To investigate if mouse choroid plexus tumors share these features, we performed array-based comparative genomic hybridization (aCGH) on normal choroid plexus, CPPs from AM mice, and CPCs from AMP and BMP mice. Although no significant chromosomal aberrations were found in CPPs, CPCs exhibited multiple losses similar to those observed in human hypodiploid CPCs (8). Specifically, we observed recurrent

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**Figure 3.**
Choroid plexus tumors are dependent on Myc. A and B, qRT-PCR showing Myc and p53 expression in CPC and CPP compared with normal mouse cerebellum (CB) and normal choroid plexus (ChP). Error bars, SEM. C, Western blot analysis showing MYC expression in a collection of human CPC and CPP specimens. D, E, Effects of Myc inhibitor 10058-F4 on viability of murine CPC cells. F, Effects of CD532 on viability of murine CPC cells. G and H, Effects of various compounds that inhibit MYC on viability of human CPC cells (CHLA-CPC02 in G and CHLA-CPC03 in H). Error bars, SEM.
Mouse CPCs exhibit large chromosomal losses. A. Array CGH analysis of normal mouse choroid plexus (ChP; n = 3), CPP (n = 5), and CPC (n = 10). B-D. High-resolution image showing DNA copy number status on mouse chromosomes 8, 12, and 19. DNA copy number status is shown in Integrative Genomics Viewer.

losses of mouse chromosome 8 (syntenic with human chromosomes 8, 13, 16, and 19), mouse chromosome 12 (syntenic with human chromosomes 2, 7, and 14), and mouse chromosome 19 (syntenic with human chromosomes 9 and 10), respectively (Fig. 4A–D).

To identify pathways that are altered during the course of choroid plexus tumor formation, we performed gene expression profiling using Affymetrix Gene Chip Mouse Gene ST 2.0 microarrays to compare CPCs with CPPs and normal choroid plexus cells. Principal component analysis (PCA) indicated that CPCs and CPPs are distinct from one another, and very different from normal choroid plexus (Fig. 5A). Hierarchical clustering confirmed the results observed using PCA (Fig. 5B), indicating that CPCs and CPPs are related but distinct tumors. Using a cutoff of fold change > 2 and P-value < 0.05, we identified 2,579 genes differentially expressed between CPCs and normal choroid plexus, 2,194 genes differentially expressed between CPPs and normal choroid plexus, and 637 genes differentially expressed between CPCs and CPPs.

We then performed pathway analysis using Metascape (metascape.org; ref. 21) and Gene Set Enrichment Analysis (GSEA; refs. 22, 23) to look for pathways enriched in mouse CPCs compared with normal choroid plexus. MYC targets, as well as regulators of ribosome biogenesis and metabolic processes, which are often associated with MYC overexpression, were highly enriched in CPCs. Cell-cycle-related genes and regulators of the E2F, AURORA B, and PLK1 pathways were also highly expressed (Fig. 5C). Genes related to cilia function, which were recently reported to play key roles in choroid plexus tumor formation (24), were significantly downregulated (Fig. 5D).

To assess whether the changes in gene expression in CPCs were due to copy number alterations, we integrated our microarray and aCGH data. Interestingly, when we looked at the downregulated genes (LogFC < 0.2, P-value < 0.05, FDR < 0.05) in murine CPCs compared with CPPs, we found that these genes were enriched on three mouse chromosomes: 8 (17.4% of all chr8 genes exhibited decreased expression), 12 (9.1% of all chr12 genes exhibited decreased expression), and 19 (8.2% of all chr19 genes exhibited decreased expression; Supplementary Fig. S5A); notably, all three of these chromosomes were deleted in CPCs based on aCGH analysis. These data suggest that chromosomal losses contribute significantly to the differences in gene expression between CPP and CPC.

Analysis of the genes exhibiting copy number driven decreases in gene expression revealed enrichment of metabolic pathways, such as lipid modification and phospholipid metabolism, carbohydrate metabolism, and alpha-amino acid metabolism (Supplementary Fig. S5B). Genes involved in phosphatidylinositol signaling, mitochondrial organization, and apoptosis were also altered in CPCs compared with CPPs. To determine if these genes are also lost in human CPC, we compared our data to copy number analysis and gene expression data from patients with CPP and CPC (8). Among the genes showing copy number driven downregulation of gene expression in murine tumors, 30 also showed copy number driven loss in human CPCs (Table 1). To test if the overlap between human and murine genes in our study is significant, we performed Monte Carlo simulation. The average number of genes predicted to overlap by chance was 9 (maximum = 26), suggesting that the overlap of genes between human and murine CPCs is significant (P < 1E–6; Supplementary Fig. S6).

The overlapping genes encode adhesion molecules (cadherin related family member 3, integrin subunit beta 8), transporters (solute carrier family 22 member 8, solute carrier family 5 member 5), kinases (NIMA related kinase 5, adenylyl kinase 7), calcium binding proteins [calmodulin 1 (phosphorylase kinase, delta)], and regulators of cilia and intracellular transport (Bardet-Biedl Syndrome 1, dynein light chain roadblock-type 2, tubulin polymerization promoting protein family member 3). Although few of these genes have well-studied roles in cancer, the fact that they are lost in both murine and human CPCs suggests that they...
Figure 5.
CPC and CPP exhibit distinct gene expression profiles. A, PCA shows normal choroid plexus (ChP), CPP, and CPC cluster separately. B, Heat map of gene expression data shows CPP and CPC share common gene expression signatures, but are distinct from each other. C and D, Pathway analysis reveals enrichment (C) or downregulation (D) of pathways in CPC compared with ChP.
might represent tumor suppressor genes that are involved in initiation or progression of choroid plexus tumors.

### High-throughput drug screening identifies potential therapies for CPCs

To identify novel therapies for CPC, we performed high-throughput drug screening on murine CPC cells using libraries containing 7,902 biologically active compounds. Preliminary screening identified 51 compounds that inhibited viability by ≥50% compared with vehicle-treated controls. Among these, 18 were also able to inhibit the growth of primary patient CPC cells. To eliminate compounds with broad toxicity, we tested these compounds on normal granule neurons. Of the 18 compounds tested, nine exhibited minimal toxicity to granule neurons and 18 were also able to inhibit the growth of primary patient CPC cells. Among these, nine exhibited minimal toxicity to granule neurons and 50% compared with vehicle-treated control cells. Among these, nine exhibited minimal toxicity to granule neurons and 18 were also able to inhibit the growth of primary patient CPC cells.

### Discussion

In this study, we generated novel mouse models of choroid plexus tumors by conditionally expressing Myc and/or deleting Tp53 in progenitor cells that give rise to choroid plexus epithelial cells. Our CPCs exhibit deletions on chromosomes 8, 12, 19, and
resemble the hypodiploid subtype of human CPC, which is characterized by large chromosomal deletions. High-throughput drug screening identified CDK inhibitors and Triptolide as potential therapies for CPC.

Our previous studies (9, 10) demonstrated that overexpression of Myc and inactivation of Trp53 in purified hindbrain progenitors allowed these cells to give rise to tumors that resemble Group 3 medulloblastoma. The fact that inducing the same events in
transgenic mice resulted in CPC instead of medulloblastoma was unexpected. One possible explanation is the timing of Myc induction. In our retrovirus-based models, Myc was overexpressed in neonatal hindbrain progenitors, which may have undergone more extensive lineage commitment and differentiation than the embryonic progenitors targeted by the Atoh1-Cre and Blbp-Cre transgenes used in this study. However, the fact that in utero electroporation of Myc at the embryonic stage results in medulloblastoma with a small percentage of CPCs (27) suggests that embryonic neural progenitors are also capable of giving rise to medulloblastoma. An alternative explanation is that higher levels of Myc are required for development of medulloblastoma than for development of CPC. Both the retroviral and electroporation-based models express very high levels of Myc, which in our CPC model, Myc expression is driven by the Rosa26 promoter, which results in relatively weak expression in the brain (28). Further studies will be necessary to determine if transgenic mice expressing higher levels of Myc develop medulloblastoma.

Our observation that overexpression of Myc leads to CPP and that concomitant deletion of Tp53 results in CPC has implications for the relationship between these diseases. Although CPP and CPC are often described as distinct diseases (29), our results suggest that loss of TP53 could result in malignant progression of CPP to CPC. In fact, there have been several reports of malignant progression of CPP to CPC (30, 31). The mechanisms by which such progression can occur remain to be determined, but it is possible that loss of TP53 could result in deregulation of proliferation, survival, or genome stability. The fact that hypodiploid CPCs lose multiple chromosomes supports the notion that genomic instability is one key factor in progression.

The finding that CPCs can be generated from Atoh1+, Blbp+, and Gfap+ cells also raises interesting questions about the origin of CPC. One interpretation of these results is that a subpopulation of progenitors in the choroid plexus is particularly susceptible to transformation, and these cells co-express Atoh1, Blbp, and Gfap+. Alternatively, it is possible that each of these genes marks a distinct cell type or stage of differentiation, and that a number of different progenitors can function as cells of origin for CPC. Finally, we cannot rule out the possibility that overexpression of Myc and loss of p53 can reprogram a variety of cell types and thereby render them susceptible to transformation. Further analysis of the progenitors marked by Atoh1, Blbp, and Gfap+ may shed light on this issue.

Our models are distinct from previously described mouse models of choroid plexus tumors. More than two decades ago, it was found that expression of simian virus 40 (SV40) large tumor antigen in transgenic mice was sufficient to induce proliferation in choroid plexus epithelial cells (32). SV40 large tumor antigen inactivates the Rb and p53 tumor suppressors, and it was subsequently shown that inactivation of Rb could also induce choroid plexus tumor formation in transgenic mice. Notably, in this model, deletion of p53 was required for tumor progression, but it did not act by promoting chromosomal instability (33). In contrast, our CPCs exhibit chromosomal instability, including losses of chromosomes 8, 12, and 19, and resemble the hypodiploid type human CPCs. In a recent cross-species genomics study using mouse CPCs generated by simultaneously knocking out p53, Rb and Pten in embryonic choroid plexus epithelial cells, a cluster of CPC oncogenes (TAF12, NYFC, and RAD54L) located on human chromosome 1p32-35.3 were identified (3). Our CPCs do not exhibit recurrent gains of these oncogenes. Interestingly, however, GSEA analysis indicates that syntenic human chromosome 1 genes are overexpressed in our CPCs, indicating that activation of some pathways may be shared between hypodiploid and hyperdiploid CPCs.

Recent studies from Shannon and colleagues (34) demonstrated that overexpression of Myc alone in Nestin-positive neural progenitor cells led to formation of CPCs and ciliary body tumors. In our model, Myc alone is not sufficient to induce CPC. The difference may be due to different levels of Myc expression. As mentioned previously, the Rosa26 promoter used in our studies often drives weak expression in the brain, whereas the CAG promoter used in Shannon and colleagues results in higher expression of Myc. Another study from El Nagar and colleagues (35) found that overexpression of MycT58A and deletion of Trp53 in Otx2-expressing cells resulted in CPC formation in all ventricles. Pathway analysis in this model pointed to cell-cycle deregulation in these tumors, similar to that seen in our model. Although DNA copy number changes were not reported in these studies, it will be interesting to find out if our models share similarities at DNA level.

Our integrated gene expression profiling and aCGH revealed decreased expression of genes on chromosomes 8, 12, and 19 associated with DNA copy number losses. These results indicate that CPCs with p53 deficiency may undergo large chromosomal losses, leading to decreased expression of tumor suppressor genes and driving tumor formation. Identification and validation of these tumor suppressor genes may help us understand more about the mechanisms of tumorigenesis and point to novel therapeutic targets.

Our high-throughput drug screening identified CDK inhibitors as promising therapeutic agents against CPCs, consistent with our finding that cell-cycle genes are significantly upregulated in CPCs. Although Dinaciclib and Flavopiridol target multiple CDKs, CDK1 and CDK2 are the only two enzymes that are common targets of these two compounds (36, 37). Furthermore, the CDK4/6 specific inhibitor Abemaciclib did not affect the viability of CPC cells (Supplementary Fig. S6A). Therefore, CDK1 and CDK2 are likely to be responsible for the efficacy of these compounds. Dinaciclib and Flavopiridol have been used in clinical trials for treating other cancers such as acute myeloid leukemia (NCT01349972), gastric cancer (NCT00991952), breast cancer (NCT01676753, NCT01624441), chronic lymphocytic leukemia (NCT02684617), and pancreatic cancer (NCT01783171). Additional studies will be necessary to determine whether Dinaciclib or Flavopiridol, or other CDK inhibitors, can benefit patients with CPC as well.

We also identified Triptolide as an effective inhibitor of CPC survival. Triptolide acts by inhibiting heat shock protein HSP70 and RNA Polymerase II. Its poor solubility in aqueous solution limits its use in preclinical and clinical studies. A water-soluble derivative of Triptolide, called Minneldide, was successfully used to treat pancreatic cancer in preclinical models (38) and is currently in clinical trials for that disease. Our studies suggest that patients with CPC may benefit from Triptolide or Minneldide, alone or in combination with CDK inhibitors. Further studies using animal models such as the ones we have described here will provide insight into the biology of choroid plexus tumors and help identify effective therapies for this disease.
Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Other (Roussel laboratory provided CPC tumours to complement those developed by the Wechsler-Reya Lab. The investigators decided that it would be best to merge the data): J. M. F. Roussel

Acknowledgments

We thank Diane Chun, Tracy Ho, and Ngn Nham for assistance with mouse genotyping; Lili Lacarra and the SBP Animal Facility for assistance with colony maintenance; Jesus Olivera and Cody Fine of the Human Embryonic Stem Cell Core at UCSD, and Yoav Altman of the SBP Flow Cytometry Shared Resource for help with FACS sorting; Fu-Yue Zeng of the Conrad Prebys Center for Chemical Genomics at SBP for help with high-throughput output screening; Subh Govindarajan of the SBP Analytical Genomics Shared Resource for help with microarray analysis; Feng Qi and Jian-Liang [Jason] Li of the SBP Bioinformatics Shared Resource for help with analysis of microarray data. We are grateful to Joanna Phillips and the UCSF Brain Tumor SPORE Tissue Core (P50CA097257) and to Jim Loukides and the SickKids Brain Tumour Research Center Biobank for providing patient samples, and to Peter Vogt from the Scripps Research Institute for assistance with the MYC inhibitor KJ-Pyr-9. This work was supported by funding from the NCI (P30CA30199, R10 CA122759, and R01 CA159859 to R. J. Wechsler-Reya, R01 CA66832 and R01 CA21765 to M. F. Roussel), the American Lebanese-Syrian Charities [to M. F. Roussel], a National Brain Tumor Society Developmental Neurobiology Grant [to R. J. Wechsler-Reya], and a Leadership Award from the California Institute for Regenerative Medicine (LA1-01747, to R. J. Wechsler-Reya).

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Published OnlineFirst March 18, 2019; DOI: 10.1158/0008-5472.CAN-18-2565

References


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