A novel TP53 somatic mutation involved in the pathogenesis of pediatric choroid plexus carcinoma

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Summary

Background: Choroid plexus carcinoma (CPC) is an uncommon, aggressive, malignant, central nervous system neoplasm that typically occurs in children, presenting with the signs and symptoms of intracranial hypertension and cerebrospinal fluid obstruction.

Case Report: We report the case of a 2.5-year-old girl with CPC. The tumor was subtotally removed by microsurgery, followed by gamma knife radiosurgery for the residual lesion. H&E staining indicated that this was a rare case of CPC. Neuropathological studies, assayed by immunohistochemical staining, showed that the tumor sample was positive to antibodies against S-100, CgA, AE1/AE3 (cytokeratin), Ki-67, INI1 and TP53, and was negative to antibodies against Nestin, GFAP, CD133, EMA and AFP. Moreover, stainings for transthyretin and vimentin were focally positive. Interestingly, direct DNA sequencing of the paraffin-embedded tumor sample identified a novel R248Q mutation in the TP53 gene. In contrast to previous reports suggesting that TP53 germline mutations were associated with the pathogenesis of CPC, here we provide a rare case of CPC with TP53 somatic mutation, as evidence that the peritumoral tissue possesses the non-mutant TP53 allele.

Conclusions: Our finding suggests that TP53 somatic mutations, in addition to its germline mutations, may also be involved in the pathogenesis of pediatric CPC.

key words: choroid plexus carcinoma (CPC) • TP53 • somatic mutation • immunohistochemical stain (IHC)
BACKGROUND

Choroid plexus tumors (CPTs) are uncommon neoplasms derived from choroid plexus epithelium and characterized by papillary and intraventricular growth. Within this family of tumors there are benign and malignant variants, typically classified as choroid plexus papilloma (CPP), atypical CPP and choroid plexus carcinoma (CPC), respectively. CPTs account for approximately 0.3% to 0.6% of all brain tumors [1]. Within the pediatric population, however, these neoplasms are more common, representing approximately 1% to 4% of all childhood brain tumors, with 10% to 20% occurring during the first year of life. Although reported in adults, 80% of CPTs occur in children, and 20% of all choroid plexus tumors in children are CPCs. CPC is a highly aggressive malignant tumor (WHO grade III) which must be distinguished from CPP (WHO grade I). The prognosis of CPP and CPC is quite different for their histopathologic features and biological behaviors [2]. Most CPP cases can be cured by total removal, but CPC cannot be cured by neurosurgical procedure alone. CPC is associated with a poor outcome because the tumor cells diffuse through the cerebrospinal fluid (CSF) pathways. Radiotherapy and/or chemotherapy should be performed after subtotal removal for CPC patients [3]. Neuropathologic features of CPC include blurring of papillary architecture, layers of neoplastic choroid plexus epithelial cells with pleomorphic nuclei, increased nuclear-to-cytoplasmic ratio, increased mitotic activity, areas of necrosis, and brain invasion [4]. Recently, some reports showed that TP53 germline mutation was associated with the pathogenesis of CPC and CPP [5,6]; few reported CPC children with the pathogenesis of CPC and CPP [5,6]; few reported CPC children with TP53 somatic mutation. Here, we document a rare case of CPC with a novel TP53 somatic mutation.

CASE REPORT

A 2.5-year-old girl presented to our Department of Neurosurgery with 4 weeks of progressive headache and vomiting, weakness of the left limbs and unstable walking. Physical examination on admission found a slight left hemiparesis, muscle powers of left limbs were grade III to IV, her head circumference was 4 cm larger than the average for children of the same age, the 2 pupils were normal size, light reflexes were normal, and no speech and language disorders were found. No other neurologic findings were found. The patient’s family was without a history of multiple malignancies, her parents and grandparents were healthy, and the girl had no siblings. Non-enhanced brain CT revealed an ovoid lesion (8×4×6 cm-sized) in the right lateral ventricle and a marked dilation of the right lateral ventricle, and necrosis was shown in the center of lesion (Figure 1A). T1-weighted MRI showed a huge tumor, enhanced homogeneously by gadolinium-diethylenetriaminedipropionic acid (Gd-DTPA), in the right lateral ventricle, and necrosis was shown in the center of lesion (Figure 1B, C). Diffusion-weighted imaging (DWI) revealed an iso-intense mass with multiple small hypo-intense “cystic-like” areas. She underwent microsurgery via right posterior temporoparietal craniotomy. The surface of the tumor was not easily separated from the surrounding normal brain tissue. The blood supply from the choroid plexus was rich and the tumor tended to bleed easily. Then, the tumor was subtotally resected (95% removed). Her intraoperative and postoperative courses were uneventful. She received radiosurgery (gamma knife) for the residual lesion 10 days after surgery; a median margin dose of 12.0 Gy (range, 11.5–15) was used for this. Chemotherapy was refused by her parents postoperatively. Four months after the operation, she presented vomiting, rhinorrhea, weight loss and high temperature (38.8°). Brain CT revealed that the tumor had recurred. The lesions were located at the right lateral ventricle and suprasellar cistern (Figure 1D). Obviously, the lesion of suprasellar cistern was a metastasis through CSF pathways. Repeat surgery was recommended. Unfortunately, her parents refused the next treatments and she died after 6 months due to disease progression.

Written informed consent was obtained from her parents, which included the tumor tissue and peritumoral brain tissue. H&E staining and immunohistochemistry (IHC) were carried out. A few antibodies were used for the IHC staining, which included S-100, CgA, AE1/AE3 (cytokeratin), INI1, TP53, transthyretin, Vimentin, Nestin, GFAP, Ki-67, CD133, EMA and AFP (the sources of the antibodies and dilutions are summarized in Table 1). The levels of immunostaining were evaluated as previously described [7]. In brief, nuclear expression is divided into grades 0 through 4 – grade 0 means no positive cells or very few, and grade 4 means well-defined areas of positive cells with strong expression (>50%). Cytoplasmic expression is evaluated as a percentage of positive cells – score 0 (<10%), score 1 (10–50%), and score 2 (>50%). A semiquantitative scale was defined as follows: –, negative (grade 0 or score 0); +, weak (grade 1–2 or score 1); and++, moderate to strong (grade 3–4 or score 2). DNA was extracted from the paraffin embedded tissues (tumor and peritumoral tissue) using a previously reported protocol [8]. Exon 5–8 of TP53 gene was further analyzed by direct DNA sequencing (ABI 3100 Prism DNA Sequencer, Applied Biosystems, CA). The primers of exon 5–8 of TP53 and PCR conditions were designed by IARC, WHO (http://www-p53.iarc.fr/p53sequencing.html).

Neuropathological findings
H&E staining revealed a blurring of papillary architecture, pleomorphic nuclei, and brain invasion (Figure 2A, B). IHC stainings (Figure 2C–F) showed AE1/AE3(+), INI1(++), TP53(++), S-100(+), CgA(+), Ki-67(+), transthyretin(–/+), Vimentin(–/+), Nestin(–), GFAP(–), CD133(–), EMA(–), and AFP(–). S-100(+), CgA(+), Ki-67(+), transthyretin(–/+), Vimentin(–/+), Nestin(–), GFAP(–), CD133(–), EMA(–), and AFP(–). S-100(+), CgA(+), Ki-67(+), transthyretin(–/+), Vimentin(–/+), Nestin(–), GFAP(–), CD133(–), EMA(–), and AFP(–).

Cyto genetic findings
Cyto genetic analysis of the peritumoral tissue and the paraffin-embedded tumor sample revealed a novel TP53 missense mutation by DNA direct sequencing. A CGG→CAG substitution at codon 248 (exon 7), creating an Arg→Gln substitution of amino acid, was found in tumor tissue. No mutation was found in peritumoral tissue, which indicated that the mutation was a somatic mutation (Figure 3A, B). Sequencing of an independently amplified fragment in the opposite direction confirmed the mutation.

DISCUSSION

In the differential diagnosis of pediatric CPC it is difficult to distinguish it from atypical teratoid/rhabdoid (AT/RT),...
primitive neuroectodermal tumor (PNET), and medulloepithelioma on CT/MRI scans or histological features. The typical image of CPC always includes a large, irregular enhancing mass surrounding the lateral ventricle or 4th ventricle on MRI, heterogeneous signal on long TR/long TE images and short-TR images, edema in adjacent brain, hydrocephalus and presence of disseminated tumor [9]. IHC staining should be done in some difficult cases. Our results of IHC staining showed positive TP53 nuclear expression; INI1 and AE1/AE3 were also strongly immunopositive. A previous study suggested that positive staining for INI1 protein is retained in the majority of CPC and lost in AT/RT [10]. CPCs express cytokeratins and positivity for S-100 and AE1/AE3 proteins, transthyretin is less frequent than in CPP, about 20% of CPC are GFAP-positive, and EMA is usually not expressed [11]. In adults, metastatic carcinomas to the choroid plexus are possible. Expression of carcinoembryonic antigen (CEA) or AFP suggests metastatic neoplasms, which can be distinguished from primary CPC [12]. Some biomarkers for stem cells and neurons, such as CD133 and Nestin, are negative in most CPCs. One CPP and almost all CPCs showed immunohistochemical positive for TP53 protein in 12 pediatric patients with CPTs [13].

CPCs usually grow rapidly and have a 5-year survival rate of approximately 40%, and two-thirds of CPCs disseminate throughout the CSF pathway [4]. Surgery should be performed when the diagnosis is established; gross total resection allows for the best chance of survival and improves the overall prognosis [3,14]. The use of adjuvant chemotherapy after subtotal resection remains controversial in children [15]. But a meta-analysis showed that chemotherapy improves the survival of patients with choroid plexus carcinoma [16]. Radiation is considered in the treatment of adult CPCs but not for children, due to severe long-term sequelae [17]. Radiosurgery (gamma knife) is recommended for treatment of the residual CPP by Kim [18]. The girl was also treated with gamma knife instead of whole brain radiotherapy after microsurgery.

The p53 tumor suppressor protein is one of the most important molecules in the biology of human neoplasia, with TP53 somatic mutations present in ~50% of analyzed cancer

Table 1. The sources and dilutions of antibodies for immunohistochemical staining.

| Antibodies | Manufacturer         | Dilutions |
|------------|----------------------|-----------|
| TP53       | Cell Genesys, USA    | 1:1000    |
| INI1       | BD Transduction Labs, USA | 1:500   |
| AE1/AE3    | Invitrogen, USA      | 1:100     |
| S-100      | Abcam, USA           | 1:200     |
| Ki-67      | Abcam, USA           | 1:200     |
| CgA        | Abcam, USA           | 1:200     |
| Nestin     | Abcam, USA           | 1:100     |
| GFAP       | Sigma, USA           | 1:500     |
| CD133      | Abcam, USA           | 1:200     |
| EMA        | Abcam, USA           | 1:200     |
| AFP        | Abcam, USA           | 1:150     |
| transthyetin | Abbiotec, USA     | 1:500     |
| vimentin   | Abcam, USA           | 1:200     |

Figure 1. Radiologic images. (A) Enhanced MRI: a huge tumor with homogeneous enhancement by Gd-DTPA in the right lateral ventricle, necrosis in the center of lesion (Axial); (B) Enhanced MRI (Coronal); (C) Enhanced CT (4 months postoperation): a metastasis in suprasellar cistern (via CSF pathways); (D) Enhanced CT (4 months postoperation): a recurred tumor located at right lateral ventricle.
cases of all types; germline mutations are present in ~70% of families with the Li-Fraumeni syndrome (LFS) of inherited cancer predisposition [19]. Accumulating evidence shows that CPP and CPC are strongly associated with LFS in families that carry TP53 germline mutations [1,4–6,20,21]. In 2008, a novel TP53 germline mutation E285V substitution was identified in a rare case of pediatric adrenocortical carcinoma and choroid plexus carcinoma [22]. More recently, Tabori et al. reported that TP53 germline mutations were found in 50% of CPCs, and patients with CPC absence of TP53 dysfunction have a favorable prognosis and can be successfully treated without radiation therapy [23]. Until now, only scattered cases with somatic TP53 mutations at codon 179 or 242 were identified in pediatric CPCs [24].

Conclusions

In the present article, we report another novel TP53 somatic mutation of an Arg->Gln substitution at hotspot codon 248 in a sporadic CPC girl (Figure 3A, B). Our finding suggests that TP53 somatic mutations, in addition to its germline mutations, may also be involved in the pathogenesis of pediatric CPC.

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Competing of interest

None to declare.

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