Trilateral retinoblastoma variant indicative of the relevance of the retinoblastoma tumor-suppressor pathway to medulloblastomas in humans

Case report

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Results of recent studies have led investigators to suggest that the retinoblastoma tumor-suppressor (rb) gene plays an underappreciated role in the genesis of brain tumors. Such tumors cause significant rates of mortality in children suffering from hereditary retinoblastoma. It has been assumed that the pineal gland, which is ontogenetically related to the retina, accounts for the intracranial origin of these trilateral neoplasms. To address this issue, the authors describe an unusual trilateral retinoblastoma variant.

The authors provide a detailed clinicopathological correlation by describing the case of a child with bilateral retinoblastoma who died of a medulloblastoma. The intraocular and intracranial neoplasms were characterized by performing detailed imaging, histopathological, and postmortem studies. Karyotype analysis and fluorescence in situ hybridization were used to define the chromosomal defect carried by the patient and members of her family.

An insertion of the q12.3q21.3 segment of chromosome 13 into chromosome 18 at band q23 was identified in members of the patient’s family. This translocation was unbalanced in the proband. The intraocular and cerebellar neoplasms were found to be separate primary neoplasms. Furthermore, the pineal gland was normal and the cerebellar neoplasm arose within the vermis as a medulloblastoma. Finally, the two neoplasms had different and characteristically identifiable cytological and immunohistochemical profiles.

The findings of the present study, taken together with those of recent molecular and transgenic studies, support the emerging concept that rb inactivation is not restricted to central nervous system regions of photoreceptor lineage and that inactivation of this tumor suppressor pathway may be relevant to the determination of etiological factors leading to medulloblastoma in humans.

KEY WORDS • retinoblastoma tumor-suppressor gene • medulloblastoma • retinoblastoma

Abbreviations used in this paper: CNS = central nervous system; CT = computerized tomography; FISH = fluorescence in situ hybridization; GFAP = glial fibrillary acidic protein; IRBP = interphotoreceptor retinoid-binding protein; MR = magnetic resonance; PNET = primitive neuroectodermal tumor; PTCH = Drosophila melanogaster patched; rb = retinoblastoma tumor-suppressor.
primary tumors later in life, particularly osteosarcomas, and \textit{rb} mutations are found in a variety of sporadic cancers that occur in adults. When second primary tumors do occur during childhood, they are almost always intracranial. For these children, who account for approximately 8\% of those with bilateral hereditary retinoblastoma, the prognosis is dismal.\textsuperscript{2,24,25}

In an attempt to understand the origin of these rare childhood brain tumors, Bader and associates\textsuperscript{1} in 1982 called attention to the relationship between the pineal gland in mammals and that in other vertebrates. In some nonmammalian vertebrates the pineal gland actually contains photoreceptors and functions as a light-sensing organ (the third eye). The fact that the normal mammalian pineal gland, which lacks photoreceptors, expresses retina-specific proteins such as IRBP,\textsuperscript{11} supports the concept that, even in humans, the pineal gland retains a capacity for photoreceptor differentiation. In fact, the expression of IRBP has been helpful in characterizing undifferentiated sporadic pineoblastomas.\textsuperscript{20} For intracranial tumors associated with bilateral retinoblastoma, Bader and associates introduced the term “trilateral retinoblastoma” in their hypothesis that these third tumors are essentially ectopic retinoblastomas arising within the pineal gland. Although these neoplasms are often, albeit not always, located in the region of the pineal gland, these researchers’ original hypothesis has been difficult to evaluate because of an absence of critical documentation of the coexistence of retinal and intracranial tumors in the same patient (reviewed by Marcus, et al.\textsuperscript{21}).

It is not known why the retina is particularly susceptible to inactivation of one \textit{rb} allele. One explanation is that outside the retina, \textit{rb} is not essential to the control of the cell cycle in the developing organism. This does not seem likely because \textit{rb} is a central player in cell-cycle control.\textsuperscript{28} Perhaps other regions of the CNS are more resistant, having available redundant tumor-suppressor genes such as the \textit{PTCH} gene in the cerebellum.\textsuperscript{31} Finally, apoptotic selection of cells harboring a defective \textit{rb} allele may not be as efficient in the developing retina as it is in other tissues. The importance of programmed cell death in preventing \textit{rb}-mediated carcinogenesis has been highlighted by the results of transgenic mice studies.\textsuperscript{13} When \textit{rb} is inactivated in the developing retina, only generalized retinal apoptosis occurs. If \textit{p53} is also inactivated, however, tumors resembling retinoblastomas do occur. This observation suggests that the ability of the retina to activate cellular programmed cell-death pathways may modulate its susceptibility to \textit{rb}-mediated carcinogenesis.

Could inactivation of \textit{rb} lead to other CNS tumors? Various \textit{rb} mutations have been detected in astrocytomas.\textsuperscript{12,27} Recently, inactivation of \textit{rb} has been shown to cause medulloblastomas in mice.\textsuperscript{22} These highly aggressive embryonal tumors account for approximately 20\% of primary brain tumors in children. In the transgenic mouse model, inactivation of \textit{p53} or \textit{rb} was not sufficient to cause neoplastic transformation. The lack of \textit{rb} in the external granular layer of the cerebellum, together with somatic or germ-line \textit{p53} inactivation, however, did lead to the development of medulloblastomas. Although the most common chromosomal abnormality in medulloblastomas is...
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the loss of chromosome 17p, the p53 gene, which resides on the same chromosome, is rarely mutated in medulloblastomas.3

Studies of hereditary syndromes are providing insight into the molecular pathogenesis of medulloblastoma (reviewed by Taylor, et al.,26 in 2000). In patients with nevoid basal-cell carcinoma syndrome, there is an increased incidence of medulloblastomas, which is usually due to mutations in the PTCH. Mutations in the Wnt signaling pathway, particularly the adenomatous Polyposis coli gene, cause a form of Turcot syndrome that is associated with an increased incidence of medulloblastoma.

In patients with hereditary retinoblastoma there is also an increased risk of brain tumors, the most common of which are believed to represent pineoblastomas and supratentorial PNET. The occurrence of these tumors in the setting of a germline rb mutation is called a trilateral retinoblastoma.5 Although there are numerous reports of supratentorial PNETs (pineal and suprasellar) in retinoblastoma patients, it has been asserted, “the occurrence of a cerebellar medulloblastoma is almost unheard of.”26 Therefore, it is not clear whether the experimentally targeted inactivation of rb in the developing murine cerebellum is relevant to the formation of medulloblastomas in humans.

To address whether intracranial tumors in children with bilateral retinoblastoma originate in the pineal gland and whether the role of rb in medulloblastomas can be discounted, we provide a detailed clinicopathological study of an unusual trilateral retinoblastoma variant.

Materials and Methods

Hematoxylin and eosin–stained paraffin sections of ocular tumor and brain were available for review. Immunohistochemical analysis for neuron-specific enolase, retinal S-antigen, IRBP, synaptophysin, GFAP, and p53 were performed, as previously described.9,10 Blood chromosome studies were conducted using standard procedures. Slides prepared and chromosomal were banded using the Giemsa-trypsin-Giemsa method. Twenty cells from each sample were examined. Karyotypes were prepared and described as recommended in the International System for Human Cytogenetic Nomenclature.14

Molecular cytogenetic analyses were performed using FISH and whole-chromosome paints for chromosomes 13 and 18 (Vysis, Downer’s Grove, IL) according to the manufacturer’s instructions. Prepared slides were examined using a microscope (Van-Ox; Olympus America, Melville, NY) equipped with epifluorescence. High-speed color or slide film was used to document metaphase cells.

Case Report

History. At 9 months of age, the patient’s right pupil seemed enlarged to her parents. Clinical examination showed that the pupil was nonreactive and dilated to 7 mm. Funduscopy demonstrated an opaque red reflex and an intraocular mass with retinal detachment. A head CT scan revealed a calcified, intraocular mass within the right eye (Fig. 1A). The eye was removed because of a presumed retinoblastoma, and this diagnosis was confirmed histopathologically (see Pathological Findings). Postoperative-

ly, the patient received radiotherapy in the form of 4500 cGy delivered in 25 doses to the right orbit through a lateral orbital portal.

One month later, an examination performed while the patient was in a state of anesthesia revealed a second primary neoplasm, approximately one disc in diameter, and located temporally to the left optic nerve head. This lesion was subjected to laser ablation, and systemic vincristine, cytoxan, and adriamycin were administered. Regular funduscopic examinations and cerebral MR imaging conducted during the next 2 years revealed no evidence of disease progression.

When the patient was 4 years old, she presented to the authors’ institution with lethargy, irritability, and anorexia. Both CT and MR images demonstrated acute hydrocephalus caused by a large, enhancing mass in the midline cerebellum (Fig. 1B–D). Intraorbital disease was not apparent on these images. Furthermore, examination of the left fundus, performed while the patient was in a state of anesthesia, revealed only an elevated, hyperemic disc with no sign of recurrent or residual retinoblastoma. A right frontal ventriculoperitoneal shunt was placed initially. Three days later the tumor was resected through a suboccipital craniectomy. Histological findings confirmed the diagnosis of medulloblastoma (see later section). Despite having received palliative chemotherapy consisting of cytoxan and etoposide, the patient presented 5.5 months later with disease disseminated throughout the craniocaudal axis. She died 2 days later at the age of 5 years. Autopsy findings confirmed the spread of the medulloblastoma into the subarachnoid space, through the leptomeninges (Fig. 1E), and into the spinal cord.

Family History and Evaluation. The patient’s family pedigree is shown in Fig. 2; the patient (arrow) had two healthy older brothers, one of whom carries a balanced chromosome 13 translocation that he inherited from his mother (see later description). The mother has a learning disability and poor eye–hand coordination. She suffered three miscarriages, in each case at approximately 2.5 months gestation. One of the proband’s maternal uncles was stillborn. The other is blind in one eye and suffers from hearing loss and a learning disability. The proband’s maternal grand-

FIG. 2. Diagram showing the patient’s family pedigree. The arrow indicates the proband. Her mother is learning impaired and has poor eye–hand coordination. The proband’s living maternal uncle is blind in one eye and suffers from hearing loss and a learning disability. One of the proband’s brothers and their mother are carriers of the balanced chromosome 13 translocation. See text for further details.
father died of cancer when he was 48 years old. The remainder of the proband’s immediate family is healthy. There is no history of cancer on the father’s side of the family or on the maternal grandmother’s side of the family. The proband was treated for gastroesophageal reflux, reactive airway disease, and multiple otitis media infections. She had dysmorphic features (low-set ears and hairline as well as cleft palate), emitted a high-pitched cry, and demonstrated developmental delay.

Pathological Findings. The histopathological features of the patient’s ocular tumor are shown in Fig. 3. The neoplasm displayed an exophytic growth pattern in which the neural retina was completely detached from the retinal pigmented epithelium. In the center of the posterior chamber, neural retina from opposite poles was observed to be in apposition and adherent to the lens capsule, creating a pupillary block (Fig. 3A). The neoplastic cells replaced regions of the choroid (Fig. 3B) and involved the optic nerve head 0.1 mm beyond the lamina cribosa (data not shown). The main tumor mass consisted of characteristic cellular cords of viable neoplastic cells surrounding individual capillaries in a collaretic arrangement (Fig. 3C). These pseudorosettes were separated by areas of necrosis and basophilic mineral deposits, the latter accounting for the intraocular radio-density (Fig. 1A). Cytologically, the tumor displayed photoreceptor differentiation ranging from anaplastic cells to various types of rosettes (Fig. 3D). Flexner–Wintersteiner rosettes and Homer–Wright rosettes were clearly evident in many areas. True fleurettes, in which cytoplasmic processes could be observed to extend into the rosette’s lumen, were present in some areas (data not shown). Immunohistochemical studies revealed cytoplasmic staining for retinal S-antigen and IRBP (Fig. 4).

The patient’s cerebellar tumor consisted of densely packed primitive-appearing cells arranged in sheets and nodules, which were interspersed by connective tissue septae (Fig. 5A and B). Cytological investigation revealed that the cells had hyperchromatic, elongated nuclei with scant cytoplasm. Structures vaguely suggestive of Homer–Wright rosettes were focally seen. The frequency of mitotic figures ranged from one to three per 10 high-power fields. The tumor cells were immunoreactive for synaptophysin. No immunoreactivity for either retinal S-antigen
or IRBP was present. Scattered, entrapped GFAP-positive astrocytes were sometimes observed. These findings are characteristic of a medulloblastoma.

At autopsy, the brain was found to be diffusely edematous with a mass of 1030 g. Coronal sections of cerebellum revealed a 1.1-cm tannish-yellow lesion within the deep vermis underlying the fourth ventricle (Fig. 5C). The lesion infiltrated adjacent parenchyma and was partially necrotic. Sections of the lower thoracic, lumbar, and sacral spinal cord demonstrated multifocal subarachnoid collections of tumor cells. In several sections, the tumor directly infiltrated the parenchyma of the spinal cord, ranging from minute superficial nodules to frank invasion of the posterior columns, as seen in Fig. 5D. The pineal gland was identified and appeared normal. Histological examination of the left eye revealed no evidence of tumor.

Cytogenetic Findings. Constitutional karyotype analysis of the patient’s blood revealed an abnormal chromosome 13 in all cells examined. The abnormality was an interstitial deletion with breakpoints at q12.3 and q21.3 (Fig. 6). A balanced rearrangement consisting of an insertion of a portion of chromosome 13 into the long arm of chromosome 18 at band q23 was found in the patient’s mother. The same balanced rearrangement was also found in her brother (Fig. 6). The girl’s father and other siblings were examined and found to have normal karyotypes.

A FISH analysis performed using whole-chromosome paint for chromosomes 13 and 18 revealed the unbalanced nature of the patient’s karyotype (Fig. 7). The FISH analyses of chromosomes in the patient’s mother and brother revealed the insertion of chromosome 13 material into chromosome 18 and documented that no chromosome 18 material was present in the abnormal chromosome 13. The final karyotype designations in the affected parties were as follows: 46,XX,der(13)del(13)(q12.3q21.3)ins(18;13) (q23;q12.3q21.3)maternal in the patient; 46,XX,ins(18;13)9q23;9q23.q12.3q21.3) in her mother; and 46,XY,ins(18;13) (q23;q12.3q21.3)maternal in her brother.

Discussion

We are aware of only one other reported case in which a cerebellar neoplasm arose in a child with hereditary retinoblastoma. Nevertheless, the evidence shows that the ocular and cerebellar neoplasms in our patient were separate primary tumors. First, the cerebellar neoplasm could not represent a direct extension of the retinoblastoma. Although retinoblastomas can extend from the globe through the optic nerve, the tumor did not involve the nerve significantly beyond the lamina cribosa. Second, although the retinoblastoma involved the choroid, a hematogenous spread of disease is not a plausible explanation, given the absence of a vascular connection between the eye and the cerebellum. Third, the intracranial tumor was contained within the cerebellum. Finally, both tumors were found to have distinct histomorphological and immunocytochemical profiles. Given the imaging, postmortem, and histological data, we conclude that both the retinoblastoma and medulloblastoma occurring in our patient were separate primary neoplasms.

As advances in the treatment of retinoblastomas have led to a reduction in the risk of metastasis to a 5-year cumulative survival rate of 91% in the United States, coexistent intracranial tumors have become significant contributors to mortality in patients suffering from hereditary retinoblastoma. Intracranial tumors will develop in 8% of
patients with hereditary retinoblastomas, and the average length of survival following diagnosis of an intracranial tumor is 6 months. Consistent with these statistics, our patient died 5.5 months after diagnosis of the cerebellar tumor. Survival time in such patients can be significantly longer when the intracranial tumor is detected before the onset of related symptoms rather than afterwards. It has therefore been suggested that in patients with hereditary retinoblastoma contrast-enhanced CT or MR imaging be performed every 6 months until the child reaches 5 years of age.

The findings in our case support the emerging concept that the origin of intracranial neoplasms in children with hereditary retinoblastoma is less related to the pineal gland than previously believed. Bader and colleagues, drawing attention to the phylogenetic and ontogenetic relationship between the pineal gland and photoreception, postulated that mutant \(rb\) predisposes to the development of neuroblastic tumors arising in cells of photoreceptor origin. It was assumed that, in such cases, ectopic CNS tumors generally arise in the infant’s pineal gland. Those arising from the diencephalon have been thought to arise from germinal matrix cells located near cells that normally give rise to the

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**Fig. 5.** Results of histopathological analysis of the medulloblastoma. A and B: Photomicrographs of the cerebellar tumor showing that it consists of sheets of undifferentiated cells with numerous mitotic figures (arrows). The histological appearance of the tumor was similar in both surgical and autopsy specimens. C: Coronal section of cerebellum showing the tumor mass with indistinct borders infiltrating the surrounding cerebellar parenchyma (arrows). D: Section of thoracic spinal cord. Multiple foci of subarachnoid tumoral infiltration (asterisks) are present throughout the lower thoracic, lumbar, and sacral cords. Parenchymal infiltration (arrows) was also seen in multiple levels of the spinal cord. H & E, approximate original magnifications × 40 (A and B), and × 2 (D).

**Fig. 6.** Partial karyotypes determined by standard cytogenetic analysis. Left: The patient has a normal chromosome 18 and a shortened chromosome 13 (arrow). Right: Her brother has a balanced insertion of a portion of chromosome 13 into chromosome 18 (bracket).
optic cup. In a 1998 reevaluation of the histogenesis of trilateral retinoblastoma, however, Marcus, et al., suggested that these intracranial tumors are more likely to arise from a germinial layer of predisposed primitive subependymal neuroblasts that are not necessarily destined for pineal or photoreceptor differentiation. In the present case, the pineal gland was normal. Although the findings in our case tend to discount a relationship between the ectopic tumor and the retina, it is interesting that a subset of medulloblastomas are known to exhibit a retinoblastoma-like phenotype4,5,18,19 that apparently does not require rb mutations.15 In the present case, we found no evidence for photoreceptor differentiation, in either our morphological study or our immunohistochemical analysis for photoreceptor proteins.

Findings in the present case suggest that disruption of rb, recently shown to be capable of causing medulloblastomas in mice, may be relevant to this tumor in humans. Amplification of c-myc and n-myc,16 upregulation of PAX, inactivation of PTCH,17 and mutations in the Wnt signaling pathway26 have been associated with medulloblastomas. Recently, it was shown that Cre-LoxP–mediated inactivation of rb and p53 in the cerebellar external granular layer leads to the development of medulloblastomas in mice. Although disruption of rb is essential for the development of medulloblastoma in this system, the tumors only developed in the absence of p53, presumably because the lack of apoptosis is necessary for the tumors to escape programmed cell death. Inactivation of the cell-death pathway may be relevant to carcinogenesis of the cerebellum because medulloblastomas comprise 16% of brain tumors in patients with Li–Fraumeni syndrome. Interestingly, inactivation of p53 is not commonly found in sporadic medulloblastomas, and thus p53 is not currently implicated in the tumorigenesis of medulloblastoma. Although we did not formally rule out the presence of p53 mutations in our case by using direct DNA sequencing, the medulloblastoma was not shown to overexpress p53 protein by our immunohistochemical analysis. In contrast with the present case, rb mutations usually are not associated with medulloblastomas in humans. The lack of the rb mutations in sporadic medulloblastomas in humans could be explained by mutations involving other components of the rb pathway. Alternatively, rb could be functionally inactivated. Consistent with this mechanism is the recent finding by Zhen, et al., that SV40 TAG is expressed and forms specific complexes with p53 and rb in 33% of medulloblastoma cases.

Conclusions

In this paper, by providing a detailed characterization of both the ocular and intracranial tumors that developed in a child with a germline rb deletion, we provide evidence for the emerging concept that rb inactivation is not restricted to CNS regions of photoreceptor lineage. Furthermore, rb, whose inactivation can lead to medulloblastoma in mice, may also be relevant to humans.

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