EDULLOBLASTOMAS and GBMs are distinct tumor types affecting patients at different ages and locations, and they also differ in prognosis. The incidence of radiation-induced brain tumors is approximately 1%, yet they are an increasingly important complication of radiotherapy to the brain. Following radiotherapy, a radiation-induced neoplasm can occur after a long latency period, and a concomitant increase in the irradiation field indicates a direct role of exposure to ionizing radiations. The transformation of tissue into radiation-induced GBM following therapeutic doses of radiation depends on many physical and biological factors, but it is believed to result mainly from various types of radiation-induced genetic changes. Nevertheless, underlying radiation-induced mutations in oncogenes and tumor suppressor genes are not well known. In this study, we investigated radiation-induced genetic changes in a GBM occurring 10 years after treatment of a cerebellar medulloblastoma.

Case report

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✓ Radiation-induced glioblastoma multiforme (GBM) is a rare complication of radiotherapy. The authors report such a case occurring 10 years after treatment of cerebellar medulloblastoma. The patient was a 15-year-old boy who had undergone a gross-total removal of a medulloblastoma and received radiation therapy at the age of 5 years. He had experienced no tumor recurrences for 10 years until a new enhancing mass was found at the original site of the medulloblastoma. Following its resection the new lesion was found to be a GBM and there was no evidence of a medulloblastoma. The second tumor developed at the same site as the previous one after a sufficient latent period and fulfilled the criteria for a radiation-induced neoplasm. The original tumor cells expressed synaptophysin without p53 overexpression, a characteristic feature of medulloblastomas. In contrast, cells from the later tumor expressed glial fibrillary acidic protein and p53 but not synaptophysin. A sequence analysis of the p53 gene showed deletion at codon 233 and a C to G transition at codon 278 in the GBM but no mutation in the medulloblastoma. A GBM specimen revealed no amplification of the epidermal growth factor receptor compared with a normal control specimen. In conclusion, the clinical features of a radiation-induced GBM are similar to that of the primary GBM, whereas its genetic alterations render it a secondary GBM. These findings indicate that radiation-induced GBM should be considered a distinct clinical entity.

KEY WORDS • medulloblastoma • radiation-induced neoplasm • p53 gene mutation • glioblastoma multiforme • pediatric neurosurgery

MEDULLOBLASTOMAS and GBMs are distinct tumor types affecting patients at different ages and locations, and they also differ in prognosis. The incidence of radiation-induced brain tumors is approximately 1%, yet they are an increasingly important complication of radiotherapy to the brain. Following radiotherapy, a radiation-induced neoplasm can occur after a long latency period, and a concomitant increase in the irradiation field indicates a direct role of exposure to ionizing radiations. The transformation of tissue into radiation-induced GBM following therapeutic doses of radiation depends on many physical and biological factors, but it is believed to result mainly from various types of radiation-induced genetic changes. Nevertheless, underlying radiation-induced mutations in oncogenes and tumor suppressor genes are not well known. In this study, we investigated radiation-induced genetic changes in a GBM occurring 10 years after treatment of a cerebellar medulloblastoma.

Abbreviations used in this paper: CT = computerized tomography; EGFR = epidermal growth factor receptor; GBM = glioblastoma multiforme; GFAP = glial fibrillary acidic protein; MR = magnetic resonance; PCR = polymerase chain reaction.

Case Report

Initial Presentation, Operation, and Adjuvant Therapy. This 5-year-old boy was first admitted to our hospital in December 1991 because of headache, vomiting, and gait disturbance, which he had experienced throughout the previous month. Neither the patient nor his family had any known genetic disease predisposing to cancer. Magnetic resonance imaging revealed an enhancing mass lesion in the cerebellar vermis compressing the fourth ventricle (Fig. 1) as well as obstructive hydrocephalus. A gross-total resection of the tumor was performed in January 1992 and the tumor was histologically diagnosed as a medulloblastoma. Postoperatively, the boy received a total of 36 Gy of radiation to the brain, an additional 20 Gy to the primary tumor site as a focal boost, and 24 Gy to the spine followed by 10 courses of chemotherapy following an eight-in-one regimen (solumedrol, vincristine, lomustine, procarbazine, hydroxyurea, cisplatin, cytoxan, and cytosine arabinoside). After these treatments, he underwent CT scanning or MR imaging at regular follow-up intervals, with no evidence of recurrence until October 2001 (Fig. 2).
Second Presentation, Operation, and Adjuvant Therapy. The boy was readmitted in December 2001, at 15 years of age, because of increased aspiration and general weakness during the previous month. A CT scan revealed a large, poorly delineated enhancing mass at the original tumor site. Magnetic resonance imaging demonstrated an irregularly well-enhancing mass lesion in the posterior fossa extending to the left cerebellopontine angle and suspicious brainstem invasion (Fig. 3). The tumor was subtotally removed. After the second operation, the boy received chemotherapy with tamoxifen, irinotecan, cisplatin, and 13-cis retinoic acid; however, he deteriorated rapidly and died of tumor regrowth in August 2002, at the age of 16 years.

Histological Examination. The first tumor was highly cellular and consisted of round to oval cells with hyperchromic nuclei, which were round to carrot shaped and remarkably ill-defined, and scanty cytoplasm. Immunohistochemically, the neoplastic cells strongly expressed synaptophysin and showed focal cytoplasmic staining for GFAP. No p53 immunoreactivity was noted. These findings are typical of medulloblastomas with focal glial differentiation (Fig. 4A–D). The histological features of the two tumors were strikingly different. The second one consisted of large pleomorphic cells with a high mitotic rate, pseudopalisading geographic necrosis, and prominent endothelial proliferation. Immunohistochemically, most cells were immunoreactive for GFAP and p53 but not for synaptophysin. No findings suggestive of medulloblastoma were observed. The histopathological diagnosis was that the tumor was a GBM (Fig. 4E–H).

The DNA Sequencing Analysis of p53. Genomic DNA of the medulloblastoma was obtained from the formalin-fixed, paraffin-embedded archival tissue and that of the GBM was obtained from the second tumor tissue frozen in liquid nitrogen by using a DNA isolation kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations.

The PCR technique was used to amplify the genomic DNA corresponding to exons 5 through 8 of the p53 gene. Amplification was performed in a 20-µl reaction mixture containing 100 ng of genomic DNA, 0.25 µM of each of the primers, 100 µM each of deoxynucleoside triphosphate, 1.5 mM MgCl₂, 0.4 U of Taq DNA polymerase (Perkin-Elmer, San Francisco, CA), and 2 µl of 10 × PCR buffer. Amplification was performed for 35 cycles, each consisting of denaturation at 95°C for 5 minutes, annealing for 1 minute (55°C for exons 5 and 8, 56°C for exon 6, and 59°C for exon 7), and extension at 72°C for 1 minute. The final extension was continued for 10 minutes and the products were stored at 4°C. The sense and antisense primers...
used for the four exons were as previously described:4 exon 5, 5'-TTCAACTCTGTCTCCTCTCCT-3' and 5'-CAGCCC TGTCGTCTCTCCAG-3'; exon 6, 5'-GCCTCTGATTCC TCACCTGAT-3' and 5'-TTAACCCCTCTCCCCAGAGA -3'; exon 7, 5'-AGGCACCTGGCCCTCATCTT-3' and 5'-TGTCAGGGCACGCAAGTGGC-3'; and exon 8, 5'-T TCTTACGTGGCTTCTGCTT-3' and 5'-AGGCATACT GCACCTTG-3'. The amplified DNA fragments were purified with a quick PCR purification kit (QIA; Qiagen) and the purified product was sequenced with an automated sequencing system (ABI PRISM 3700; Perkin-Elmer). All mutations detected by direct sequencing were confirmed by a second PCR on fresh DNA samples.

On direct sequencing of the medulloblastoma, no p53 mutations were detected. On the other hand, direct DNA sequencing analysis revealed two mutations in the PCR products derived from the GBM: deletion at codon 233, exon 7, and a C to G missense at codon 278, exon 8, which leads to an amino acid change from proline to alanine (Fig. 5).

Real-Time Quantitative PCR Analysis of EGFR. Real-time quantitative PCR analysis was performed on the genomic DNA of the GBM. A portion of neocortex, obtained from a 2-year-old boy with intractable epilepsy, and a sample of a medial temporal lobe ganglioglioma served as control samples. The neocortex was histologically confirmed to correspond to bases 3964 through 4052 of the complementary DNA sequence. The reactions were performed in a 25-μl reaction volume containing 12.5 μl of 2 × SYBR Green Master Mix (Applied Biosystems), 9 μM forward primer, 2.5 μl reverse primer, and 10 ng/μl of genomic DNA with a sequence detection system (ABI PRISM 7700; Applied Biosystems).

The thermal profile for all PCRs done with SYBR Green was 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. When using SYBR, the human GADH gene (Applied Biosystems) was used for standardization. The cycle threshold values of normal brain and GBM tissues were calculated and compared.

Real-time quantitative PCR performed using a GBM specimen revealed no amplification of EGFR compared with a normal control specimen. Relative quantitation of EGFR transcripts from normal brain tissue and GBM tissue was 1 and 0.21, respectively.

Discussion

Medulloblastomas account for 20 to 25% of all childhood intracranial malignancies. With primary surgery and adjuvant radiotherapy, the 5-year survival rate for most pediatric patients is approximately 58%. Nevertheless, curative central nervous system irradiation in young children may result in significant delayed side effects such as cognitive or motor dysfunction and radiation-induced neoplasm.

In the present case, the later tumor was different from the initial medulloblastoma histologically, immunohistochemically, and from the standpoint of molecular biology. Furthermore, this patient survived the period of risk for recurrence without relapse and should have been considered free of risk for recurrence according to the Collins law.55 Since the first case of radiation-induced GBMs published in 1978 by Kleriga, et al.,40 more than 10 cases of such gliomas have been reported (Table 1).5,6,10,12-14,16,17 Those cases and ours fulfill the criteria for radiation-induced neoplasm described by Cahan:4,15 1) the occurrence of a second tumor within the field of irradiation used to treat the primary disease; 2) a prolonged latency period between radiotherapy and the detection of the second tumor (usually several years); 3) a histological difference between the primary and subsequent tumor; 4) the rarity of the tumor in patients who have not been treated with radiotherapy; and 5) no known genetic or other conditions predisposing to secondary malignancy.

The appearance of radiation-induced GBMs following therapeutic doses of irradiation depends on many physical
Fig. 4. Photomicrographs depicting the two tumor types. A: The first tumor is highly cellular and is composed of round to oval cells having carrot-shaped hyperchromatic nuclei and scanty cytoplasms, characteristics typical of medulloblastomas. B–D: Immunohistochemically, the primary tumor shows strong immunoreactivity for synaptophysin and very focal cytoplasmic staining for GFAP. No p53 immunoreactivity is noted. E: Histological features of the second tumor are strikingly different from those of the original tumor. The tumor consists of large pleomorphic cells with a high mitotic rate, consistent with a GBM. F–H: The second tumor shows strong immunoreactivity for both GFAP and p53 but not for synaptophysin. H & E (A and E); synaptophysin immunostaining (B and F); GFAP immunostaining (C and G); and p53 immunostaining (D and H). Original magnifications × 100 (A) and × 200 (B–H).
and biological factors, but they are believed to result from various types of radiation-induced genetic changes. Radiation-induced genomic instability accumulates gene mutations and gross chromosomal rearrangements, both of which has been thought to play a role in radiation-induced tumorigenesis.\textsuperscript{22} Radiation-induced initial DNA damage in surviving cells is memorized. After irradiation, potentially unstable chromosomal regions are transmitted over many generations through the progeny of surviving cells.\textsuperscript{21} This transmission of memory causes delayed DNA breakage, which in turn plays a role in the induction of delayed phenotypes. This process offers a possible explanation of the delayed loss of the wild-type $p53$ allele.\textsuperscript{22} With ultraviolet irradiation, for example, it has been shown that loss of the wild-type $p53$ allele can account for the late development of skin cancer in the elderly, from a single cell with an early mutation due to exposure to the sun in childhood.\textsuperscript{24} The tumorigenic effects on the target tissues also depend on the proliferative state of the target tissue at the time of radiation exposure. It has been suggested that radiation-induced neoplasms are caused by imperfect repair of ionizing radiation-induced DNA strand breaks in tumor suppressor genes or protooncogenes.\textsuperscript{3}

The spectrum of genetic alterations in radiation-induced GBMs is currently thought to be similar to those of the primary GBM;\textsuperscript{3} for example, both have less common $p53$

### Table 1

<table>
<thead>
<tr>
<th>Authors &amp; Year</th>
<th>Age (yrs), Sex</th>
<th>Dose of Radiation</th>
<th>Latency (yrs)</th>
<th>Histological Diagnosis</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kleriga, et al., 1978</td>
<td>1, M</td>
<td>50 Gy head, 25 Gy spinal cord</td>
<td>11</td>
<td>malignant astrocytoma</td>
<td>lt cerebellum</td>
</tr>
<tr>
<td></td>
<td>5, M</td>
<td>30 Gy whole brain &amp; 10 Gy pst fossa, 20 Gy spinal cord</td>
<td>13</td>
<td>glioblastoma</td>
<td>pt parietal lobe</td>
</tr>
<tr>
<td>Cohen, et al., 1981</td>
<td>4, F</td>
<td>35 Gy whole brain &amp; 10 Gy pst fossa, 30 Gy spinal cord</td>
<td>14</td>
<td>malignant astrocytoma</td>
<td>lt frontal lobe</td>
</tr>
<tr>
<td>Schmidbauer, et al., 1987</td>
<td>13, M</td>
<td>60 Gy whole brain</td>
<td>6</td>
<td>glioblastoma</td>
<td>rt cerebellum</td>
</tr>
<tr>
<td>Safneck, et al., 1992</td>
<td>2, M</td>
<td>44 Gy whole brain &amp; 10 Gy pst fossa, 30 Gy spinal cord</td>
<td>9</td>
<td>malignant astrocytoma</td>
<td>lt optic nerve</td>
</tr>
<tr>
<td>Osumi, et al., 1994</td>
<td>14, F</td>
<td>50 Gy whole brain, 28 Gy spinal cord</td>
<td>9</td>
<td>low-grade astrocytoma</td>
<td>rt cerebellum</td>
</tr>
<tr>
<td>Furuta, et al., 1998</td>
<td>8, M</td>
<td>40 Gy whole brain &amp; 15 Gy pst fossa, 30 Gy spinal cord</td>
<td>15</td>
<td>malignant astrocytoma</td>
<td>lt cerebellum</td>
</tr>
<tr>
<td>Nakamizo, et al., 2001</td>
<td>11, M</td>
<td>30 Gy whole brain &amp; 24 Gy pst fossa, 30 Gy spinal cord</td>
<td>9</td>
<td>malignant astrocytoma</td>
<td>rt cerebellum</td>
</tr>
<tr>
<td></td>
<td>18, F</td>
<td>30 Gy whole brain &amp; 20 Gy pst fossa, 30 Gy spinal cord</td>
<td>9</td>
<td>malignant astrocytoma</td>
<td>4th ventricle</td>
</tr>
<tr>
<td>present case</td>
<td>5, M</td>
<td>36 Gy whole brain &amp; 20 Gy pst fossa, 24 Gy spinal cord</td>
<td>10</td>
<td>glioblastoma</td>
<td>lt cerebellum</td>
</tr>
</tbody>
</table>

* Pst = posterior.
mutations. One study showed that complete genomic DNA sequence analysis of the p53 gene revealed a single point mutation in one of nine radiation-induced gliomas sequenced.\textsuperscript{3} Crucial differences do exist between radiation-induced and primary GBMs, however. First, no radiation-induced GBMs studied showed PTEN mutations.\textsuperscript{3,23} In contrast, 32\% of primary GBMs studied showed a PTEN mutation and a homozygous PTEN deletion.\textsuperscript{23} Second, radiation-induced GBMs showed a slightly lower percentage of EGFR and p16 alterations than did primary GBMs.\textsuperscript{3,7} Third, radiation-induced gliomas occur in a younger patient population than would be expected for primary, high-grade gliomas.\textsuperscript{19} Fourth, almost all radiation-induced gliomas are astrocytic (that is, they are not oligodendroglial or ependymal) in their histological differentiation, compared with 70\% of primary GBMs.\textsuperscript{3,11}

In the present case, the secondary GBM showed clinical features similar to that of the primary GBM: rapid progression and poor prognosis. Molecular genetic analysis of the radiation-induced GBM showed p53 mutations, however, including a missense mutation and no EGFR amplification, suggesting secondary GBM.\textsuperscript{3,4} The sudden occurrence and rapid growth of the later tumor can be explained by clonal expansion of tumor cells with genetic mutations and radiation-induced, delayed loss of the wild-type p53 allele.\textsuperscript{18,22} Although the p53 mutation is not solely responsible for radiation-induced GBM, it is possible that radiation-induced genetic instability is accelerated after the mutation of the p53 gene.\textsuperscript{3,7,9,23}

Conclusions

The clinical features of the secondary radiation-induced GBM we discuss are similar to those of the primary GBM, whereas its genetic alterations render it a secondary GBM. These findings support the notion that radiation-induced GBM should be considered a distinct clinical entity. Further understanding of multiple genetic changes after radiation will help protect patients who have already been cured of their original malignancy.

References


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