Ganglioglioma in a patient with Turcot syndrome

Case report

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TURCOT syndrome (also known as BTP syndrome) is a heritable disorder characterized by an association of primary neoplasia of the CNS and colorectal cancer.21 The mode of genetic transmission of the syndrome still remains unclear because Turcot syndrome is a rare disorder. The development of molecular genetic analyses, however, has led to progress in resolving the genomic abnormality of this syndrome. At present, patients with Turcot syndrome seem to encompass a heterogeneous group that can be classified into two clinical types based on clinical features and genomic disorders.2,4,15 The first type (BTP syndrome Type 1) consists of patients having glioma, colorectal adenoma without polyposis, and DNA replication errors. The second type (BTP syndrome Type 2) consists of patients having medulloblastoma, FAP (or FAP kindred), and germline mutation of the \( \text{APC} \) gene.15

The vast majority of CNS neoplasms associated with Turcot syndrome are glioma, and in particular, glioblastoma multiforme, and medulloblastoma.15 Although other types of CNS tumors related to Turcot syndrome have been noted in a few case reports, there is no report of ganglioglioma in Turcot syndrome. To the authors’ knowledge, this is the first report of a patient with Turcot syndrome who had a ganglioglioma in the CNS and an adenocarcinoma in the colon.

Abbreviations used in this paper: APC = adenomatous polyposis coli; BTP = brain tumor–polyposis; CNS = central nervous system; FAP = familial adenomatous polyposis; NFP = neurofilament protein.

Case Report

History. This 33-year-old woman had a sister in whom colon cancer and a brain tumor had been diagnosed. The detailed histological diagnosis in her sister’s case was unknown, but her parents had no history of cancer. In 1996, the patient was diagnosed as having colon cancer and underwent a subtotal colectomy at another hospital. Results of pathological examination of the resected specimen revealed a few polyps, but not polyposis, and the cancer was diagnosed as an adenocarcinoma with a serosal extension and local lymph-node metastasis. Because the patient suffered episodes of unconsciousness after her colectomy, she consulted clinicians at our department in March 1997.

Examination. The patient exhibited no neurological deficit and no skin pigmentation such as café-au-lait spots. An electroencephalogram indicated an epileptic pattern on the left frontal leads. Results of computerized tomography scanning studies revealed a large low-density lesion at the left frontotemporal lobe, similar to an arachnoid cyst, without a high-density area suggesting calcification. Results of magnetic resonance imaging studies confirmed a large, well-demarcated intracerebral lesion in the same area. The tumor appeared as a hypointense signal on the \( T_1 \)-weighted image, with no enhancement after administration of contrast material, and as a hyperintense signal on the \( T_2 \)-weighted image (Fig. 1). The fluid attenuation inversion recovery image best distinguished the solid nature of the lesion. Angiography results showed no remarkable findings.
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Operation. In August 1997, the patient initially underwent a stereotactic biopsy for histological diagnosis, the results of which characterized the lesion as a low-grade glioma. Two weeks later, the patient underwent a standard frontotemporal craniotomy with subtotal resection of the tumor. The tumor was entirely composed of soft, gray, solid matter, with no cystic portion. The patient experienced neurological deficits after the operation.

Findings of Pathological Study. The tumor tissue was composed of astrocytic and neuronal components. Hematoxylin and eosin staining visualized a diffuse growth of astrocytic cells in a fibrillary background. The astrocytic component, which did not contain areas of pronounced hypercellularity, vascular proliferation, necrosis, or mitotic figures, resembled a low-grade fibrillary astrocytoma (Fig. 2A). Based on the results of immunohistochemical analysis, the astrocytic cells and fibrillary background stained diffusely positive for glial fibrillary acidic protein (Fig. 2B). In the fibrillary backgrounds, large polygonal cells with abundant eosinophilic cytoplasm and eccentric large nuclei were scattered haphazardly. These cells were entirely, but unevenly, distributed in the resected specimens and were grouped in clusters without a constant direction (Fig. 2C). Results of morphological analysis showed that the cells were compatible with ganglion cells, and binucleated ganglion cells, a specific feature of neoplastic neuron cells, were recognized (Fig. 2D arrow). Synaptophysin immunoreactivity study results appeared as a diffuse granular precipitate along the surfaces of the cell membranes in large ganglion cells and, occasionally, in small cells (Fig. 2E). The synaptophysin-positive small cells were compatible with the cells having neuronal differentiation suggested by electron microscopy. The addition of antibody for NFP (68 kD) revealed cytoplasmic stainings in ganglion cells (Fig. 2F). The occasional linear stainings for synaptophysin and NFP in the background presumably denoted entrapped axons.

Electron Microscopic Findings. Electron microscopic evaluation of the tumor tissues also revealed two populations: astrocytic cells and neuronal cells. The astrocytic cells had ovoid nuclei with coarse chromatin, a moderate amount of cytoplasm, and cytoplasmic projections. Common organelles included dense bodies of varying size, mitochondria, and free polyribosomes; bundles of intermediate filaments were occasionally recognized (Fig. 3A). Although the tissue processed for electron microscopy did not contain typical ganglion cells, we did find smaller cells having round nuclei with irregular infoldings, scant cytoplasm, and long slender cytoplasmic projections. Fasicles of microtubules, characteristic cytoskeletal structures indicating neuronal differentiation, were variably represented within the perikarya (Fig. 3B). Dense core granules, the most distinctive organelles in neoplastic neuronal cells, were not identified within these cells.

In accordance with the microscopic, immunohistochemical, and electron microscopic findings, the tumor was diagnosed as a ganglioglioma Grade II.

Molecular Genetic Analysis. The germline status of the APC gene was determined using peripheral blood leukocyte RNA and DNA obtained in the patient. The APC gene has 15 exons that encode 2844 amino acids and two noncoding exons. Mutations of the APC gene found in patients with FAP are located widely in the entire coding region and have no hot spots, unlike the p53 gene.13,14 Because almost all mutations truncate the gene product by frame shift due to deletion or nonsense mutation,13,14,16,17 the APC gene was analyzed using a protein truncation assay created by Powell and colleagues16 and developed by Prosser and coworkers.17 This system needed six sets (systems A–F) of primers to cover the entire APC coding region (exons 1–15), but showed no abnormality in the APC gene in the patient (Fig. 4).

Postoperative Course. The patient did not undergo radiotherapy or chemotherapy after tumor resection. Her episodes of unconsciousness have been controlled by a regimen of anticonvulsant medications. One year after surgery, results of follow-up magnetic resonance images demonstrated no evidence of tumor recurrence.

Discussion

Data from several large series of gangliogliomas have served to delineate the general characteristics of this tumor.1,3,9,19,22 Patient ages varied widely; 63 to 83% of patients were younger than 20 years at the time of diagnosis, with peak occurrence during the second decade.3,5,9 Hirose, et al.,3 noted that the most common presenting symptom was long-term seizures, which occurred in 73% of their patients, and that more than half of the tumors were located in the temporal lobes.

It is imperative to distinguish ganglioglioma from infiltrative glioma, particularly fibrillary astrocytoma because...
of differences in treatment and prognosis. Based on histological results, gangliogliomas are composed of an intimate admixture of neoplastic astrocytes and atypical ganglion cells.\textsuperscript{3,9,19,22} When ganglion cells are present in only small numbers, it is difficult to distinguish neoplastic cells from normal neurons engulfed by infiltrative glioma. Bi-nucleated ganglion cells are an accepted marker of the neoplastic nature of the neuronal cells, but they are not always present in ganglioglioma.\textsuperscript{3,9} Miller and associates\textsuperscript{9} defined four important features for the correct diagnosis: 1) clusters of large cells potentially representing neurons; 2) no perineuronal clustering of glial cells around the alleged neoplastic neurons; 3) fibrosis; and 4) calcification. Immunohistochemical analysis performed using neuronal and glial markers is important in diagnosing gangliogliomas. Synaptophysin, NFP, chromogranin A, class 3 β-tubulin, and neuropeptide Y have been reported to be neuronal markers.\textsuperscript{1,3,9,19} Some authors especially recommend synaptophysin immunostaining for detecting neoplastic neurons within gangliogliomas because such cells exhibit dense surface perikaryal staining, whereas normal neurons exhibit no stain.\textsuperscript{3,9,22} However, other recent investigators have cautioned that normal neurons are also immunopositive for synaptophysin; they reported finding synaptophysin-positive neurons and irregular white-matter synaptophysin immunostaining in the normal brain\textsuperscript{18} and synaptophysin-positive neurons in the spinal cord.\textsuperscript{23} The fact that synaptophysin-positive neurons are found in gangliogliomas is not definitive proof; other histological and ultrastructural evidence must be considered for an accurate diagnosis.

The most characteristic finding in electron microscopy studies of the neuronal component of gangliogliomas is the presence of large and polygonal neuronal cells containing accumulation of dense core granules, a well-developed Golgi apparatus, and a rough endoplasmic reticulum.\textsuperscript{3} Although we did not detect typical large ganglion cells in our tissue samples viewed by electron microscopy, we did find small cells containing microtubules, which indicated neuronal differentiation within the perikarya. These cells were believed to be compatible with synaptophysin-positive cells in immunostaining. Such cells have also been reported by other authors. Miller and associates\textsuperscript{9} reported that four of 63 cases in their series contained, in addition to large neoplastic ganglion cells, clusters of small round cells resembling oligodendroglioma. These cells were surrounded by a neuropil-like background of synaptophysin immunopositivity. Wolf and associates\textsuperscript{22} noted small or intermediate-sized neurons in their series of 61 cases. In viewing 27 gangliogliomas through an elec-

![Fig. 2. Photomicrographs depicting the results of H & E staining (A, C, and D) and immunostaining (B, E, and F) of the tumor. Original magnification × 200. A: Large ganglion cells scattered in the glial component. B: Astrocytic cells diffusely stained with glial fibrillary acidic protein. C: Clusters of ganglion cells. D: Rare binucleated ganglion cells (arrow). E: Synaptophysin-positive diffuse granular precipitate along surfaces of cell membranes of large ganglion cells. F: Reaction to NFP (68 kD) antibody in some ganglion cells.](Image)
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electron microscope, Hirose, et al., noted that some neuronal cells with small nuclei and scant cytoplasm resembled neurocytes. The authors speculated that this tumor originates from pluripotent progenitor cells. These reports suggest that the presence of small cells resembling glioma cells with neuronal differentiation, in addition to the presence of large ganglion cells (especially binucleated ganglion cells), might be a feature of and thus facilitate the diagnosis of ganglioglioma.

Because the description by Turcot and colleagues of the association between neuroepithelial tumors of the CNS and adenomatous polyposis of the large intestine, the eponym “Turcot syndrome” has been used for patients having these two conditions. Investigators disagree on whether this association is a random occurrence of two separate diseases or constitutes a distinct genetic disorder. However, the distinct category of Turcot syndrome has been clarified by recent molecular genetic findings. Paraf, et al., reviewed 151 BTP cases referred to as Turcot syndrome in the literature. The following brain neoplasms were reported: glioma (55%), including astrocytoma Grades II to IV; ependymoma and polar spongioblastoma; medulloblastoma (24%); pituitary adenoma; primary CNS lymphoma; meningioma; and craniopharyngioma. In addition to Paraf’s review, a case of malignant ependymoma and a combined case of medulloblastoma and glioblastoma have been recently reported. To our knowledge, our patient is the first to harbor a ganglioglioma as part of Turcot syndrome.

According to recent clinical and molecular reports, patients with Turcot syndrome are classified into two distinct groups based on the phenotype of the polyps, the presence of colorectal cancer, the type of brain neoplasm (glioma or medulloblastoma), the presence of skin lesions and café-au-lait spots, and consanguinity. The first group, BTP syndrome Type 1, is composed of patients who have glioma and colorectal adenomas without polyposis (non-FAP cases) and siblings with glioma or colorectal adenoma or both. Brain tumor–polyposis syndrome Type 1 should be recognized as true Turcot syndrome because it corresponds to the description provided in the initial report by Turcot and colleagues. In their study, the brain neoplasms were almost exclusively glioma (98%; 81% were malignant astrocytomas), and most patients (62%) had a colorectal phenotype of Group 1, as defined in the classification scheme of Itoh, et al. This group of patients also demonstrated a higher prevalence of colorectal adenocarcinoma. Furthermore, the distribution of skin lesions was also significantly different; there was a higher prevalence (53%) especially of café-au-lait spots, which were present in only 6% of FAP patients. Eighty percent of the patients with malignant glioma were younger than 20 years (prevalence occurs in an older age group in the general population), which strongly supports the existence of an underlying genetic cause. The neoplasms in these patients exhibited DNA replication errors, which suggests a relationship to hereditary nonpolyposis colorectal cancer, a disease characterized by germline alterations in DNA mismatch repair genes. Some authors have pointed out that patients with this syndrome harbored germline mutations of the genes hMSH2 (human mutS homolog 2), hMLH1 (human mutL homolog 1), and hPMS1 and hPMS2 (human postmeiotic segregation 1 and 2). Taylor, et al., reported a case of Turcot syndrome that featured a germline mutation of exon 5 of the hPMS2 gene. Two metachronous glioblastomas, both with distinct oligodendrogliomas, developed in this patient, revealing that the same genetic defect was not commonly involved in sporadic oligodendrogliomas or glioblastomas. The mechanism of DNA replication errors and DNA repair system has not been clarified, however, and further studies are necessary to understand the mechanism of carcinogenesis.

The second group, BTP syndrome Type 2, is typically composed of patients who harbor both a brain neoplasm, usually a medulloblastoma (58%), and colorectal polyposis and who belong to a family with FAP. In this phenotype, occurrence of colorectal cancer without polyposis is rare and skin lesions are infrequent. These patients have a germline mutation in the APC gene. Hamilton and co-workers revealed a 92-fold increase in the relative risk of developing a medulloblastoma among families with FAP. This suggests a direct association between FAP and the development of brain tumors. It may also represent a pleiotropic manifestation of the APC gene defect or a predisposition to the development of brain tumors as a result of a subsequent somatic mutation that causes the loss of...
the normal allele at the \textit{APC} gene locus.\textsuperscript{2,12} Our patient had colon cancer without polyposis and a ganglioglioma; she also had a sibling who had had colon cancer and a brain tumor. Results of the protein truncation assay showed no germline mutation in our patient’s \textit{APC} gene. Thus, our patient belongs to BTP syndrome Type 1 of Turcot syndrome, which suggests a strong possibility that she had germline alterations in her DNA mismatch repair genes.

It is difficult to establish strict guidelines to screen for CNS lesions in patients with a hereditary colon adenoma or cancer. However, care should be taken for patients and families in whom there has been a diagnosis or suspicion of Turcot syndrome. Symptoms or signs suggesting a CNS tumor require prompt and careful investigation in patients with FAP, hereditary nonpolyposis colorectal cancer, or multiple tumors with DNA replication errors and in at-risk offspring or siblings. At-risk members of families with FAP who have brain tumors can be tested for a mutant \textit{APC} gene. They should also be evaluated for colorectal adenomas or cancer.

\textbf{Acknowledgments}

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\textbf{References}

evidence for linkage to the adenomatous polyposis coli (APC) locus. Neurology 44:1083–1086, 1994

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