Detection of JC virus DNA sequences in brain tumors in pediatric patients

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Object. The JC virus is a human neurotropic polyomavirus that causes progressive multifocal leukoencephalopathy and is closely related to simian virus 40. Several recent reports have indicated a possible association between the JC virus and the development of various human brain tumors. The authors examined the presence of JC virus DNA sequences in primary brain tumors in pediatric patients to evaluate the hypothesis that particular brain tumors can arise in the pediatric population as a consequence of infection with the JC virus.

Methods. Genomic DNA sequences were isolated from 62 brain tumors (32 medulloblastomas, 18 ependymomas, five choroid plexus papillomas, and seven pilocytic astrocytomas) and analyzed for the presence of JC virus DNA by Southern blot hybridization and direct sequencing. The JC virus DNA sequence was detected in five ependymomas and one choroid plexus papilloma. Immunohistochemical studies revealed nuclear expression of the large T-antigen in a choroid plexus papilloma. None of the medulloblastomas or pilocytic astrocytomas contained JC virus DNA.

Conclusions. The results of this study provide molecular evidence of the association between JC virus and the development of certain ependymomas and choroid plexus papillomas.

Key Words • JC virus • T-antigen • brain neoplasm • pediatric neurosurgery

Viruses have been known to induce experimental tumors and some play an important role in the development of particularly human malignancies such as cervical carcinomas and lymphomas. The first of such discoveries was the report of the Rous sarcoma virus in 1912.13 The JC virus belongs to the polyomavirus group and was isolated in 1971 from the brain tissue of a patient with progressive multifocal leukoencephalopathy.31 The JC virus is related to SV40 and contains a genome of closed, circular, double-stranded DNA that contains the following three functional domains: the early coding region, the late coding region, and the transcriptional control region (Fig. 1).12,19 The JC virus Tag in the early coding region has been found to interact with p53 or retinoblastoma tumor-suppressor proteins and block their inhibitory effects on cell proliferation, thus resulting in tumor development.4,8,14,15,25 For many decades since its discovery, SV40 was studied for its possible involvement in the process of oncogenesis in several human brain tumors.1,18,23,27,34 More recently, several reports have indicated that the JC virus may be associated with oncogenesis in various brain tumors.6,7,24 However, this potential association has not been determined conclusively. In the present experiment we investigated the presence of the JC virus DNA in various pediatric CNS tumors to determine whether the JC virus is actually involved in tumorigenesis. We used four sets of primers to detect the presence of the JC virus DNA via PCR experiments and confirmed these results by Southern blot hybridization and direct sequencing. Immunohistochemical staining was used to detect the expression of Tag and viral capsid protein in tumor tissue samples.

Materials and Methods

Clinical Samples and DNA Extraction

The associated records for 32 medulloblastomas, 18 ependymomas (including nine supratentorial and nine infratentorial ependymomas), five choroid plexus papillomas, and seven pilocytic astrocytomas were retrieved from the files of the Department of Neurosurgery at Saga University and Okayama University. The patients harboring these lesions had been treated between 1975 and 2003. Each institution’s respective research review ethics committee approved the study. Tumors were histologically classified according to the World Health Organization criteria.32 Several paraffin sections (10 μm thick) from each sample were deparaffinized using xylene. The DNA was extracted using a DNeasy Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. A fresh blade was used for each specimen to prevent DNA contaminations in each sample.

Abbreviations used in this paper: CNS = central nervous system; PCR = polymerase chain reaction; SV40 = simian virus 40; Tag = T-antigen; VP = viral protein.
Polymerase Chain Reaction Amplification

Sample DNA was amplified by PCR in a final volume of 50 μl that contained the following: 10 mM Tris-HCl, 50 mM KCl, 1 mM MgSO4, 0.2 mM deoxynucleoside triphosphate, 0.2 μM of each specific primer, and 1 U of Tag polymerase. Hot-start PCR with the aid of a thermal cycler (Biometra, Göttingen, Germany) was performed for 45 cycles, each of which consisted of denaturation at 95°C for 20 seconds, annealing at each temperature for 20 seconds, and extension at 72°C for 20 seconds. This was followed by a final 7-minute extension at 72°C. The samples amplified in the absence of template DNA served as negative controls. The JC viral genome (strain MAD-1) was purchased from American Type Culture Collection (Manassas, VA). Inclusion of the JC viral genome served as a template and positive control for each procedure. All samples were screened with four sets of primers that have been previously described (Fig. 1).9,32 The primer pairs JCE1 (CCCCATACCAACATTAGCTTTC) and JCE2 (CCAGATTTGTAAGGCAGATAG) were used to amplify the JC virus Tag sequence (242 bp, annealing temperature 55°C); JCC1 (TTTCCCTCATATTCAGCACTT) and JCC2 (TTCCTCCCTATTCAGCACTT) were used for the control sequences (381 bp, 55°C); and VP2 (CTAGTTGTCGTCGTCTCTGC) and VP3 (CTAGTTGTCGTCGTCTCTGC) were used to amplify the JC virus-specific VP1 capsid gene sequences (212 bp, 54°C). The primer pairs AGNO1 (GTTCTTCGCCAGCTGTCAC) and AGNO2 (GTCTGCTCAGTCAAACCACTG) were used to amplify sequences within the coding region of the JC virus agnoprotein, which is a 71-amino acid protein in the late region that may modulate viral gene transcription and DNA replication (179 bp, 57°C).33

Southern Blot Hybridization

The PCR products were separated by electrophoresis in 2% agarose gel. The gels were transferred to a nylon membrane (Hybond; Amersham, Little Chalfont, UK) in 0.4 M NaOH. The membranes were prehybridized for 30 minutes in Church phosphate buffer, followed by hybridization in the same buffer containing 5 x 10° cpm/ml [y-p]adenosine triphosphate end-labeled oligonucleotide which is specific for the JC virus. Blots were washed twice in 2 x sodium do-decyl sulfate/0.1% standard saline citrate for 10 minutes and twice in 0.5 x sodium dodecyl sulfate/0.1% standard saline citrate for 10 minutes. The probes used for Southern blotting included an early genome probe (ATCCCTCTGAATGGGCACG) for fragments amplified with the JC1 primers, a VP probe (AGCCAGGCAGGGCAGCAGG) for those amplified with VP1 primers, and an agnoprotein probe (AAAGACAGAGACACAGTGGTT) for sequences amplified with agnoprotein primers (Fig. 1).

Sequence Analysis

All PCR products that were detected as positive bands via PCR/Southern blot hybridization were sequenced. Direct genomic DNA sequencing was performed using the Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer Applied Biosystems Division, Foster City, CA) and analyzed by the Applied Biosystems 310 Genetic Analyzer (Perkin-Elmer Applied Biosystems Division).

Immunohistochemical Analysis

In all paraaffin-embedded samples, Tag and VP1 were detected immunohistochemically on 4-μm-thick tumor sections. The streptavidin-biotin complex method was applied. Primary antibody incubation was performed with monoclonal antibody against SV40 Tag, which cross-reacts with the JC virus Tag (clone 416, Oncogene Research Products, San Diego, CA) or the JC virus polyclonal antibody (DAKO, Glostrup, Denmark), which recognizes VP1 of the JC virus.

Results

The PCR/Southern Blot Hybridization and Sequence Analyses

The PCR amplifications with four sets of primers were performed to detect the JC virus DNA sequences in the following 62 CNS tumors: 32 medulloblastomas (median patient age at diagnosis 6.7 years), 18 ependymomas (5.1 years), five choroid plexus papillomas (1.9 years), and...
In total, we identified 14 positive DNA fragments in 62 pediatric brain tumors with the aid of PCR by using primers derived from the Tag (a), VP1 (b), and agnoprotein (c). + = positive control; − = negative control; Lanes 1–3, medulloblastoma cases; Lanes 4–6, ependymoma cases (Cases 13–15); Lane 7, choroid plexus papilloma case (Case 19); Lanes 8 and 9, pilocytic astrocytoma cases. The size of the different JC viral PCR products is indicated.

![Image](https://via.placeholder.com/150)

**Fig. 2.** Representative PCR/Southern blot analysis for the detection of JC viral DNA from 62 pediatric brain tumors with the aid of PCR by using oligonucleotide probes that are specific for the JC virus DNA by Southern blot hybridization. Figure 2 provides representative data for the Southern blot hybridization with oligonucleotide probes that are specific for the JC virus. Results from multiple experiments demonstrated that none of the 32 medulloblastomas and seven pilocytic astrocytomas contained any of the JC virus sequences. On the other hand, JC virus DNA sequences were detected in five of 18 ependymomas and in one of the five choroid plexus papillomas. We detected the presence of JC virus sequences that corresponded to the Tag coding region in four (22.2%) of 18 ependymomas and one (20%) of five choroid plexus papillomas (Fig. 2a). The viral DNA sequence corresponding to the VP1 coding region was found in three (16.7%) of 18 ependymomas and one (20%) of the five choroid plexus papillomas (Fig. 2b). The viral sequences that corresponded to the segments of the agnoprotein coding region were detected in three (16.7%) of 18 ependymomas and one (20%) of the five choroid plexus papillomas (Fig. 2c). One choroid plexus papilloma sample (Case 19) demonstrated all four sequences that corresponded to the JC virus DNA. The results for the ependymomas and choroid plexus papillomas are summarized in Table 1. In total, we identified 14 positive DNA fragments in 62 CNS tumor samples by using Southern blot hybridization. Fragments were excised and sequenced by automated fluorescent DNA cycle sequencing. Results from direct sequencing revealed that all of the DNA fragments contained the corresponding sequences to the JC virus DNA (data not shown).

**Immunohistochemical Analysis**

Immunohistochemical analyses were performed to detect the JC virus large Tag and VP1 in all paraffin-embedded tumor samples. Only one choroid plexus papilloma showed positive immunostaining for the large Tag; however, none of the specimens displayed positive immunostaining for VP1 (Fig. 3).

**Discussion**

Although many epidemiological studies appear in the literature, few define the risk factors for pediatric brain tumors. Inherited syndromes, such as neurofibromatosis, tuberous sclerosis, and Turcot syndrome, are known to be associated with CNS tumors. Therapeutic doses of ionizing radiation administered to the CNS to treat leukemia and brain tumors have been unequivocally identified as an environmental risk factor; however, there is little evidence that low doses of radiation from diagnostic radiographs do not increase the risk for the fetus or the child.2,3 Other possible factors have been reported, such as chemicals, medications, and viruses. Recent epidemiological studies of the contamination of polio vaccines with polyomaviruses, which occurred in the late 1950s and early 1960s, concluded that recipients of the contaminated vaccines did not display a highly increased risk for carcinogenesis.11,29 Several studies reported a small measurable increase in the frequency of nervous system tumors, however, particularly among the offsprings of women vaccinated during pregnancy, which suggests a possible role for polyomavirus in the pathogenesis of human brain tumors in pediatric patients.10,17

The JC virus is a human neurotropic polyomavirus infecting more than 80% of the human population. Initial infection with the JC virus is largely asymptomatic and occurs predominantly in childhood, after which the viruses persist in kidney and B lymphocytes.28 Brain tumors including medulloblastomas and ependymomas have been induced by injecting several animal species with the JC vi-

### TABLE 1

Summary of the JC virus sequences detected in human brain tumors in pediatric patients*

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Tumor Type (location)</th>
<th>Age (yrs)†, Sex</th>
<th>Control Region</th>
<th>Early Genome</th>
<th>VP1</th>
<th>Agno</th>
<th>Tag</th>
<th>VP1</th>
</tr>
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<tr>
<td>3</td>
<td>ependymoma (IT)</td>
<td>11, F</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
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<td>5</td>
<td>ependymoma (IT)</td>
<td>2, M</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>9</td>
<td>ependymoma (ST)</td>
<td>8, F</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>13</td>
<td>ependymoma (IT)</td>
<td>1, M</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>14</td>
<td>ependymoma (IT)</td>
<td>3, M</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>19</td>
<td>choroid plexus papilloma (ST)</td>
<td>1, F</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

* Agno = agnoprotein; IHC = immunohistochemical analysis; IT = infratentorial; ST = supratentorial; + = positive; − = negative.
† Age at time of operation.
The JC virus in brain tumors in children

In 1973 Walker and colleagues\(^{16}\) demonstrated that malignant gliomas could be induced in hamsters by using the JC virus. Several investigators have since shown that the JC virus is involved in the oncogenesis of various human brain tumors by demonstrating the JC viral genome in tumor cells.\(^{4,7,9,14,20,26,32}\) Krynska, et al.,\(^{24}\) reported that 11 (47.8%) of 23 medulloblastomas in their series contained the JC virus DNA. More recently, Del Valle, et al.,\(^{7}\) examined 85 brain tumor samples obtained from the UK, Greece, and the US; they reported that the JC viral DNA sequence was commonly involved in various CNS tumors. The results of their studies revealed that four (57.1%) of seven oligodendrogliomas, 10 (76.9%) of 13 astrocytomas, four (80%) of five pilocytic astrocytomas, five (62.5%) of right oligoastrocytomas, two (66.6%) of three anaplastic oligodendrogliomas, five (83.3%) of six anaplastic astrocytic tumors, 12 (57.1%) of 21 glioblastomas multiforme, and five (83.3%) of six ependymomas contained the JC viral early gene sequence. In all, 57.6% of the CNS tumor samples contained the JC viral genome. Immunohistochemical analysis revealed the expression of JC viral Tag in 32.9% of the samples.

On the other hand, there have been negative results regarding the presence of JC viral DNA in human brain tumors.\(^{1,16,18,37}\) Weggen, et al.,\(^{37}\) reported only one case involving a meningioma that contained JC viral DNA among the 131 meningiomas, 116 medulloblastomas, 25 ependymomas, and two subependymomas examined in Germany. Kim, et al.,\(^{23}\) reported no JC viral sequences in 15 North American patients with a medulloblastoma. In accordance with their results, we also failed to detect JC viral sequences in medulloblastomas and pilocytic astrocytomas from Japanese pediatric patients. These findings together indicate that there may be no great correlation between the JC virus and the pathogenesis of medulloblastomas and pilocytic astrocytomas in Japan.

In contrast, JC viral DNA sequences were detected in one of five choroid plexus papillomas and five of 18 ependymomas. Immunohistochemical studies revealed nuclear expression of the JC viral Tag in a choroid plexus papilloma. To the best of our knowledge, this is the first case of an association of the JC virus with choroid plexus papilloma.

The JC virus Tag is transcribed before DNA replication, whereas VP1, VP2, VP3, and agnoprotein are transcribed after viral DNA replication.\(^{14}\) Gallia, et al.,\(^{14}\) indicated that, after infection of oligodendrocytes, JC virus DNA expres-

Fig. 3. Case 19. Histological and immunohistochemical findings of a choroid plexus papilloma. Left: Staining with H & E. Center: Immunostaining with an anti-Tag antibody. Right: Immunostaining with anti-VP1 antibody. Original magnification × 100.

sion leads to progressive multifocal leukoencephalopathy when Tag expression causes events leading to viral replication, virion formation, and subsequent lysis and destruction of the host cell. When the JC viral Tag is expressed without viral replication, it works as an oncogenic factor to bind to tumor suppressor proteins. In our series, Tag was expressed in one choroid plexus papilloma; however, immunohistochemical staining demonstrated that VP1 was not expressed. This result might indicate that in this case Tag worked as an oncogenic factor without viral replication.

Our study of ependymomas revealed that five tumors contained JC virus DNA without expression of Tag, which is indicative of an alternative oncogenic pathway that is independent of Tag-mediated inactivation of the tumor suppressor genes. The exact mechanism of alternative oncogenesis of the JC virus remains to be elucidated. The JC virus Tag has been demonstrated to possess mutagenic activity and induce chromosomal instability in lymphocytes. Further evidence of the ability of Tag to induce polyplody and chromosomal damage in human fetal glial cells has been observed.\(^{30,35}\) On the other hand, it has been shown that tumors produced in transgenic mice with inducible SV40 Tag expression eventually lose their dependence on Tag for maintenance of the transformed state. A more recent study on JC virus–induced mouse medulloblastoma has demonstrated the presence of a Tag negative subpopulation of tumor cells. These results suggest that the JC virus Tag may promote the occurrence of mutations in various important genes and chromosomal instability as an earlier phase for tumorigenesis. Once dysregulation of cell-cycle profiles or induction of chromosomal instability occurs, the uncontrolled proliferation of cells may become independent of Tag in a later phase of multistep transformation strategy.

Conclusions

There have been several reports describing the positive and negative association of the JC virus with CNS tumors. It is important to accumulate data concerning JC viral DNA and proteins in CNS tumors. In this study we have demonstrated that JC virus DNA was found in one of five choroid plexus papillomas and five of 18 ependymomas; however, none of the 32 medulloblastomas or seven pilocytic astrocytomas contained any of the JC virus sequences. Our present results provide molecular evidence of the possible association between the JC virus and tumorigenesis of certain ependymomas and choroid plexus papillomas; however, no such association between the JC virus and medulloblastomas or pilocytic astrocytomas was detected. Further investigations with wide-ranging samples and epidemiological studies are necessary to elucidate fully the role of the JC virus in human CNS tumors in pediatric patients.

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References

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