Detection of JC Virus in the Brains of Korean Glioblastoma Multiforme Patients

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Objective: The ubiquitous human polyomavirus, JC virus (JCV) is the etiologic agent of the fatal demyelinating central nervous system (CNS) disease, progressive multifocal leukoencephalopathy (PML). Recent studies have reported the detection of the JCV in samples derived from several types of human neural tumors and suggested the possible association of JCV with CNS tumors. Here we report for the first time, the presence of JCV in Korean glioblastoma multiforme (GM) patients.

Methods: Two Korean GM patients were assayed for JCV. To detect JCV, we performed immunohistochemical analysis using anti-JCV and anti-glial fibrillary acidic protein (GFAP) serum and polymerase chain reaction (PCR) using primers.

Results: JCV antigen was detected in cytoplasm abundantly in cells of this tumor case. Also, GFAP immunoreactivity was predominantly observed in cytoplasm of the cells that were morphologically bizarre appearing astrocytes in GM. In addition, both of the large T antigen gene and the VP1 gene were detected and this result correspond with previous result of immunohistochemistry.

Conclusion: Although it is not certain that GM is associated with the JCV, we are attempted to elucidate the possible implication of JCV in the tumorigenesis of certain human malignant gliomas.

KEY WORDS: JC virus - Glioblastoma multiforme - Brain.
including GM patients that were supposed to be associated with polyomaviruses, JCV, BKV, and SV40, but it has not been reported in Korea, yet. Thus we investigated the polyomaviruses in Korean GM patients. Here, we report detection of JCV in the brains of Korean glioblastoma multiforme patients and discuss its role in carcinogenesis with a literature review.

Materials and Methods

Immunohistochemical analysis of tissue material

Immunohistochemical staining for JCV and glial fibrillary acidic protein (GFAP) was performed on formalin-fixed, paraffin-embedded tumor using an avidin-biotin complex technique. Briefly, duplicated serial 6 μm sections were cut from blocks, and dewaxed with xylene, hydrated with graded ethanol and treated with 0.3% H2O2 in methanol to block endogenous peroxidase. The nonspecific protein binding was blocked by 10% normal donkey serum in phosphate-buffered saline solution (pH 7.4). Sections were reacted with mouse anti-JCV serum (1:20, Novocastra laboratories, UK) or mouse anti-GFAP serum (1:500, Dako, Denmark) followed by biotinylated donkey anti-mouse serum and an avidin-biotin complex peroxidase detection system (Vectastain elite ABC, Vector Laboratories, USA). For double labeling of anti-JCV and anti-GFAP, sections were exposed to the monoclonal anti-JCV serum for overnight at 4°C followed by biotinylated donkey anti-mouse (Jackson ImmunoResearch, UK) at 1:800 and ABC Elite PK-6100 and visualization with DAB. The second primary rabbit anti-GFAP was applied for 1 hour at room temperature, followed by alkaline phosphatase conjugated donkey antirabbit serum and visualization with NBT/BCIP substrate kit (Vector Laboratories, USA).

Polymerase chain reaction (PCR) amplification of viral DNA

Tissues were obtained from GM cancer. Total DNA was extracted using DNA extraction kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. DNA samples were used in PCR amplification as an 5μl DNA aliquot of the each sample with using the primers of JCV large T antigen (JCV T Ag)12), JCV portion of the late region VP1 gene (JCV VPN)4), and glyceraldehydes-3-phosphate dehydro-genase (GAPDH)9).

Used primers were followed as; JCV T Ag (T1, 5‘AATA-GTGGTTTACCTAAAG-3‘, T2, 5‘-TGAATAGGGAGGATCCCATG-3‘), JCV VPN(VPN3, 5‘TTTTTGGGACACCTAACGGAG-3’, VPN4, 5‘-GTCAACGTATCTCATCAGT-3’) and GAPDH (sense, 5‘-TGGTATCATGTTGGAAGGACTCATGAC-3’, antisense, 5‘-ATGCCAGTGAAGCTCCTTCA-3’). PCR reactions for each DNA samples were carried out in a final volume of 50μl in a buffer with 10 mM of each dNTP mixture (Promega), 2.5 units of Taq DNA polymerase (Promega), and 50 μM of each oligonucleotide primer.

The PCR conditions were followed as; for JCV T Ag and JCV VPN, an initial denaturation step of 95°C for 5 min, 40 cycles of 95°C for 45 sec, 52°C for 45 sec, and 72°C for 1 min and final extension at 72°C for 10 min. For GAPDH an initial denaturation step of 95°C for 3 min, 30 cycles of 95°C for 1 min, 58°C for 1 min, and 72°C for 2 min and final extension at 72°C for 10 min. PCR products were electrophoresed on 1% agarose gel 5V/cm for 1 hour then observed at illuminator (Biorad).

The purification of PCR products for sequencing was done using a PCR purification kit (QIAGEN, Valencia, CA). The sequencing was carried out on an ABI 377 automatic sequencer using a Taq dideoxy terminator cycle sequencing kit (ABI, Foster city, CA). Nucleic acid sequences of JCV virus were assembled and edited using a combination of the ABI 377 DNA sequencer Data Analysis Program and Sequence Navigator Software.
Results

Immunohistochemical evaluation of GM harboring JCV

To check whether JCV was infected in tumor tissue or not, immunohistochemistry was performed. As expected, JCV antigen was detected in cytoplasm abundantly large in cells of this tumor case (Fig. 1A, B). To identify cell types that were stained for anti-JCV serum, we used the serial sectioning manner. JCV-immunoreactive cells were usually colocalized with the GFAP positive cells. Also, GFAP immunoreactivity was predominantly observed in cytoplasm of the cells that were morphologically bizarre appearing astrocytes in glioblastoma multiforme (Fig. 1C, D).

To clarify the presence of JCV antigen in the GFAP-positive cells, we performed double labeling immunostaining manner with anti-JCV and anti-GFAP serum. As Fig. 1, most of the cells that were showed positive reaction with anti-JCV serum were usually colocalized with anti-GFAP (Fig. 2).

Detection of the JCV genome in GM by PCR

Two segments of the JCV genome were targeted for detection: the large T antigen gene (519bp) from the early region and the structural protein VP1 gene (180bp) from the late region (Fig. 3). Both of the segments are critical for viral replication and multiplication. Kidney tissue was used for positive control and sequence analysis was performed to confirm the genome as a JCV DNA (data not shown). Both of large T antigen gene and VP1 gene were detected and these results correspond with previous results of immunohistochemistry (Fig. 1, 2). GAPDH was used as a marker of housekeeping gene.

Discussion

The human neurotropic JC virus (JCV) is a member of the polyomavirus family, which includes the human BK virus (BKV) and the simian virus 40(SV40). Both JCV and BKV are thought to infect greater than 70% of the human population worldwide during early childhood, but does not induce any obvious clinical symptoms, and to establish latency in the kidney.

The JCV is icosahedral nonenveloped DNA viruses with capsid diameters of approximately 45nm. The genome consists of three functional domains, the early and the late coding regions seperated by the transcriptional control region, w-hich has been sh-own to be the m-ajor determinant of the tropism of this virus for n-euroectodermally derived tissues.

The late region encodes capsid p-proteins produced late in the JCV lytic cycle, whereas the early region encodes the multifunctional oncoprotein, and large tumor anti-gen (Tantigen).

The JCV was first identified as the etiologic agent of the fatal de-myelinating disease, PML, which usually occurs in individuals with defects in cellmediated immunity, including acquired immune deficiency syndrome (AIDS) and frequently presents with rapidly progressing dementia and weakness. The histopathological changes in PML consist of lytic destruction of oligodendrocytes with resultant foci of demyelination and relative sparing of neuronal cell bodies and axons, intranuclear inclusions in oligodendrocytes, and bizarrely appearing astrocytes.

The increased incidence of AIDS and the use of immuno-suppressive chemotherapy have dramatically raised the incidence of PML. The coincidental occurrence of malignant astrocytes and oligodendrocytes in PML patients provides intriguing speculation on the association between JCV and CNS malignancies.

Recently, otherwise, several studies, including GM tumor study, have been reported the detection of JCV in some types of neural as well as non-neural human tumors. Although, relation of JCV and GM takes a small part of the putative pathogenesis of GM, it is still important in the meaning of possibility to find the way to cure the brain tumor patients.

Previously, there was several report that JCV is postulated to be related with GM pathogenesis, but in Korea there was not any report of detection of JCV in GM patients. In our study, we detected JCV antigen, the large Tantigen gene and the VP1 gene in Korean GM patients using by immunohistochemical analysis and PCR. These observations suggest a potential etiological role for JCV in multiple types of human brain tumors and it also suggesting the possibility that it may be applicated in Korean GM pati-ents, either.

Although evid-ence for the role of JCV in human CNS neoplasms is not clear, yet, the oncogenic p-otential of this
human virus has been well established in several experimental animal models\(^6\)–\(^8\). Intracerebral inoculation of JCV into owl and squirrel monkeys results in the development of astrocytomas\(^7\),\(^8\). Injection of JCV into the brain of newborn rats caused undifferentiated neuroectodermal origin tumors in the brain of 75% of the animals\(^5\). Intracerebral inoculation of newborn Golden Syrian hamsters with JCV induced a broad range of tumor including medulloblastoma, astrocytoma, GM, primitive neuroectodermal tumors and peripheral neuroblastomas in more than 85% of inoculated animals\(^6\). Whereas no evidence for the lytic infection of the tumor cells with JCV was observed, expression of the viral oncoenic protein, T-antigen, was prominent in the tumor cells\(^5\),\(^6\).

The T-antigen of JCV has multiple regulatory functions, which are required for the successful progression of the virus through the lytic cycle. When JCV early gene expression is followed by viral DNA replication and late gene expression, that result in a productive infection. As the cases with PML, JCV-infected oligodendrocytes, and the myelin-producing cells of the CNS, those kind of injury may be permissive for viral infection and process these viruses to lytic pathway then lead to demyelination and finally disturb the brain system\(^5\).

Activation of the viral early promoter under circumstances that are not optimal for efficient lytic infection cycle can result in the accumulation of T-antigen in the brain. This, in turn, may trigger a cascade of events that results in uncontrolled proliferation of CNS cells. In this condition, the expression of T-antigen may lead to inactivation of tumor suppressor genes, dysregulation of signaling pathways, or DNA instability, which could contribute to transformation and subsequent tumorigenesis\(^3\). The factors of contributing the permissiveness to viral infection of a given cell type may be determined with its tissue of origin, a stage of development, or a phase of differentiation\(^5\). For example, perhaps it may be the mature oligodendrocyte that can fully support a viral lytic infection while an undifferentiated oligodendrocyte maybe only susceptible to T-antigen and leading to the development of the oligodendroglioma.

The most of information of the potential mechanisms that T-antigen may lead to cellular transformation has been obtained by in vitro analysis as well as through the study of tissue from T-antigen-induced tumors in experimental animal models\(^5\). Such studies may provide better understanding of cellular regulatory pathways involved in tumorigenesis and lead to unveil the therapeutic targets of JCV-transformed cells, potentially even the targeting of T-antigen itself.

Taken together, exploring the correlation of human polyomaviruses and human brain tumor is important because it may suggest that those viruses may be associated with the development of some, though not all, of the various types of brain tumors. Indeed, a large scale multi-institutional study is required to establish the association of JCV with CNS tumors.

**Conclusion**

Although it is not certain that GM is associated with the JCV, we are attempted to elucidate the possible implication of JCV in the tumorigenesis of certain human malignant gliomas. Since current study was the first case of reporting the Korean GM patients which were detected the JCV, it still remains a lot of task for the further studies.

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**References**