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An overview: Human polyomavirus JC virus and its associated disorders

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JC virus (JCV) is a polyomavirus infecting greater than 80% of the human population early in life. Replication of this virus in oligodendrocytes and astrocytes results in the fatal demyelinating disease progressive multifocal leukoencephalopathy (PML) in immunocompromised individuals, most notably acquired immunodeficiency syndrome (AIDS) patients. Moreover, recent studies have pointed to the association of JCV with a variety of brain tumors, including medulloblastoma. The JCV genome encodes for viral early protein, including large and small T antigens and the newly discovered isoform T′, at the early phase of infection and the structural proteins VP1, VP2, and VP3 at the late stage of the lytic cycle. In addition, the late gene is responsible for the production of a small nonstructural protein, agnoprotein, whose function is not fully understood. Here, we have summarized some aspects of the JCV genome structure and function, and its associated diseases, including PML and brain tumors. *Journal of NeuroVirology* (2003) **9(suppl. 1),** 3–9.

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Progressive multifocal leukoencephalopathy (PML) is a neurodegenerative disease of the human central nervous system resulting from the lytic infection of oligodentrocytes by a human polyomavirus, JC virus (JCV). This disease mostly develops in patients with underlying immunosuppressive conditions including Hodgkin's lymphoma and lymphoproliferative diseases, as well as those undergoing antineoplastic therapy and those with acquired immunodeficiency syndrome (AIDS) (Berger and Concha, 1995; Berger *et al*, 1987; Brooks and Walker 1984). In addition, in a small number of cases, PML was also found to affect individuals with no underlying disease. PML is a subcortical white matter disease of the brain, exhibiting signs and symptoms indicative of involvement of multiple regions (Berger *et al*, 1987; Gordon and Khalili, 1998; Major *et al*, 1992; Walker *et al*, 1973). Figure 1 illustrates cranial T1-weighted magnetic resonance imaging (MRI) of a PML pateint depicting hypointense signal abnormalities of the white matter (Figure **1A**), and a coronal slice of PML brain through both cerebral hemispheres showing the areas of lesions (Figure **1B**). Demyelination typically occurs as a multifocal process that is rarely unifocal and can develop in any location in the white matter (Brooks and Walker 1984) (Figure **1C**). Clinically, the most common signs and symptoms of PML at the time of presentation include visual deficit, motor weakness, and mental deficit (emotional liability, difficulty in memory, and dementia) (Berger *et al*, 1987; Frisque and White 1992). Lytic infection of oligodentrocytes by JCV results in the destruction of myelin-producing cells. The primary function of these glial cells is to myelinate the axons that project from the neuronal cell bodies of the overlying cortex. Destruction of these cells initially leads to microscopic lesions (Berger *et al*, 1987; Major *et al*, 1992), but as the disease progresses, demyelinated areas become enlarged and eventually may coalesce, making them visible on gross examination of the cut sections

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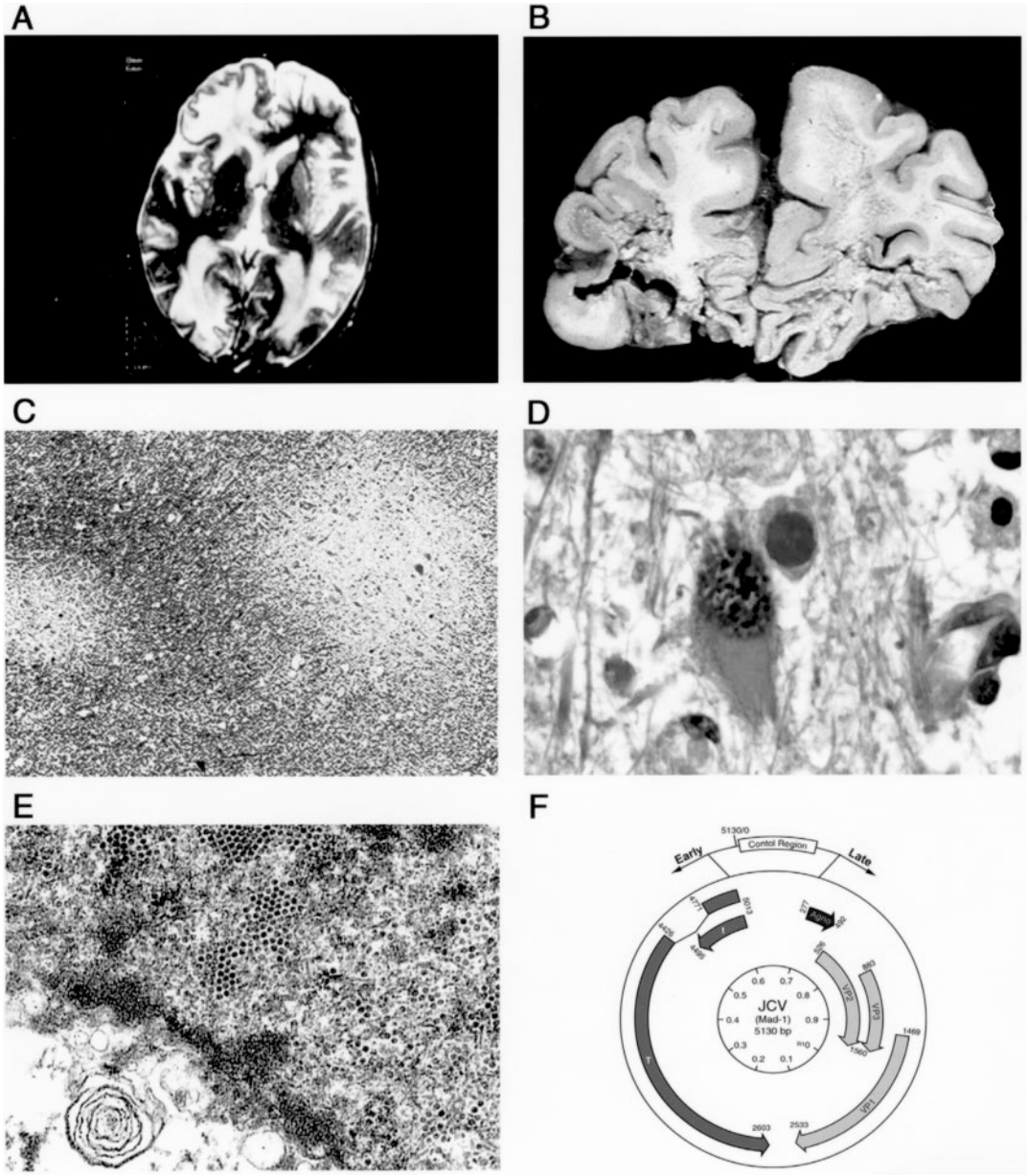

Figure 1 Progressive multifocal leukoencephalopathy, from MRI to JCV genome. **A**, Magnetic resonance image of brain from a PML patient. **B**, Macroscopic section of the brain depicting affected areas of cavitation. **C**, Luxol Fast blue staining of brain illustrating demyelinated plaques. **D**, Hematoxylin and eosin staining depicting bizarre astrocytes and enlarged oligodendrocytes with inclusion bodies. **E**, Electron micrograph of the nucleus of an oligodendrocyte highlighting icosahedral structure of JCV. **F**, Genomic organization of JCV DNA illustrating the various regions of the viral genome, including the early and late genes as well as the control region.

(Astrom *et al*, 1958). In most cases, astrocytes, the other member of the glial cells, are also affected by infection exhibiting enlarged, lobulated, and bizarre-looking nuclear structures (Richardson, 1961) (Figure **1D**). Electron microscopic evaluation of nuclei of oligodendrocytes shows accumulation of icosahedral particle, a characteristic of polyomaviruses (Figure **1E**).

PML was previously considered a rare complication of middle-aged and elderly patients with lymphoproliferative diseases. In recent years, however, due to increasingly widespread use of immunosuppressive chemotherapy and the AIDS epidemic, it is now a commonly encountered disease of the central nervous system (CNS) in patients of different age groups. Particularly, considerably higher incidence of PML in AIDS patients than with other immunosuppressive disorders has led us to believe that human immunodeficiency virus (HIV) infection in the brain directly or indirectly participates in this process. The direct link between HIV infection and increased incidence of PML in AIDS patients came from

the experimental evidence that HIV-encoded transregulatory protein, Tat (transactivator of transcription) can cross-transregulate JCV transcription from the viral late promoter through a Tat-responsive cis-element (TAR) present in the regulatory region of JCV (Chowdhury *et al*, 1992; Tada *et al*, 1991). Tat is a potent transactivator of transcription from HIV long terminal repeats (LTRs) and plays a critical role in HIV replication as well (Cullen, 1990; Frankel, 1992). Tat can also be secreted from HIV-infected cells (Ensoli *et al*, 1990; Frankel and Pabo, 1988). Once released, it can be taken up by neighboring uninfected cells and/or infected cells and modulate the expression of Tat-responsive cellular and resident viral genes. In addition to its direct effect, Tat may also indirectly participate in the transregulation of JCV gene expression, perhaps through Tat-induced up-regulation of cytokines (Genis *et al*, 1992) and other soluble factors whose effect on JCV gene regulation has yet to be demonstrated.

JCV is a small, human DNA virus with a double-stranded covalently linked circular genome (Figure **1F**). The nucleotide sequence analysis of JCV has revealed that it is composed of three functional regions, including the viral early and late coding regions and viral noncoding regulatory region (Frisque *et al*, 1984). The viral early coding region encodes two regulatory proteins, small t and large T-antigens. Although little is known about the function of small t-antigen, the large T-antigen was shown to be a multifunctional phosphoprotein involved in both the initiation of viral DNA replication and T-antigen–mediated activation of JCV late genes (Khalili *et al*, 1987; Lashgari *et al*, 1989; Swenson *et al*, 1996). More recent studies have revealed expression of novel isoforms of T-antigen, called T′, which are produced due to alternative splicing of the early transcript (Frisque, 2001).

The viral late coding region encodes the structural proteins, VP1, VP2, and VP3. These three proteins form capsids for the viral genome and function in the attachment, adsorption, and penetration of the virus to the host cells. The leader sequences of the late transcripts also contain an open reading frame encoding a small protein called agnoprotein. The function of this 71–amino acid protein in the viral life cycle is not fully understood, although our recent observations indicate that it appears to have regulatory roles in JCV gene transcription and replication (Safak *et al*, 2001). The regulatory region of JCV is composed of the origin of DNA replication, promoter elements for both early and late genes, and cis-acting enhancer elements.

JCV is closely related to the other polyomaviruses, BK virus (BKV) and simian virus 40 (SV40). These three viruses share significant sequence homology, particularly in their coding regions (Frisque *et al*, 1984). JCV and BKV share approximately 80% homology in their early coding regions and close to 70% homology in their late coding regions. JCV and SV40 also share a similar degree of homology in those respective regions. However, sequences in the regulatory regions of these three viruses are significantly divergent, which are believed to lead to narrow species- and tissue-specific expression of JCV genome (Kenney *et al*, 1984; Tada *et al*, 1989, 1991). Data from promoter-swapping experiments performed between SV40 and JCV in transgenic mice have indicated that cis-acting promoter/enhancer elements of JCV and tissue-specific cellular factors are the two main determinants for narrow host range for JCV (Feigenbaum *et al*, 1992). Reporter gene expression studies utilizing the regulatory region of the JCV in *in vivo* and *in vitro* transcription assays have revealed that expression of the viral early promoter is substantially higher in glial cells than nonglial cells (Feigenbaum *et al*, 1987; Lashgari *et al*, 1989; Lynch and Frisque, 1990, 1991; Lynch *et al*, 1994; Tada *et al*, 1989). Additionally, somatic cell hybridization studies between JCV T-antigen–transformed hamster glial cells and mouse fibroblast cells have suggested the presence of positive regulatory factors in glial cells and negative regulatory factors in nonglial cells, which support the findings from the above-mentioned reporter gene expression studies (Beggs *et al*, 1988; 1990). Altogether, these studies suggested that the narrow species- and tissue-specific expression of JCV genome lies at least in part in the regulatory region of JCV.

As observed from numerous PML patients, the hallmark of the PML is the demyelination of axons projecting from neuronal cell bodies in the CNS caused by the lytic infection of oligodentrocytes by JCV. In order to analyze the pathogenesis of JCV-induced demyelination, transgenic mouse models were created with the regulatory and coding sequences for JCV T-antigen (Small *et al*, 1986a, 1986b). Electron microscopic (EM) analysis of tissue sections from the brains of those animals revealed hypo- and dysmyelination of the axons, which has been attributed to a direct or indirect involment of T-antigen in this process by altering the expression levels of CNS specific genes, including myelin basic protein (MBP), proteolipid protein (PLP), and myelin associated glycoprotein (MAG).

In addition to its demyelination activity in the CNS, like other polyomaviruses, JCV has also been shown to have oncogenic potential in experimental animals in tissues of neural origin. The type of the tumors induced by JCV in animal models depends on the animal type, age, and site of viral inoculation. For example, intracerebral, intraperitoneal, and subcutanous inoculation of live virus into newborn Syrian hamsters resulted in the development of glioblastomas, medulloblastomas, pineocytomas, and other neuroectodermal origin tumors (Varakis *et al*, 1978; Walker *et al*, 1973). In contrast, intraocular inoculation of the virus into neonatal hamsters induced abdominal neuroblastomas (Gordon and Khalili, 1998; Varakis *et al*, 1978). Interestingly among its close counterparts, SV40 and BKV, JCV

is the only polyomavirus shown to induce tumors in nonhuman primates. Intracerebral inoculation of JCV into owl and squirrel monkeys caused malignant cerebral tumors similar to astrocytomas seen in humans (London *et al*, 1978, 1983). Tumor induction by JCV was also studied in transgenic mice by constitutively expressing JCV T-antigen under the control of its own promoter. They exhibited tumors of neuronal origin (Franks *et al*, 1996; Krynska *et al*, 1999; Small *et al*, 1986b). Besides direct experimental evidence of the ability of JCV to induce tumors in animal models, there are a number of reported cases that also link polyomaviruses to the association of tumor induction in humans. Among several cases, the first evidence was reported by Richardson (1961), who, at autopsy, in addition to a PML case, incidentally discovered the presence of oligodentroglioma in an elderly patient. In another PML case, EM analysis of the specimens revealed the presence of JCV in concomitant glioma (Castoigna *et al*, 1974). Further, our laboratory and others also reported the association of JCV with a variety of human tumors in patients without PML, including oligoastrocytoma (Rencic *et al*, 1996), colon and colorectal cancer (Laghi *et al*, 1999), and medullablastomas (Krynska *et al*, 1999). Additionally, JCV may also induce tumors by causing DNA damage and genomic instability (Neel, 1999). Although the mechanisms by which JCV induces tumors in experimental animals and perhaps in humans remains to be elucidated, it is postulated that large T-antigen of JCV inactivates the function key cell cycle regulatory proteins, including p53 and pRb, by perturbing normal cell cycle progression (Bollag *et al*, 1989; Dyson *et al*, 1989; Haggerty *et al*, 1989; Howard *et al*, 1998).

Transcriptional regulation of the JCV early and late promoters in the lytic cycle appears to be rather complex and requires both participation of viral and cellular factors. Similar to that of SV40, the JCV lytic cycle begins with the expression of early genes encoding the regulatory large T and small t-antigens. Large T-antigen initiates viral DNA replication and orchestrates the transition from early to late gene transcription by activating late gene expression and by suppressing its own early promoter (Khalili *et al*, 1987; Lashgari *et al*, 1989). Following the onset of DNA replication, the virus enters the late phase of infection, during which capsid proteins are encoded, virions are matured, and finally the host cell is lysed (Raj and Khalili, 1995). Over the years, a number of cellular transcription factors have been identified and characterized for their involvement in JCV gene transcription, including nuclear factor kappa B (NF-κB) (Ranganathan and Khalili, 1993; Safak *et al*, 1999b), Tst-1 (Renner *et al*, 1994; Wegner *et al*, 1993), NF-1 (Amemiya *et al*, 1989), Sp-1 (Henson *et al*, 1992), GBP-i (Raj and Khalili, 1994), YB-1 (Kerr *et al*, 1994; Safak *et al*, 1999a), and Purκ (Chen and Khalili, 1995; Chen *et al*, 1995; Safak *et al*, 1999c). Figure 2 represents some of the transcription factors that associate with the JCV regulatory region. At the initial stages of infection, only host cellular factors are responsible for expression of early genes in the absence of T-antigen.

As mentioned earlier, similar to other polyomaviruses including BKV and SV40, the late genome of JCV contains a short open reading frame positioned in the leader sequences encoding a small peptide called agnoprotein (Frisque *et al*, 1984). The function of this protein in JCV biology remains largely unknown. Our earlier studies have revealed that the level of Agnoprotein increases as the infection progresses, and the protein is mainly localized to the cytoplasmic compartment of the cells, with a relatively increased accumulation to the perinuclear region. Occasionally protein was found in the nucleus as well.

The assumption that JCV agnoprotein may play functional roles in the viral lytic cycle is inferred from the studies performed on SV40 agnoprotein. Published reports indicated that SV40 agnoprotein may have regulatory roles in many aspects of SV40

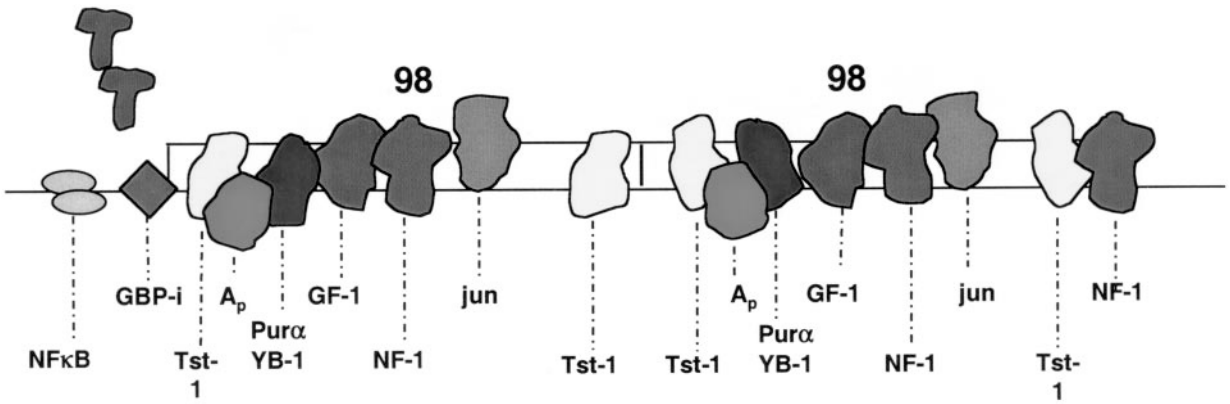

Figure 2 Control region of JCV and the associated transcription factors. Schematic representation of the JCV Mad-1 regulatory region depicting some of the cellular factors that potentially interact with the viral DNA sequence.

viral growth, including transcription, translation (Alwine *et al*, 1982; Haggerty *et al*, 1989; Piatak *et al*, 1981; Varakis *et al*, 1978), viral assembly (Carswell and Alwine, 1986; Carswell *et al*, 1986), and maturation (Margolskee and Nathans, 1983; Mertz *et al*, 1983; Ng *et al*, 1985). Our recent data indicate that, like T-antigen, JCV agnoprotein also appears to have regulatory roles in the JCV lytic cycle through its interaction with both viral and cellular factors. In this respect, we have demonstrated that agnoprotein physically and functionally interacts with the viral early regulatory protein, T-antigen, and suppresses both T-antigen–mediated viral gene transcription and viral DNA replication (Safak *et al*, 2001) Our most current data also indicate that agnoprotein also exhibits negative regulatory effects on YB-1–mediated activation from JCV promoters (Safak *et al*, 2002).

To gain more insight into the biological role of agnoprotein, we have created several stable cell lines that constitutively express agnoprotein (Figure 3). We have demonstrated that in the absence of other viral proteins, expression of agnoprotein can inhibit cycle progression by deregulating progression of several key regulators, including cyclin A and cyclin B. Cells with constitutive expression of agnoprotein were found to be largely accumulated at the G2/M stage. In addition, agnoprotein showed the ability to augment p21 promoter activity in transient transfection assays and a noticeable increase in the level of p21 is detected in cells stably expressing agnoprotein. Results from binding studies revealed the interaction of agnoprotein with p53 through the N-terminal region of the agnoprotein spanning residues 1 to 36. Coexpression of p53 and agnoprotein further stimulated transcription of the p21 promoter. Thus, the interaction of p53 and agnoprotein can lead to a higher level of p21 expression and contribute to the deregulation of cell cycle progression.

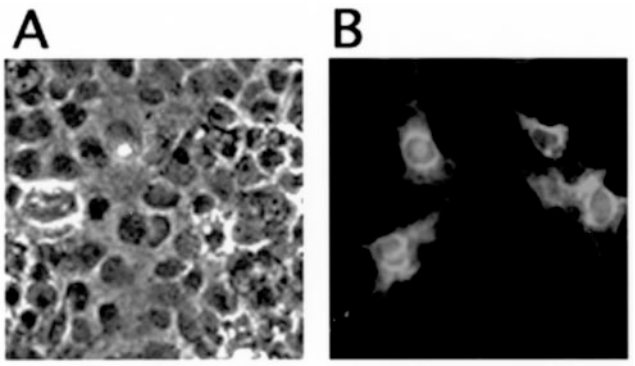

Figure 3 Expression and subcellular localization of agnoprotein. **A**, Phase microscopy images of SVGA cells after 5 day infection with JCV. **B**, Immunofluorescence staining of JCV-infected SVGA cells after incubation with anti-agnoprotein antibody showing cytoplasmic perinuclear staining of agnoprotein; low level agnoprotein was also evident in the nuclei.

Currently, studies are in progress to assess the role of small t-antigen, as well as the products of two small open reading frames of JCV in the pathogenesis of PML and their role in the oncogenic activity of JCV.

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