Oncogenic Potential of Human Neurotropic Virus: Laboratory and Clinical Observations

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Abstract

Cancer is a multi-step disease involving a series of genetic alterations that result in the loss of control of cell proliferation and differentiation. Such genetic alterations could emerge from the activation of oncogenes and the loss or malfunctioning of tumor suppressor gene activity. Our understanding of cancer has greatly increased through the use of DNA tumor viruses and their transforming proteins as a biological tool to decipher a cascade of events that lead to deregulation of cell proliferation and subsequent tumor formation. For the past ten years our laboratory has focused on the molecular biology of the human neurotropic papovavirus, JCV. This virus causes progressive multifocal leukoencephalopathy, a fatal neurodegenerative disease of the central nervous system in immunocompromised patients. JCV is a common human virus that infects more than 80% of humans but does not induce any obvious clinical symptoms. The increased incidence of acquired immune deficiency syndrome and the use of immunosuppressive chemotherapy have dramatically raised the incidence of PML. The coincidental occurrence of malignant astrocytes and oligodendrocytes in PML patients, coupled with the induction of glioblastoma in JCV-infected non-human primates, provides intriguing speculation on the association between JCV and CNS malignancies. In this report we discuss clinical data and laboratory observations pointing to the direct involvement of JCV in cancer.

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The human neurotropic JC virus is the etiological agent of progressive multifocal leukoencephalopathy, a fatal demyelinating disease of the central nervous system. JCV is a member of the papovavirus family of DNA tumor viruses, which includes BK virus and the well-known Simian virus 40. JCV co-exists within the human population, as evidenced by JCV-specific antibodies in more than 80% of adults worldwide [1]. It is believed that infection with the virus is sub-clinical, occurs in early childhood, and remains latent until it reactivates under immunosuppressive conditions to result in PML.

Prior to the AIDS epidemic, PML was considered a rare disorder associated with immunocompromising diseases such as lymphomas, or was seen in renal transplant and chemotherapy patients as a complication of immunosuppressive therapies. However, recent reports indicate that 70% of all human immunodeficiency virus-1 infected patients will exhibit neurological disorders and at least 5% (and more likely closer to 10%) of all HIV-1 infected patients will develop PML [2]. PML is characterized by demyelination due to the cytolytic destruction of oligodendrocytes, the myelin-producing cells of the CNS. Other hallmarks of PML include giant bizarre astrocytes and hyperchromatic oligodendrocyte nuclei with multiple foci of demyelination [1].

The viral genome consists of a closed, circular, double-stranded DNA that is separated into early and late coding sequences by the viral regulatory region [Figure 1]. The regulatory region encodes the viral origin of DNA replication and contains a bidirectional promoter composed of two 98 base-pair repeats that control transcription. The viral early genes, large and small T-antigen, are transcribed before DNA replication, and the viral late genes, capsid proteins VP1, VP2, and VP3 as well as the accessory Agno protein, are transcribed after DNA replication [3]. The lytic cycle of JCV explains some of the pathological features seen with PML – during its normal course of replication, JCV infects oligodendrocytes, replicates within an infected cell, and then lyses and destroys the oligodendrocyte and consequently the myelin sheath. JCV exhibits very limited tissue specificity, replicating most efficiently in primary human fetal glial cells but remaining latent in the kidney. Several recent studies support its ability to replicate in B cells [4]. However, due to the species specificity of the DNA polymerase, JCV can only replicate in primates; humans are presumed to represent the natural viral host [3].

Several reports point to an association between JCV and human brain tumors. The first to observe this link was Richardson [5], who reported the incidental finding of an oligodendroglioma during autopsy of a 58 year old man with chronic lymphocytic leukemia and PML. Cases of PML associated with multiple astrocytomas have also been reported [6,7]. Castaigne et al. [6] described an 18 year old male with long-lasting immunodeficiency syndrome who was found at

JCV = JC virus
PML = progressive multifocal leukoencephalopathy
CNS = central nervous system

HIV = human immunodeficiency virus

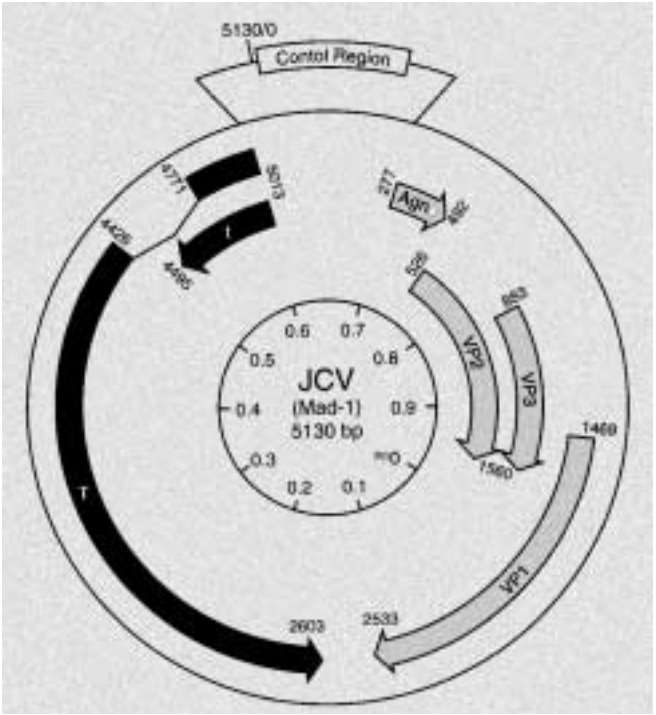

Figure 1. Schematic presentation of the JCV genome. The JCV early regions that encode the viral early protein, T-antigen, are shown on the left side of the control region whereas on the right side the positions of the viral late genes, agno and VP1, VP2, and VP3 are depicted

autopsy to have, in addition to PML demyelinating lesions, numerous neoplastic foci of anaplastic astrocytes. Ultrastructural analysis of these foci by electron microscopy demonstrated viral particles in the oligodendrocytes but not in the neoplastic astrocytes. In a similar description by Sima and co-workers [7] of a PML patient with multiple malignant astrocytomas presumably originating from the PML lesions, ultrastructural analysis of these lesions demonstrated viral particles in both oligodendrocytes and astrocytes within the PML foci but not in the neoplastic astrocytes. Interestingly, no type of immunodeficiency was revealed in this 68 year old man either clinically or at autopsy. Gulotta et al. [8] also reported the concomitant occurrence of PML and CNS gliomas; postmortem examination revealed the presence of gliomatous foci in a 30 year old HIV-1-negative PML patient. The authors noted a pleomorphic astrocytoma in the brain and diffuse neoplastic infiltration in the brainstem, but in neither of these lesions was JCV detected by *in situ* hybridization.

In addition to the cases of concomitant PML and cerebral neoplasm, JCV has been associated with human brain tumors in the absence of any PML lesions. Rencic and colleagues [9] detected DNA from the JCV non-coding region and T-antigen DNA, RNA, and protein in tumor tissue from an immunocompetent HIV-1-negative patient with an oligoastrocytoma. Moreover, this patient did not clinically or microscopically exhibit any features of PML. Interestingly, Boldorini et al. [10] noted the presence of JCV DNA in the brain of a 9 year old immunocompetent child with a pleomorphic xanthoastrocytoma. Both cases demonstrated the presence of JCV in human brain tumors of immunocompetent non-PML patients. Although a causal relationship between JCV and the development of human brain tumors may currently be considered speculative, these intriguing findings raise the question of whether or not JCV is involved in tumor pathogenesis in the CNS.

While evidence for the role of JCV in human CNS neoplasms is mounting, the oncogenic potential of polyomaviruses, including JCV, has been well established in several animal models. Specifically, intracerebral inoculation of JC virus into non-human primates, owl and squirrel monkeys, resulted in the development of astrocytomas 16 to 24 months after inoculation [11,12]. Further analysis of the monkey tumor tissue revealed the expression of the JCV early protein, T-antigen, but virion antigens were not detectable in the animals. With one exception, tissue from JCV-infected monkeys was unable to release virus when grown in cell culture [1], indicating that primate-origin cells may not be permissive for JCV infection.

In another series of studies, newborn Golden Syrian hamsters were shown to develop a broad range of tumors approximately 6 months after inoculation with JCV, the most common being medulloblastoma, astrocytoma, glioblastoma, primitive neuroectodermal tumors, and peripheral neuroblastomas [13]. Tumors of the central nervous system were detected in more than 85% of newborn hamsters inoculated intracerebrally with JCV, clearly demonstrating its oncogenic potential in neural origin tissue. Again, as in the primate studies, JCV T-antigen was detected in tumor tissue but there was no evidence of viral replication or persistent infection. Similarly, injection of JCV into the brains of newborn rats induced undifferentiated neuroectodermal origin tumors in the cerebrum of 75% of the animals [14,15].

Perhaps some of the most interesting observations on the oncogenicity of JCV have come from studies on several lines of transgenic mice that have been generated to contain the entire gene for JCV T-antigen under the control of its own promoter. Since these mice do not contain any viral late genes, the phenotypes observed are solely dependent on the expression of JCV T-antigen. Earlier studies by Small and colleagues [16,17] resulted in T-antigen transgenic mice that developed one of two phenotypes – the appearance of adrenal neuroblastomas, or less frequently, abnormal formation of myelin sheaths in the CNS (dysmyelination). We have generated additional mice utilizing the same JCV T-antigen transgene and established a line of mice that exhibit tumors of primitive neuroectodermal origin [18]. Two interesting observations can be derived from these studies: first, that T-antigen in the absence of JCV, and therefore viral replication, can alter myelin formation to induce dysmyelination; and second, that JCV T-antigen is able to transform cells and induce tumors of neural origin.

We recently described the development of a transgenic animal model using the JCV early gene. Several founder mice containing the JCV sequences showed signs of illness at 9–13

months of age, manifested as poor grooming, paresis of rear limbs, and hunched posture. Gross examination of the various organs including the brains of transgenic animals showed no distinct signs of abnormality that could cause the illness in the affected animals. Microscopic examination of the brain from the ill or healthy JCV T-antigen-positive mice showed no evidence of abnormalities in myelin, oligodendrocytes or astrocytes. Of particular interest, however, was the detection of primitive neuroectodermal tumors in the hindbrains of these animals, which resembled human medulloblastomas. The tumors consisted of sheets of cells with a high nuclear to cytoplasmic ratio, occasional Homer-Wright rosettes, frequent pyknotic nuclei, and approximately one mitotic figure per 40x high powered field. Figure 2A illustrates an island of tumor cells in a cerebellar hemisphere. The tumor cells abutting the cerebellar folia showed microscopic interdigitations in the molecular layer and early subarachnoid spread [Figure 2B]. Since the tumors were larger in three other animals it was not possible to determine the anatomic region of their origin.

Medulloblastoma, a malignant invasive tumor of the cerebellum, represents one of the most common neoplasms of the nervous system in children, with an annual incidence of approximately 1/200,000 [19]. Nearly 70% of medulloblastomas occur in children under the age of 16 [20] and are rarely seen in patients over 50 years old. While both genders are affected, there is a slight predominance of male patients (65% male). Histologically, medulloblastomas are comprised of densely cellular tumors with frequent apoptotic cells and mitotic figures. Vascular proliferation and hemorrhage are occasionally observed. Although a small percentage of these embryonal neuroblastic tumors are related to genetically defined heritable syndromes associated with a predisposition toward tumorigenesis, the majority of medulloblastomas are sporadic and their etiology remains unknown. Previous results from molecular and cytogenetic studies have pointed to the possible involvement of chromosomes 1, 17, and to a lesser degree, 6, 9, 10, 11, and 16 in the development of medulloblastoma [21,22]. Loss of heterozygosity in portions of chromosome 17 (17p) has been reported in 30–45% of medulloblastomas [23]. The presence of the gene responsible for the production of the tumor suppressor protein, p53, on chromosome 17 led to the early speculation that this protein may play a key role in the development of medulloblastoma. While the etiology of medulloblastomas in humans remains unknown, results from several experiments, as described above, indicate that JCV is able to induce cerebellar neoplasms in rodents that exhibit a phenotype similar to that of human medulloblastomas.

These observations from transgenic mice prompted us to examine pediatric medulloblastoma for the presence and expression of the viral early protein, T-antigen, in tumor tissue. Toward this end, a collection of medulloblastoma (23 specimens) was assembled from various institutions in the United States and, after histological assessment, were examined for the presence of JCV DNA sequences by polymerase chain reaction and production of JCV early and late proteins by immunohistochemistry. Results from these studies revealed that of 23 well-characterized human medulloblastomas, 20 samples (87%) were positive for the N-terminal region of JCV T-antigen, 13 samples (56.5%) contained sequences corresponding to the JCV T-antigen C-terminal region, and 20 samples (87%) possessed sequences of the VP1 region. Furthermore, it was evident that 11 samples of the tumor tissue (48%) contained the DNA sequences of the JCV genome, which corresponded to all three amplified regions, including the N- and C-terminals of T-antigen and the viral capsid VP1. Results from immunohistological analysis of human medulloblastoma for detection of the JCV early protein showed the presence of T-antigen in only 25% of the samples that were positive for JCV early gene sequence. Although these observations provide supporting evidence for the oncogenecity of JCV, the pathomolecular mechanisms responsible for JCV-induced tumors, including medulloblastomas, remain to be explored.

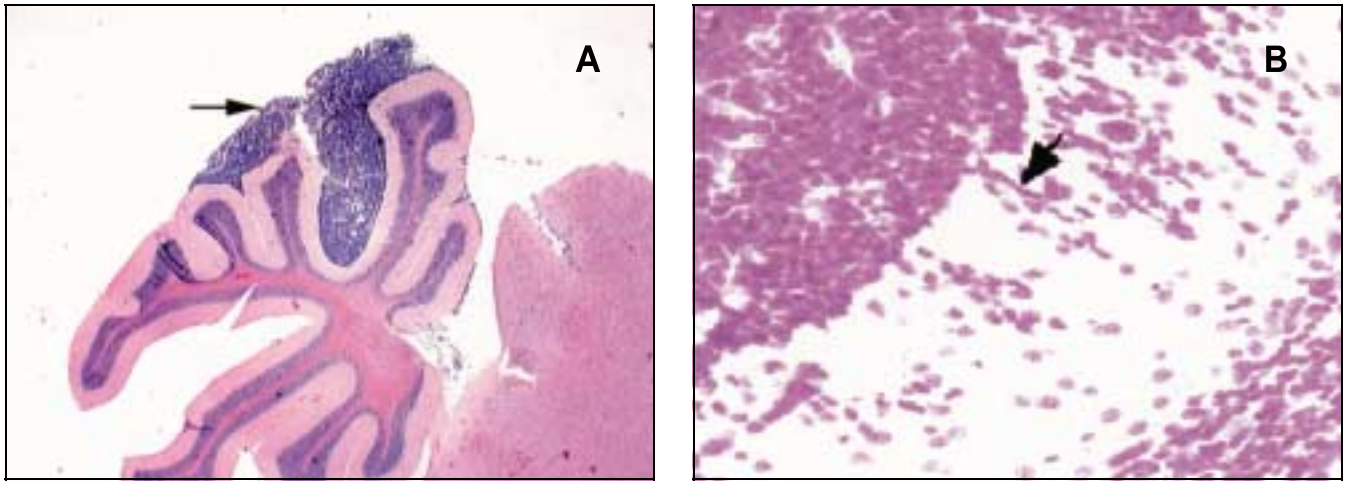

Figure 2. Microscopic evaluation of JCV T-antigen induced medulloblastoma in mouse brain. **[A]** Cerebellar location of the tumor in a transgenic animal (arrow) at low power. **[B]** Invasion of the molecular layer (arrow) at high power.

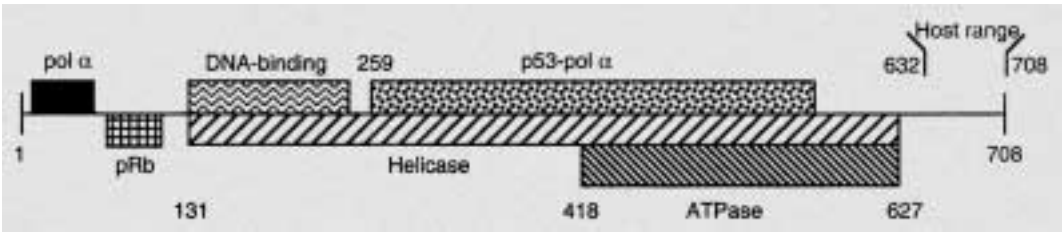

Figure 3. Structural organization of polyomavirus T-antigen. Linear structure of SV40 large T-antigen depicting the various domains of the viral oncogenic protein. The positions of the protein regions that interact with the tumor suppressor proteins, pRb and p53, are shown.

Studies in transgenic mice indicate the direct involvement of JCV T-antigen in tumor formation. Postulated cellular events that may lead to a transformed phenotype include the interaction of JCV T-antigen with specific cellular proteins such as tumor suppressor gene products. The T-antigen of JCV is composed of 695 amino acids with 65–70% homology with SV40 large T-antigen. This homology has led to the speculation that like SV40, JCV T-antigen has multiple functional domains [24] essential for the viral lytic cycle and for cell transformation [Figure 3]. JCV T-antigen has been demonstrated to form stable complexes with p53 in T-antigen transformed cells *in vitro*, as well as in cell lines derived from JCV-inoculated animals [3]. Interestingly, JCV and SV40 T-antigens physically interact with the same region of p53 [25,26]. JCV T-antigen has also been demonstrated to bind to other tumor suppressor proteins such as pRb and p107 [27,28]. Moreover, JCV T-antigen has been shown to interact with these cellular tumor suppressor gene products in tumor tissue derived from transgenic mice expressing JCV T-antigen [29]. Although the ability of JCV T-antigen to functionally block the inhibitory effects of these regulatory proteins is yet undescribed, future *in vivo* studies utilizing JCV T-antigen mutants will address the relevance of these interactions in the pathogenesis of JCV-induced tumors.

The p53 tumor suppressor protein has been shown to play an important role in cell cycle checkpoint control [30]. Evidently, p53 function is required for G1 arrest in response to DNA damage induced by agents such as ionizing radiation, anticancer drugs, and perturbations in the nucleotide pool [31]. In addition, p53 function is required in some cell types for optimal programmed cell death (apoptosis) induced by DNA damage [32] and other agents [33,34]. Several experimental data have led to the belief that the p53-mediated G1 checkpoint is controlled by a key downstream target of p53, i.e., p21WAF, which has the ability to bind cyclin-dependent kinase-2 (cdk2) and cyclin E. cdk2/cyclin E are the essential components for G1 progression into the S phase of the cell cycle [35]. Thus, according to one model, the association of JCV T-antigen with p53 may block the ability of p53 to induce p21WAF, a protein that inhibits cyclin:cdk activity. Deregulation of the participant cyclins, particularly cyclins E and A and their associated kinases, may, in turn, lead to the phosphorylation of pRb and the liberation of E2F-1. The release of E2F-1 from the pRb:E2F-1 complex may also be achieved via the association of T-antigen with pRb. As the level of E2F-1 increases in the cells, E2F-1 may induce its own gene expression as well as those from other S-phase specific promoters such as PCNA and thereby stimulate rapid entry of cells into the S-phase [Figure 4].

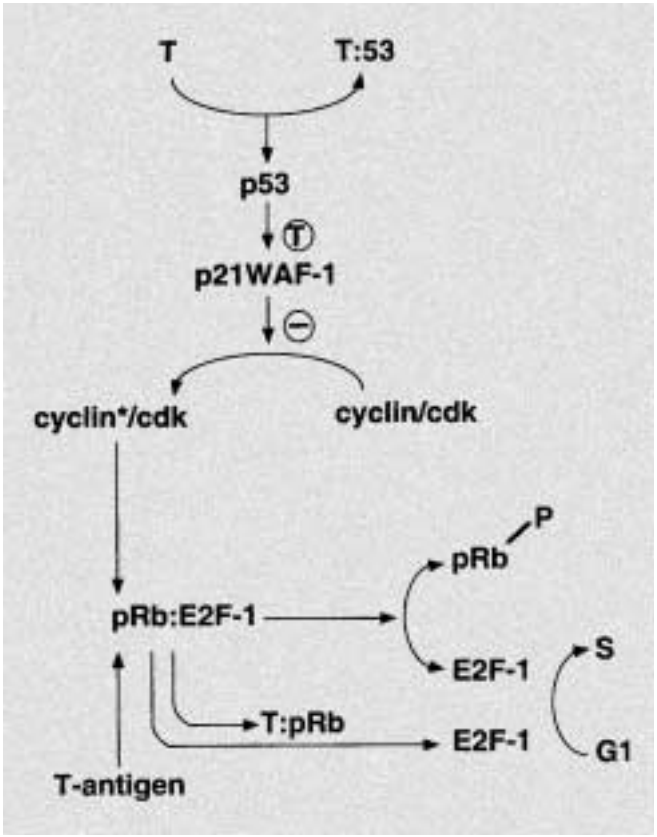

Figure 4. Proposed pathway by which JCV T-antigen induces tumors in experimental animals. Wild-type p53 has the capacity to augment transcription of p21WAF-1, an inhibitor of cyclin kinases, including cyclins E and A and their associated kinases. A decrease in kinase activity of cyclin:kinase maintains pRb in a hypophosphorylated state, which in turn sequesters the transcription factor E2F-1. The association of JCV T-antigen with p53 abrogates the ability of p53 to exert its regulatory action via p21WAF-1. In addition, the association of JCV T-antigen with pRb may liberate E2F-1 from the pRb:E2F-1 complex and permit E2F-1 to induce transcription of S-phase-specific genes.

SV40 = Simian virus 40

As mentioned above, JCV T-antigen has the ability to form a complex with p53 both *in vitro* and *in vivo*. To further examine the role of T-antigen and p53 in the control of medulloblastoma cell proliferation, we derived several cell lines from a mouse medulloblastoma. It was evident that while a majority of tumor cells produced T-antigen, there exist some tumor cells with no evidence of T-antigen expression. Examination of p53 expression in T-antigen-positive and T-antigen-negative tumor cell lines revealed that wild-type p53 is produced in T-antigen-positive cells which is found in association with T-antigen. In T-antigen-negative cells, p53 was found to be mutated and containing a deletion between residues 35 and 123, leading to expression of a smaller p53 transcript [36]. Thus, it is likely that at the early stage, expression of T-antigen and its association with p53 functionally inactivates p53. Inactivation of p53 may result in a broad range of deregulating events at cell cycle checkpoints and can induce genomic instability. The latter event may eventually lead to mutations in the p53 genome, causing a deletion at residues 35 to 123. At this stage, expression of T-antigen may no longer be required for maintenance of tumor cell characteristics.

In conclusion, recent publications on the detection of sequences similar to the polyomavirus SV40 in human tumors, including ependymomas and mesotheliomas, have pushed the human polyomavirus JCV into the spotlight, as this virus is widespread among the human population during childhood and its oncogenic potential is established in experimental animals. Recent efforts by several laboratories have suggested the association of JCV with human medulloblastoma [37]. Thus, it is likely that reactivation of the JCV promoter in CNS and other cell types results in expression of the viral oncogenic protein T-antigen, which in turn leads to the development of neoplasia. All these observations suggest that under different physiological conditions, the human polyomavirus JCV may be associated with two distinct diseases – fatal demyelination of CNS and the development of cancer.

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