Human neurotropic JC virus and its association with brain tumors

Kamel Khalili
Center for Neurovirology and Cancer Biology, College of Science and Technology, Temple University, 1900 North 12th Street, 015-96, Room 203, Philadelphia, PA 19122, USA
Tel.: +1 215 204 0678; Fax: +1 215 204 0679; E-mail: kkhalili@astro.temple.edu

JC virus (JCV) is a human polyomavirus known as the causative agent of the fatal demyelinating disease, Progressive Multifocal Leukoencephalopathy (PML). Further, in experimental animals this virus causes a broad range of tumors of central nervous system origin. Recent studies have suggested the association of JCV with several human tumors most notably malignant brain tumors of childhood, medulloblastoma. The development of tumors by JCV is most likely through mechanisms involving inactivation of tumor suppressors and de-regulation of signaling pathways such as Wnt by the viral early protein, T-antigen. The neurotropic nature of JCV along with the overwhelming evidence for its oncogenic potential in laboratory animals and its detection in significant numbers of human medulloblastomas invite the re-evaluation of the role for JCV in the development of human brain tumors.

Primary tumors of the central nervous system (CNS) can originate from various cells and tissues in brain. In adults, astrocytomas, oligodendrogliomas, and glioblastomas emerge from glial cells; while ependymoma and meningioma, choroid plexus papilloma initiate from ependymal cells and supporting tissues, respectively. In children, the most common tumors, including medulloblastoma, neuroblastoma, and chordoma, originate from primitive neuroectodermal cells. During the past two decades numerous analytical epidemiological studies have been performed to determine the etiology of these diverse human brain tumors. Thus far, all attempts to identify a specific exposure or causative environmental agent in the development of brain tumors have been unsuccessful. Recently much attention has been paid to the human ubiquitous polyomavirus, JCV, and its role in the development of brain tumors. JCV is a neurotropic virus which is considered as the etiologic agent of Progressive Multifocal Leukoencephalopathy (PML), a fatal demyelinating disease of the central nervous system (CNS). JCV is a member of the polyomavirus family of DNA tumor viruses which includes BK virus and the well-known Simian Virus 40 (SV40). JCV co-exists within the human population, as greater than 80% of adults worldwide exhibit JCV-specific antibodies [18]. Infection with the virus is subclinical and occurs in early childhood. The virus remains at the latent stages until the immune system is impaired due to illness such as lymphoproliferative and myeloproliferative disorders, and more recently, AIDS.

The viral genome consists of a closed, circular, double-stranded DNA which is separated into early and late coding sequences by the viral regulatory region which contains the viral origin of DNA replication and several ubiquitous and inducible regulatory modules. Replication of JCV and expression of the viral genome occurs preferentially in CNS cells, and the viral regulatory region is responsible for cell type-specific expression of the viral genome (Fig. 1). The viral early genes, large and small T-antigens, 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 [10].

Several reports point to an association between JCV and human brain tumors. For example, the postmortem exam of a 58 year old man with chronic lymphocytic leukemia and PML revealed the development of an oligodendroglioma [22]. The association of multiple astrocytoma, anaplastic astrocytoma, and PML have also been reported [3,12,24].

In addition to the cases of concomitant PML and cerebral neoplasm, JCV has been associated with human brain tumors in the absence of PML. Rencic et al. [21] reported detection of JCV DNA and expression of the viral early protein, T-antigen, in oligoastrocytoma from an immunocompetent HIV-1-negative patient. Interestingly, Boldorini et al. [2] reported the presence of JCV DNA in a pleomorphic xanthoastrocytoma developed in a 9-year-old immunocompetent patient.
While evidence for the role of JCV in human CNS neoplasms is mounting, the oncogenic potential of JCV has been well established in several animal models. Intracerebral inoculation of non-human primates, including owl and squirrel monkeys, with JCV induced the development of astrocytomas [15,16]. Expression of the JCV early protein, T-antigen, but not capsid proteins was evident in the tumor cells. Newborn Golden Syrian hamsters developed a broad range of tumors including medulloblastoma, astrocytoma, glioblastoma, primitive neuroectodermal tumors, and peripheral neuroblastomas, approximately 6 months after inoculation with JCV [28]. The emergence of CNS tumors in greater than 85% of newborn hamsters inoculated intracerebrally with JCV and expression of JCV T-antigen, clearly verify the oncogenic potential of this virus in neural origin tissue. Injection of JCV into the brains of newborn rats has induced undifferentiated neuroectodermal origin tumors in the cerebrum of 75% of the animals [19,20].

Perhaps some of the most interesting observations on the oncogenicity of JCV relate to studies on several lines of transgenic mice expressing 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. In earlier studies Small et al. reported the appearance of adrenal neuroblastomas in some JCV T-antigen transgenic mice [26]. Recently, we have described the development of a transgenic animal model using the early region of JCV encoding JCV T-antigen [14,25]. Mice developed cerebellar tumors which closely parallel human medulloblastomas in location, histological appearance, and expression of differentiation markers. One potential cellular event which may lead to a transformed phenotype includes the interaction of JCV T-antigen with specific
cellular proteins such as tumor suppressor gene products. 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 [10,17,27]. JCV T-antigen has also been demonstrated to bind to other tumor suppressor proteins such as pRb and p107 [5,6]. Results from biochemical and histological studies revealed that in mouse medulloblastoma, JCV T-antigen interacts with these cellular tumor suppressor gene products in tumor tissue derived from transgenic mice expressing JCV T-antigen [13]. Further examination of JCV-induced medulloblastoma revealed a novel mutation in the p53 genome that causes alternative splicing of the p53 transcript and expression of a smaller p53 protein. Thus, it is clear that expression of T-antigen can inactivate p53 by either physical association and/or introduction of mutation in its genome. In JCV-induced brain malignancy, p53 may not be the only pathway which is affected by T-antigen as examination of the Wnt signaling pathway revealed de-regulation of β-catenin and LEF-1, two major components of this pathway in tumors from mouse medulloblastoma. Thus, a model is proposed which suggests de-regulation of p53 and Wnt pathway by JCV T-antigen which may affect cell cycle progression, cell proliferation, and tumor development (Fig. 2).

Human medulloblastoma is considered a malignant invasive tumor of the cerebellum representing one of the most common neoplasms of the nervous system in children with an annual incidence of approximately one per two hundred thousand [9]. Nearly seventy percent of medulloblastomas occur in children under sixteen years of age [23] and are rarely seen in patients over fifty years of age. While both sexes are affected, there is a slight predominance of male pa-
Fig. 3. Immunohistochemical detection of β-catenin in medulloblastoma cell lines. Two mouse medulloblastoma cell lines were immunolabeled for T-antigen and β-catenin. BSB8 cell line shows intense nuclear immunostaining for T-antigen (Panel A, original magnification 100 x) and both nuclear and cytoplasmic reactivity for β-catenin (Panel B, original magnification 100 x). BS1b culture shows no evidence of T-antigen (Panel C, original magnification 100 x), but β-catenin is present in the perinuclear area of the cytoplasm (Panel D). Two human medulloblastoma cell lines exhibit intense nuclear immunoreactivity for T-antigen, D-283 (Panel E) and DAOY (Panel G, original magnification 100 x) and they exhibit intense nuclear and cytoplasmic staining for β-catenin (Panels F and H, respectively, original magnification 100 x; Panel H original magnification 40 x).

tients (65% male). Histologically, medulloblastomas are comprised of densely cellular tumors with frequent apoptotic cells and mitotic figures. Infrequently, vascular proliferation and hemorrhage are 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 one, seventeen, and to a lesser degree, six, nine, ten, eleven, and sixteen in the development of medulloblastoma [1,11]. Loss of heterozygosity in portions of chromosome seventeen (17p) has been reported in 30–45% of medulloblastomas [4]. The presence of the gene responsible for the production of the tumor suppressor protein, p53, on chromosome seventeen led to the early speculation that this protein may play a key role in the development of medulloblastoma. Furthermore, recent studies showed a mutation in β-catenin in greater than 40% of human medulloblastoma cases [7], suggesting that similar to murine medulloblastoma, Wnt signaling pathway may participate in the pathogenesis of this type of brain tumor in humans. While the etiology of medulloblastomas in humans remains unknown, results from several animal experiments, as described above, have indicated that the human neurotropic JC virus (JCV) is able to induce cerebellar neoplasms in rodents which exhibit a phenotype similar to that of human medulloblastomas. Interestingly, by utilizing PCR techniques, more recently, we have demonstrated that eleven out of twenty-three samples of human medulloblastomas contain DNA sequences corresponding to three different regions of the JCV genome. More importantly, we have demonstrated the presence of DNA sequences encoding the N- and C-terminal regions of the JCV oncogenic protein, T-antigen, in eleven out of twenty-three samples and the production of the viral early protein, T-antigen, in the nuclei of four samples of tumor tissue. These observations provide evidence for the possible association of JCV with human medulloblastomas. With this notion, our next effort will be the examination of several regulatory pathways which may participate in the genesis of medulloblastoma and the development of rapid and convenient assays for detection of JCV expression at the early stage of disease, and indeed, development of effective therapeutic strategies including a vaccine against JCV and the associated medulloblastoma.

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