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Case Report

PCR detection of JC virus DNA in the brain tissue of a 9-year-old child with pleomorphic xanthoastrocytoma

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Pleomorphic xanthoastrocytoma (PXA) is a rare cerebral tumor of young adults with a slow growth and a good prognosis. Due to its peculiar histopathological findings, the tumor resemble to the lytic phase of progressive multifocal leukoencephalopathy (PML), a JC Virus (JCV) induced disease. For these reasons, the presence of JCV genoma and viral particles were searched for by means of nested polymerase chain reaction (nPCR) and electron microscopy (EM) in a 9-year-old child with PXA. Although EM did not reveal any viral particles, nPCR did reveal genomic sequences of the LT, R, and VP1 regions of JCV. Sequence analysis showed that the R region was mutated with respect to the archetypal form thus yielding the *Mad 4* variant of JCV previously reported as being oncogenic in animals. We suggest that JCV may have played a role in the development of this tumor.

Keywords: JC virus; pleomorphic xanthoastrocytoma; PCR; brain

JC virus (JCV) is a widely diffused human polyomavirus with a narrow host range and tissue tropism, which restricts its viral replication to the glial cells of the central nervous system (CNS) (Major *et al*, 1992). Serological evidence indicates that JCV infects more than 70% of the population in early childhood (Walker and Padgett 1983). The virus reaches its target organs by the hematogenous route and becomes latent in kidney and brain, where it can reactivate and replicate under conditions of immunologic impairment (Sundsfjord *et al*, 1994), and thus cause progressive multifocal leukoencephalopathy (PML). Different strains of JCV have been identified by means of polymerase chain reaction (PCR), on the bases of the structure of the non-coding regulatory region (Yogo *et al*, 1994). Archetypal strains have been found in the urine and renal tissue of healthy individuals (Yogo *et al*, 1990) as a result of genomic rearrangements, and it is known that archetypal strains can generate viral variants with mutated tissue tropism and pathogenic behavior (Loeber and Dorries 1988).

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JCV shares a high degree of genomic homology with other polyomaviruses, such as BKV and SV40, which can induce tumors in laboratory animals and are capable of transforming human cells in culture (Major *et al*, 1992). Moreover, a few reports have described the presence of SV40-viral sequences in human brain tumors (Dorries *et al*, 1987; Lednicky *et al*, 1995; Bergsagel *et al*, 1992), and the association of gliomas and CNS lymphoma with PML in immunocompromised subjects (Sima *et al*, 1983; Gullotta *et al*, 1992). Recently, JCV DNA and RNA have been isolated from a case of oligoastrocytoma in which the neoplastic oligodendroglial cells expressed viral oncoprotein T antigen (Rencic *et al*, 1996).

We here report a case of a pleomorphic xanthoastrocytoma (PXA) occurring in an immunocompetent child, in which JCV DNA and its sequence analysis was obtained from tumor tissue by means of PCR.

A 9-year-old, immunocompetent boy, with a two-year history of seizures was admitted to hospital in June 1996. Computed tomography of the head revealed a firm, centrally cystic, superficial left temporal mass of about 3 cm, in diameter (Figure 1). Complete surgical resection of the tumor was carried out, and the patient was discharged two weeks later without any therapy. The patient

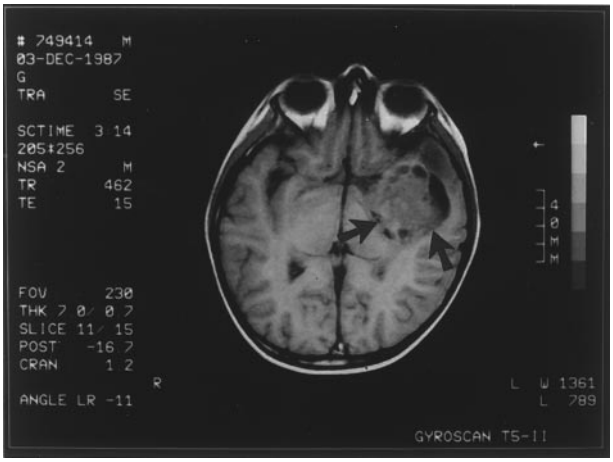

Figure 1 Brain computed tomography shows a round cystic mass of about 3 cm in left temporal lobe of the patient (arrows).

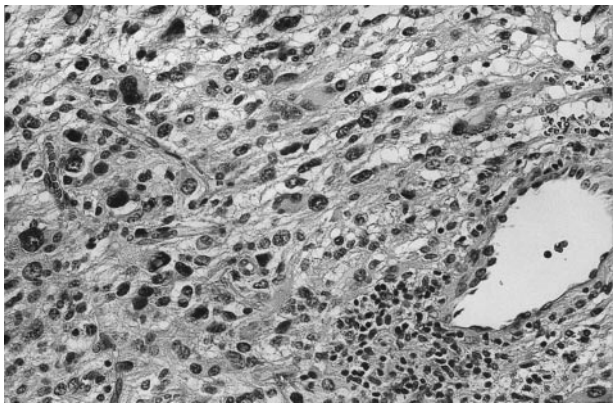

Figure 2 Light microscopical findings of pleomorphic xanthoastrocytoma: closely packed pleomorphic cells with abundant cytoplasm and a small focus of perivacular lymphocytes. (Haematoxylin-eosin, 200×).

currently is alive and well. The surgical specimens were processed for both light and electron microscopy. For the former they were fixed in 10% formalin and embedded in paraffin. Some of the sections were stained with haematoxylin and eosin (HE), Masson trichrome, periodic acid Schiff (PAS), phosphotungstic acid haematoxylin (PTAH) and silver reticulin; others were stained using the streptavidin biotin method for glial fibrillary acid protein (GFAP, Dako, Copenhagen, Denmark), S100 (Dako), CD68 (Dako) and neurofilaments (Pabisch, Milan, Italy). For EM, fresh tissue was fixed in 2.5% glutaraldehyde, post fixed in 1% osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by means of transmission electron microscope (TEM).

On LM, the tumor showed increased cellularity and pleomorphism and occasionally, mitosis. The pattern was biphasic: spindle-shaped cells with elongated nuclei were found in some areas, whereas markedly pleomorphic rounded cells (sometimes with a foamy appearance and large 'bizarre' nuclei) were prevalent in other areas (Figure 2). The tumor was highly vascularized and a small collection of lymphocytes and plasmacells was seen around the small vessels and among the neoplastic cells. The cytoplasm of the spindle-shaped and round cells (and occasionally of the foamy cells) was stained with GFAP. The neoplastic cells were not stained with neurofilaments, but these were present in normal neurons entrapped within the tumor. TEM examination revealed that the cytoplasm of the neoplastic cells contained abundant filaments of about 100 Å. In some cells, there were a large number of lipid vacuoles, mitochondria, and occasional perinuclear Golgi complexes and rough endoplasmic reticulum. The cells were similar to fibrillary astrocytes, but both the individual cells and cell groups were surrounded by a prominent basal lamina. Despite a careful search, no papovavirus particles were identified in the neoplastic cells.

In order to identify the presence of polyomavirus DNA sequences, nested PCR (nPCR) was used. The DNA was extracted from paraffin-embedded sections of three different samples of the neoplasm by means of phenol-chloroform method. Moreover, we analysed a sample of urine and heparinized peripheral blood. Blood was separated by gradient centrifugation with Ficoll-Hypaque (Pharmacia Biotech, Uppsala, Sweden) and aliquots of 2.5×10^6 peripheral lymphocytes (PBL) were suspended in 200 μl of lysis buffer containing 50 mM Kcl, 10 mM Tris-HCl, pH 8.3, 2.5 mM $MgCl_2$, 1% Tween 20, 0.5% NP40, 100 μg/ml proteinase K (Boehringer Mannheim, GmbH, Germany). After incubation at 56°C for 1 h, the enzyme was inactivated at 95°C for 15 min. Twenty microliters of lysate was added to the nPCR reaction mixture.

Fresh urine sample was centrifuged for 10 min at 1500 r.p.m. and 5 μl of the urinary sediment was added to the nPCR reaction mixture. In the first round, a long PCR common for JCV, BKV and SV40 genome was performed using primers JRE1 (5′-CCTCCCTATTCAGCACTTTGT-3′, nt 4989–5009) and JC2 (5′-GCTTCAGACAATGGTTTGGG-3′, nt 4573–4592).

Afterwards, we made three inner amplifications specific for the large T antigen (LT), the VP1 late coding region, and non-coding regulatory region (R). The LT region was amplified using PEP1 (5′-AGTCTTTAGGGTCTTCTACC-3′; 4255–4274) and PEP2 (5′-GGTGCCAACCTATGGAACAG-3′; 4408–4427) primers, the VP1 region using VP3 (5′-TTTTGGGACACTAACAGGAG-3′; 2107–2126) and VP4 (5′-GTCAACGTATCTCATCATGT-3′; 2481–2500) primers, and the R region using JRI1 (5′-CTCCACGCCCTTACTACTTCT-3′; 5087–5107)

and JRE2 (5′-TACGTGACAGCTGGCGAAGAA-3′; 281–301) primers. All PCR reactions were performed with one negative and one positive control. The negative control contained all the PCR components except the template, whereas as the positive control we used BKV DNA, obtained from a brain tumour-derived cell. It was provided by the Institute of Histology and General Embryology of the University of Ferrara (Italy). PCR positive samples were analysed by direct automated sequencing.

Direct nucleotide sequencing was performed using the AutoLoad Solid Phase Sequencing kit, and the biotinilated amplified products were run on ALFexpress DNA Sequencer (Pharmacia Biotech, Uppsala, Sweden). The sequence analyses were compared using the DNAsis® for Windows® program of Hitachi Software Engineering Co. (San Bruno, CA, USA).

Sequences of the LT, R and VP1 regions of JCV-DNA were found only in one sample of paraffin embedded tumour tissue. PBL and urine specimens were negatives. Sequence analysis of the R region showed some rearrangements in the 98 bp tandem repeats that are present in the *Mad-1* prototypal strain (Major *et al*, 1992). In our isolated strain, the second TATA box was lost and both of the penta regions in the 98 bp repeat with the sequence 5′-AGGGAAGGGA-3′ were mutated (Figure 3). The same rearrangement has been previously detected by Martin *et al* (1985), who called it *Mad-4*. Sequence analysis of the VP1 and LT regions showed the characteristics nucleotide sites of *Mad-1*.

No human brain tumors have yet been shown to be surely caused by viral infection, but human polyomaviruses JCV and BKV are known to induce a variety of tumors after the intracerebral inoculation of juvenile hamsters. BKV causes ependymoma and choroid plexus papilloma (Corallini *et al*, 1978), whereas JCV induces glioblastomas, medulloblastomas, meningiomas, ependymomas and other unclassified primitive tumors (Walker *et al*, 1973).

BKV and SV40 DNA sequences have been isolated from various types of human brain tumor. Dorries *et al* (1987) examined 24 human brains for the presence of BKV, JCV and SV40 by means of Southern blot analysis and found BKV DNA sequences in 11, including meningiomas (five), neurinomas (three), glioblastomas (one), oligodendroglioma (one) and an unclassified malignant glioma (one). No JCV or SV40 genomic sequences were detected in their specimens. Lednicky *et al* (1995) detected SV40 sequences in 14 out of 17 samples of choroid plexus tumors and ependymomas in children.

An association between PML, (a JCV-induced demyelinating disease) and brain tumors has been

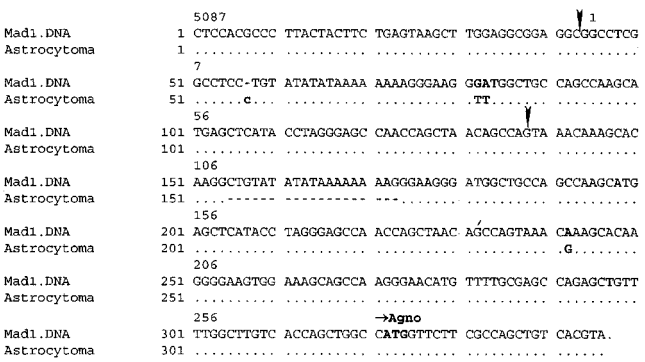

Figure 3 Nucleotide sequence of JCV regulatory region obtained from PXA tissue. The letters in bold represent the pentanucleotide activator present in *Mad-1* strain. The arrows define the boundaries of 98 bp tandem repeats. Numbering is based on *Mad-1* strain.

reported by a number of authors (Sima *et al*, 1983); Gullotta *et al*, 1992). Moreover, Rencic *et al* (1996) demonstrated the presence of JCV DNA and RNA by means of PCR and T large antigen by means of immunohistochemistry in the oligodendroglial cells of an oligoastrocytoma in an imunocompetent patient.

To our knowledge, no authors have previously evaluated the presence of human papovavirus DNA in PXAs. These are rare tumors occuring in children and young adults, that generally have a good prognosis; the histopathological findings resemble those observed during the lytic phase of PML (Gildenberg *et al*, 1993), and the latter is frequently mistaken for PXA, especially in small brain biopsies. The present case showed the typical light and electron microscopical features of PXA (Kepes *et al*, 1979) and PCR analysis revealed the presence of JCV genomic sequences. Sequence analysis of the isolated JCV showed an R region that was mutated with respect to the archetypal form: the deletion of the distal TATA box found in the present case yields the JCV variant called *Mad-4* (Martin *et al*, 1985). Major *et al* (1984) have reported finding of JCV *Mad-4* in the tumor cells of an astrocytoma taken from a monkey inoculated with the JCV archetype, suggesting that these genomic rearrangements make the virus potentially oncogenic.

In our case, no viral particles were found in the tumor cells; this may have been due to the integration of viral DNA into the cellular genoma.

The PCR isolation of JCV DNA from brain tissue, and the characterization of its genomic sequence, suggests that the virus may have been related to the development of PXA in our case.

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