Detection of Merkel Cell Polyomavirus Large T-Antigen Sequences in Human Central Nervous System Tumors

Farzin Sadeghi,[1,2] Mostafa Salehi-Vaziri,[1] Ahad Alizadeh,[3] Seyed Mohammad Ghodsi,[4] Farah Bokharaei-Salim,[5] Abolfazl Fateh,[1] Seyed Hamidreza Monavari,[5] and Hossein Keyvani[5]*

[1]*Department of Virology, Tehran University of Medical Sciences, School of Public Health, Tehran, Iran*
[2]*Department of Immunology and Microbiology, Babol University of Medical Sciences, School of Medicine, Babol, Iran*
[3]*Department of Epidemiology and Biostatistics, Tehran University of Medical Sciences, School of Public Health, Tehran, Iran*
[4]*Department of Neurosurgery, Tehran University of Medical Sciences, Shariati Hospital, Tehran, Iran*
[5]*Department of Virology, Iran University of Medical Sciences, School of Medicine, Tehran, Iran*

Despite decades of epidemiological investigation, little is known about the etiology of the central nervous system (CNS) tumors, and few well-established risk factors have been recognized. This study tested the presence of Merkel cell polyomavirus (MCPyV), the only member of the Polyomaviridae family convincingly linked to human cancer, in diverse CNS malignancies. In total, 58 CNS tumor biopsies were analyzed for the MCPyV large T-antigen (LT-Ag) gene by quantitative real-time PCR. Merkel cell polyomavirus LT-Ag DNA load was determined as viral copies per cell and viral copies per microliter of purified genomic DNA from CNS tumor samples. The MCPyV LT-Ag sequence was detected in 34 (58.6%) of the 58 tested samples. Viral LT-Ag was quantified in 19.0% of schwannomas, 13.8% of meningiomas, and 5.2% of pituitary adenomas. The difference between MCPyV positivity in different types of CNS malignancies was not statistically significant ($P = 0.066$). The mean LT-Ag copy number in 34 positive samples was 744.5 ± 737.7 and $0.056 \times 10^{-3} \pm 0.091 \times 10^{-3}$ per microliter and per cell, respectively. Among MCPyV-positive CNS tumors, the mean MCPyV copy number was higher in meningiomas (993.8 ± 853.2 copy per microliter and $0.098 \times 10^{-3} \pm 0.108 \times 10^{-3}$ copy per cell). Multiple linear regression analysis revealed statistically significant difference in MCPyV copy number between meningioma and other CNS tumor types, when the model was adjusted for age and sex ($P = 0.024$). This study shows the first evidence of the detection of MCPyV LT-Ag sequence at a low copy number in human CNS tumors. ***J. Med. Virol.***

KEY WORDS: merkel cell polyomavirus; large T-antigen; central nervous system tumors

INTRODUCTION

Central nervous system (CNS) tumors cause significant morbidity and mortality worldwide and account for 2% of all human adulthood neoplasms [Siegel et al., 2013]. Statistics on the incidence of CNS tumors have revealed an estimated rate of 3.9 and 3.2 per 100,000 person-years in males and females, respectively [Ferlay et al., 2010]. Despite decades of epidemiological investigations, little is known about etiologies or causative agents for CNS tumors, and few consistent risk factors have been identified, including genetic predisposition, exposure to ionizing radiation, neurocarcinogens, allergic diseases, and persistent infection with oncogenic viruses [Bondy et al., 2008].

In terms of viral etiologies of CNS tumors, the largest area of inquiry comes from studies of polyomaviruses [Saddawi-Konefka and Crawford, 2010].

Conflicts of interest: The authors declare that they have no conflicts of interest.

Grant sponsor: Tehran University of Medical Sciences; Grant number: 92-01-30-20990.

*Correspondence to: Hossein Keyvani, Department of Virology, Iran University of Medical Sciences, School of Medicine, Tehran, Iran. E-mail: keyvanlab@yahoo.com

Accepted 30 January 2015

DOI 10.1002/jmv.24178
Published online in Wiley Online Library (wileyonlinelibrary.com).

Polyomaviruses are a family of DNA tumor viruses with near-ubiquitous infection in the human population. Currently, twelve human polyomaviruses are known [Moens et al., 2014], all of which have the capacity to induce tumors because all encode the tumor antigen (T-Ag) oncoproteins [Moens et al., 2011]. The tumor antigen encoded by many polyomaviruses readily transforms cells from different species and induces CNS tumors in experimental animals [Croul et al., 2003].

Several recent reports have indicated possible associations between CNS tumors and human polyomaviruses, JC polyomavirus (JCPyV), and BK polyomavirus (BKPyV) and their genomic sequences have been identified in CNS cancer tissue [Flaegstad et al., 1999; Delbue et al., 2005; Tsekov et al., 2011; Sadeghi et al., 2014]; but the members of this family that discovered more recently have yet to be investigated in association with human CNS tumors. In 2008, the fifth human polyomavirus, designated Merkel cell polyomavirus (MCPyV), was isolated from 80% of Merkel cell carcinomas, a neuroendocrine cancer of the skin [Feng et al., 2008]. Merkel cell polyomavirus is the first polyomavirus that demonstrates a strong association with human cancer. Although there is limited information regarding MCPyV's mode of transmission, isolation of MCPyV genomic sequences in Merkel cell carcinomas indicates a tropism for neuroepithelial cells. Several investigations have examined the presence of MCPyV in other human tumors. Although MCPyV DNA was detected in a subset of patients with chronic lymphocytic leukemia [Haugg et al., 2011; Teman et al., 2011], non-small-cell lung cancer [Hashida et al., 2013], cervical cancer, and non-melanoma skin cancer [Kassem et al., 2009; Imajoh et al., 2012a], it remains unclear whether MCPyV plays a role during the development of these malignancies. So far, only two pilot studies have investigated the presence of MCPyV in CNS tumors [Giraud et al., 2009; Sastre-Garau et al., 2009], all from young children and, according to the available information, the presence of MCPyV in diverse types of CNS malignancies has not been examined. The facts reviewed above encouraged us to investigate the presence of MCPyV in different types of CNS neoplasias.

The aim of this study was to assess the possibility of MCPyV neuropersistence and to investigate the presence of MCPyV DNA sequences in tumor samples taken from Iranian patients diagnosed with CNS malignancy.

MATERIALS AND METHODS

Patients and Samples

From November 2012 to January 2014, a total of 58 fresh CNS tumor biopsy samples were obtained from patients who underwent surgical operations of the CNS at the neurosurgery department of Shariati Hospital affiliated to Tehran University of Medical Sciences. At the time of surgical procedure, each fresh biopsy specimen was excised and placed immediately into RNALater solution (Sigma R0901, St. Louis, MO). Samples were stored at −80°C until the time of examination. Central nervous system tumor histopathologic diagnosis was established as part of the routine examination by the pathology department of Shariati Hospital. In total, 56 patients had intracranial brain tumor and meningioma and two patients had spinal cord tumor. Apart from one case, all of the patients included in this study showed normal white blood cell (WBC) count (more than 4,500 cells/μl before surgical operation. All of the patients were tested negative for human immunodeficiency virus type 1 (HIV-1) infection. None of the subjects had received immunosuppressive therapy except for 8 mg of intravenous dexamethasone before surgery to decrease swelling and pressure in the normal tissue around the tumor. This study was approved by the Ethical Committee of Tehran University of Medical Sciences and, for all subjects, written informed consent was obtained.

DNA Extraction

DNA was extracted from 10 mg of each tissue sample, using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality and quantity of extracted DNA was specified with a nanodrop spectrophotometer (Thermo Scientific, Wilmington, DE) at the end of the extraction procedure. Sterile tubes containing only reaction mixtures were processed in parallel with the tissue samples as an extraction negative control.

Quantitative Real-Time Polymerase Chain Reaction

Quantitative real-time PCR was performed using a Rotor-Gene Q real-time PCR system (Qiagen, Hilden, Germany) using primer sets and TaqMan probes specific for the MCPyV LT-Ag gene and the human RNase P gene [Becker et al., 2009; Imajoh et al., 2012b]. Each reaction consisted of 500 ng of extracted DNA, 12.5 μl Maxima Probe qPCR Master Mix (Fermentas, Glen Burnie, MD), 0.3 μM of each primer, and 0.2 μM of dual-labeled probe in a 25 μl total reaction volume. To generate standard curves, real-time PCR was performed on a 10-fold dilution series of each purified plasmid (pMCPyV TAg and pRNase P) ranging from 2×10^1 to 2×10^6 copies/μl. Viral gene copy numbers per cell were calculated by dividing the virus copy number by half of the RNase P copy number, because each diploid cell contains two copies of RNase P [McNees et al., 2005].

Real Time Reverse Transcription Polymerase Chain Reaction

Total cellular RNA was collected from MCPyV positive CNS tumors using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's

instructions. Transcripts for the MCPyV LT-Ag were analyzed by the use of qualitative Real Time Reverse Transcription PCR (Real Time RT-PCR). Real Time RT-PCR was done on a Rotor-Gene Q real-time PCR system (Qiagen) by the use of the primer sets as previously described [Sastre-Garau et al., 2009]. Amplifications were obtained using 100 ng total RNA, 12.5 μl One Step SYBR RT-PCR buffer 4 (Takara Bio, Shiga, Japan), 1 μl PrimeScript 1 step Enzyme Mix 2 (Takara Bio), and 10 μM of each primer in a 25 μl total reaction volume. Total RNA extracted from MCC tumor was used as a positive control. To confirm amplification specificity the PCR products were subjected to a melting-curve analysis.

Statistical Analysis

The statistical analyses were performed using SPSS version 16 software (SPSS, Chicago, IL). Statistical differences between groups were assessed by χ^2-test (exact *P*-value was estimated by the Monte Carlo method). Normal distribution of the variables was analyzed by the Kolmogorov–Smirnov test. Analysis of continuous variables was carried out using the independent samples *t*-test/Mann–Whitney U test and ANOVA/Kruskal–Wallis test. A multiple linear regression model was applied to estimate effects of explanatory variables on quantitative response. *P*-value of ≤ 0.05 was considered to be statistically significant.

RESULTS

Demographic and Histopathologic Characteristics

This investigation included 58 CNS tumor cases. The mean age of the patients (male 28, female 30) was 46.0 ± 17.6 years (range 5–83 years). With respect to WBC count, the mean was $8{,}438.8 \pm 2{,}645.5$ cells/μl (range 3,400–16,700 WBC/μl). Considering tumor origin, 54 (93.1%) were primary CNS tumors and four (6.9%) metastatic CNS tumors. Of the 54 primary CNS tumors, schwannoma (24.1%), meningioma (20.7%), and glioblastoma multiform (12.1%) were the three most common histopathologic types. Of the four tumors with CNS metastasis, three were diagnosed as adenocarcinoma originating from lung cancer and one was diagnosed to be squamous-cell carcinoma with an unknown primary site of origin.

Detection of MCPyV DNA and Quantification of Viral Loads by Real-Time PCR

In the present study, specimens from CNS tumors were examined for the presence of MCPyV LT-Ag sequence by quantitative real-time PCR. Of the 58 tested samples, the MCPyV LT-Ag sequence was detected in 34 (58.6%) cases. Of those 34 positive cases, 17 patients were male. No significant differences were found between the MCPyV positivity with age ($P = 0.846$) or sex ($P = 0.629$). The mean WBC count of MCPyV-positive subjects was $8{,}458.4 \pm 2{,}642.8$ cells/μl (range 3400–16,700), while the mean WBC count was $8{,}411.2 \pm 2{,}705.9$ cells/μl (range 5360–15,490) for MCPyV-negative subjects. There was no significant difference in mean WBC count between MCPyV-positive and -negative subjects ($P = 0.586$). In detail, from 58 tested samples, MCPyV LT-Ag was quantified in 19.0% of schwannomas, 13.8% of meningiomas, and 5.2% of pituitary adenomas. No statistically significant association between MCPyV positivity and various types of CNS malignancies was observed ($P = 0.066$) (Table I).

In the current study MCPyV LT-Ag load was determined as viral LT-Ag copies per microliter of purified genomic DNA from CNS tumor samples. Also, MCPyV LT-Ag load was measured as viral LT-Ag copies per RNase P gene copy (a proven single copy gene), which described the viral copy number per cell. Amplification of a cellular RNase P gene also acts as a control for the presence of sufficient amplifiable DNA. The mean MCPyV LT-Ag copy number in 34 positive cases was 744.5 ± 737.7 and $0.056 \times 10^{-3} \pm 0.091 \times 10^{-3}$ /μl of purified genomic DNA and per cell, respectively. In MCPyV-positive CNS tumors, the mean MCPyV copy number was higher in the meningioma subjects (993.8 ± 853.2 copy/μl and $0.098 \times 10^{-3} \pm 0.108 \times 10^{-3}$ copy per cell,) (Fig. 1). In the case of two MCPyV-positive metastatic adenocarcinomas, retrospective review was carried out in the archives of the pathology department of Shariati Hospital for the primary tumor samples. Formalin-fixed paraffin-embedded samples were found for one patient only (a 60-year-old woman). After quantitative real-time PCR analysis, MCPyV LT-Ag sequence was quantified in the patient's primary tumor specimen at a low copy number (122 copies/μl and 0.002×10^{-3} copies per cell).

Analysis using multiple linear regression showed a statistically significant difference in MCPyV copy number between meningioma and other CNS tumor types when the model was adjusted for age and sex ($P = 0.024$). In addition, according to the regression model, MCPyV copy number was significantly higher in the group of "others" (consisting of MCPyV-positive tumors with metastatic adenocarcinoma, hemangioblastoma, pineoblastoma, chordoma, and cavernoma) in comparison with different types of CNS tumor ($P = 0.044$) (Table II). Statistical analysis of WBC counts and MCPyV copy numbers in viral-positive CNS tumors revealed that WBC count was not correlated with MCPyV copy number per microliter of purified genomic DNA ($R = 0.136$, $P = 0.308$) or per cell ($R = 0.036$, $P = 0.788$).

Expression of MCPyV LT-Ag Transcript

To evaluate the association between MCPyV and CNS tumors, viral DNA positivity alone is not sufficient. Therefore, the expression of the MCPyV LT-Ag in MCPyV DNA positive CNS tumors was examined at the RNA level using Real Time RT-PCR. Merkel cell

TABLE I. Statistical Associations Between the Presence of MCPyV LT-Ag Sequence and Demographic and Histopathologic Parameters in Iranian Patients With CNS Neoplasias

Patients		MCPyV LT-Ag Sequence			
		Positive	Negative	Total	*P*-value
	No of patients	34 (58.6%)	24 (41.4%)	58	
	Age (years)	46.3 ± 16.1 (16.0–83)	45.4 ± 19.7 (5.0–74.0)	46.0 ± 17.6 (5.0–83.0)	0.846
	Gender Male	17 (29.3%)	11 (19.0%)	28 (48.3%)	0.754
	Female	17 (29.3%)	13 (22.4%)	30 (51.7%)	
	WBC[a] Counts per microliter	8,458.4 ± 2,642.8 (3,400–16,700)	8,411.2 ± 2,705.9 (5,360–15,490)	8,438.8 ± 2,645.5 (3,400–16,700)	0.586
Histopathology	Schwannoma	11 (19.0%)	3 (5.2%)	14 (24.1%)	0.066
	Meningioma	8 (13.8%)	4 (6.9%)	12 (20.7%)	
	Glioblastoma multiform	2 (3.4%)	5 (8.6%)	7 (12.1%)	
	Astrocytoma	2 (3.4%)	1 (1.7%)	3 (5.2%)	
	Pituitary adenoma	3 (5.2%)	0 (0.0%)	3 (5.2%)	
	Epidermoid Tumor	0 (0.0%)	3 (5.2%)	3 (5.2%)	
	Adenocarcinoma(Metastatic)	2 (3.4%)	1 (1.7%)	3 (5.2%)	
	Hemangioblastoma	1 (1.7%)	1 (1.7%)	2 (3.4%)	
	Pineoblastoma	1 (1.7%)	1 (1.7%)	2 (3.4%)	
	Oligodendroglioma	2 (3.4%)	0 (0.0%)	2 (3.4%)	
	Oligoastrocytoma	0 (0.0%)	1 (1.7%)	1 (1.7%)	
	Chordoma	1 (1.7%)	0 (0.0%)	1 (1.7%)	
	Squamous cell carcinoma (Metastatic)	0 (0.0%)	1 (1.7%)	1 (1.7%)	
	Cavernoma	1 (1.7%)	0 (0.0%)	1 (1.7%)	
	Medulloblastoma	0 (0.0%)	1 (1.7%)	1 (1.7%)	
	Xanthoastrocytoma	0 (0.0%)	1 (1.7%)	1 (1.7%)	
	Ependymoma	0 (0.0%)	1 (1.7%)	1 (1.7%)	

[a]White blood cell.

polyomavirus LT-Ag transcript was detected in two out of 34 viral DNA positive samples (one schwannoma and one metastatic adenocarcinoma).

DISCUSSION

The principal site of persistence for polyomaviruses has been difficult to identify. Many polyomaviruses, including JCPyV, BKPyV, and MCPyV, share overlapping sites of persistence and have been found in urine [Markowitz et al., 1993; Husseiny et al., 2010], respiratory tract secretions [Sundsfjord et al., 1994; Bialasiewicz et al., 2009], and lymphoid tissues [Dorries et al., 1994; Shuda et al., 2009]. Since many studies have found a possibility of neuropersistence for JCPyV and BKPyV, a neurotropic potential for

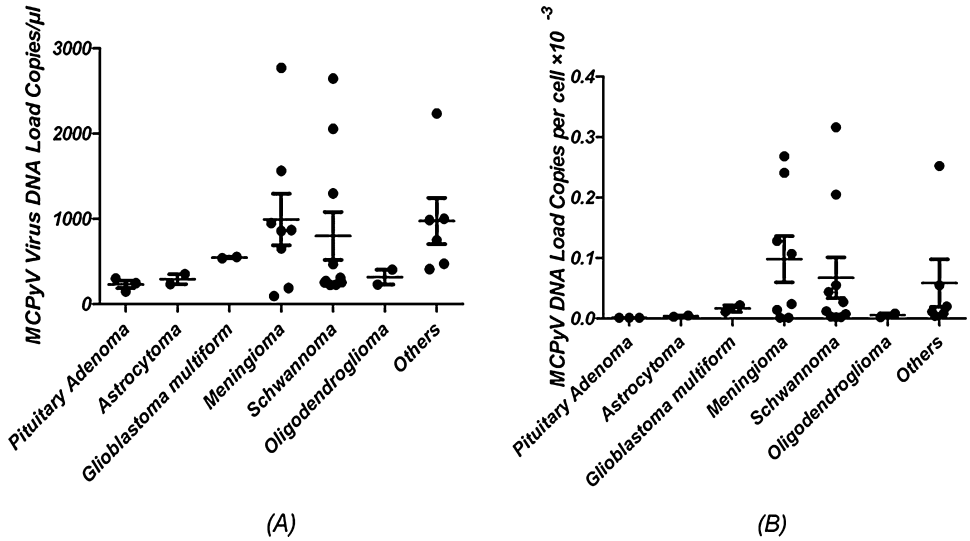

Fig. 1. Merkel cell polyomavirus LT-Ag DNA copy number in different groups of CNS tumor samples. (**A**) Merkel cell polyomavirus LT-Ag DNA copy number per microliter of purified genomic DNA from CNS tumor samples. (**B**) Merkel cell polyomavirus DNA copy per cell $\times 10^{-3}$ of CNS tumor samples. Horizontal lines indicate the mean. Error bars indicate standard error.

TABLE II. Multiple Linear Regression Analysis of MCPyV LT-Ag DNA Load in Different Groups of CNS Tumors. Dependent Variable is MCPyV LT-Ag DNA Load (Copies/μl). Pituitary Adenoma is Taken as a Reference Category (Smallest Mean in Terms of MCPyV LT-Ag DNA Load)

Parameter*		B	95% Confidence interval		*P*-value
			Lower	Upper	
(Intercept)		271.702	63.296	1,166.303	<.001
CNS tumor types	Others	3.319	1.033	10.658	0.044[a]
	Oligodendroglioma	1.407	0.351	5.640	0.629
	Schwannoma	2.261	0.785	6.510	0.131
	Meningioma	3.356[b]	1.172	9.615	0.024[a]
	Glioblastoma multiform	2.196	0.546	8.829	0.268
	Astrocytoma	1.321	0.325	5.364	0.697

*Model was adjusted for age and gender.
[a]Statistically significant.
[b]For a given age and gender by switching from pituitary adenoma to meningioma 3.356 times increase in MCPyV LT-Ag DNA load was expected.

MCPyV is also conceivable. Merkel cell polyomavirus is the only member of the polyomaviridae family convincingly linked to human neuroendocrine cancer of the skin, Merkel cell carcinoma. To date, only two research groups, with limited sample sizes, have investigated the presence of MCPyV in CNS tumors and neuroblastomas [Giraud et al., 2009; Sastre-Garau et al., 2009]. In the present study, a larger variety of human CNS malignancies were examined and fresh biopsy samples were analyzed using a quantitative real-time PCR technique. Additionally, MCPyV LT-Ag gene copy numbers were normalized to cell numbers in this study. This ratio might be informative when determining viral loads in tumor tissues [McNees et al., 2005].

The most frequent types of CNS malignancy in this cross-sectional study were schwannoma, meningioma, and glioblastoma, which agree with the pattern of CNS tumors in Iran [Mehrazin et al., 2006]. Merkel cell polyomavirus LT-Ag sequence was found with a low viral gene copy number per cell ratio in 58.6% of CNS tumor specimens. This finding is inconsistent with the results of previous reports, which have indicated an absence of MCPyV sequences from childhood CNS tumors and neuroblastomas [Giraud et al., 2009; Sastre-Garau et al., 2009]. It should be noted that the current study included no neuroblastoma patients and only two cases of childhood CNS tumor (one medulloblastoma and one xanthoastrocytoma), both of which were negative for the MCPyV LT-Ag sequence. Although recent work on autopsy brain tissues collected from 30 individuals who died from diseases unrelated to CNS showed negative results for MCPyV sequences by nested PCR assay [Lam et al., 2010], this finding does not exclude the possible role of MCPyV in CNS tumor formation.

In the group of primary CNS tumors, the MCPyV LT-Ag sequence was recognized more frequently in schwannomas, meningiomas, and pituitary adenomas. In the four metastatic CNS tumors, the MCPyV LT-Ag sequence was quantified in two patients with brain adenocarcinomas (originating from lung cancer). A primary tumor sample (formalin-fixed paraffin-embedded) was available for only one of these two patients: evaluation of the patient's primary lung tumor revealed a low copy number of the MCPyV LT-Ag sequence. A recent study has provided evidence that MCPyV may be associated partly with the pathogenesis of lung cancer, and that lung tumor cells harbor an integrated form of the viral genome [Hashida et al., 2013]. In addition, several studies have demonstrated that different DNA tumor viruses may play a role in the induction of tumor cell migration and promote a metastatic phenotype [Gou et al., 2003; Behren et al., 2005]. From this point of view, the existence of MCPyV in both the primary lung tumor and its CNS metastasis may strengthen the hypothesis of a pathogenic role for MCPyV in primary tumor induction in lung. However, a low level of viral DNA in both primary and metastatic tumors may indicate simple latent replication.

The Merkel cell polyomavirus genome is present generally at more than one DNA copy per Merkel cell carcinoma tumor cell, supporting a direct carcinogenic mechanism and tumor-viral clonality [Moore and Chang, 2010]. In the present study, a low copy number of MCPyV LT-Ag gene per cell was detected in primary and metastatic CNS tumors. A number of investigations have indicated that the expression of the MCPyV LT-Ag is crucial for the viral carcinogenesis [Shuda et al., 2008; Houben et al., 2010; Sihto et al., 2011]. In this context, the presence of the MCPyV LT-Ag transcript in MCPyV DNA positive CNS tumors was examined. Two out of 34 MCPyV-DNA-positive tumors had detectable levels of MCPyV LT-Ag transcript. Given the lack of detection for MCPyV LT-Ag transcript in the majority of MCPyV DNA positive tumors, and the immunocompetence of the subjects, low copy numbers of viral LT-Ag might be explained by a few possibilities. The first explanation could be a low level of MCPyV neuropersistence as a passenger virus with no apparent pathology associated consequence. Second, low copy numbers of LT-Ag gene might

contribute to tumor initiation by indirect carcinogenic mechanisms like hit-and-run, but not full progression to malignancy. According to the multiple linear regression model, the MCPyV copy number was significantly higher in meningiomas than other tumor types. The etiology of meningiomas is still unknown. Individuals with certain mutations in the neurofibromatosis type 2 (NF2) gene on human chromosome 22 have increased risk for meningioma [Wiemels et al., 2010], but none of the subjects in the present study had an NF2 genetic disorder. Interestingly, NF2 protein is a positive regulator of p53 in terms of tumor suppressor activity and LT-Ag can inhibit NF2's tumor suppressor function by physical interaction in an LT-Ag transgenic mouse model [Shollar et al., 2004; Del Valle et al., 2008]. This finding might be relevant to the current study results.

The findings of the present study should be interpreted with caution due to the lack of matching non-neoplastic or normal CNS tissues as a control. Also, assessment of the infection status of patients' cerebrospinal fluid (CSF) may shed more light on results found in CNS tissues, but unfortunately, access to CSF samples from the patients in this study was not possible.

Taken together, the present study is a preliminary work that provides the first evidence of the detection of the MCPyV LT-Ag sequence in both primary and metastatic human CNS tumors. Current research suggests that MCPyV persists at a low viral copy in CNS tumors and that continuous presence of this oncogenic virus in CNS may be correlated with cancer development in this region. These findings should incite further worldwide epidemiological and virological investigations to distinguish the possible role of MCPyV in tumor induction from simple persistent viral replication.

ACKNOWLEDGMENTS

We acknowledge the support of the directors and staff in Neurosurgery Department of Shariati Hospital affiliated to Tehran University of Medical Sciences for their collaboration in sample collection.

REFERENCES

Becker JC, Houben R, Ugurel S, Trefzer U, Pfohler C, Schrama D. 2009. MC polyomavirus is frequently present in Merkel cell carcinoma of European patients. J Invest Dermatol 129:248–250.

Behren A, Simon C, Schwab RM, Loetzsch E, Brodbeck S, Huber E, Stubenrauch F, Zenner HP, Iftner T. 2005. Papillomavirus E2 protein induces expression of the matrix metalloproteinase-9 via the extracellular signal-regulated kinase/activator protein-1 signaling pathway. Cancer Res 65:11613–11621.

Bialasiewicz S, Lambert SB, Whiley DM, Nissen MD, Sloots TP. 2009. Merkel cell polyomavirus DNA in respiratory specimens from children and adults. Emerg Infect Dis 15:492–494.

Bondy ML, Scheurer ME, Malmer B, Barnholtz-Sloan JS, Davis FG, Il'yasova D, Kruchko C, McCarthy BJ, Rajaraman P, Schwartzbaum JA, Sadetzki S, Schlehofer B, Tihan T, Wiemels JL, Wrensch M, Buffler PA. 2008. Brain tumor epidemiology: Consensus from the brain tumor epidemiology consortium. Cancer 113:1953–1968.

Croul S, Otte J, Khalili K. 2003. Brain tumors and polyomaviruses. J Neurovirol 9:173–182.

Delbue S, Pagani E, Guerini FR, Agliardi C, Mancuso R, Borghi E, Rossi F, Boldorini R, Veggiani C, Car PG, Ferrante P. 2005. Distribution, characterization and significance of polyomavirus genomic sequences in tumors of the brain and its covering. J Med Virol 77:447–454.

Del Valle L, White MK, Khalili K. 2008. Potential mechanisms of the human polyomavirus JC in neural oncogenesis. J Neuropathol Exp Neurol 67:729–740.

Dorries K, Vogel E, Gunther S, Czub S. 1994. Infection of human polyomaviruses JC and BK in peripheral blood leukocytes from immunocompetent individuals. Virology 198:59–70.

Feng H, Shuda M, Chang Y, Moore PS. 2008. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science 319:1096–1100.

Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. 2010. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127:2893–2917.

Flaegstad T, Andresen PA, Johnsen JI, Asomani SK, Jorgensen GE, Vignarajan S, Kjuul A, Kogner P, Traavik T. 1999. A possible contributory role of BK virus infection in neuroblastoma development. Cancer Res 59:1160–1163.

Giraud G, Ramqvist T, Pastrana DV, Pavot V, Lindau C, Kogner P, Orrego A, Buck CB, Allander T, Holm S, Gustavsson B, Dalianis T. 2009. DNA from KI, WU and Merkel cell polyomaviruses is not detected in childhood central nervous system tumours or neuroblastomas. PLoS ONE 4:e8239.

Gou XM, Chen Y, Chen XY, Arrand JR. 2003. Effects of Epstein-Barr virus latent membrane protein 1(EBV-LMP1) on related factors of metastasis of nasopharyngeal carcinoma cell line CNE1. Ai Zheng 22:481–485.

Hashida Y, Imajoh M, Nemoto Y, Kamioka M, Taniguchi A, Taguchi T, Kume M, Orihashi K, Daibata M. 2013. Detection of Merkel cell polyomavirus with a tumour-specific signature in non-small cell lung cancer. Br J Cancer 108:629–637.

Haugg AM, Speel EJ, Pantulu ND, Pallasch C, Kurz AK, Kvasnicka HM, Cathomas G, Wendtner CM, zur Hausen A. 2011. Fluorescence in situ hybridization confirms the presence of Merkel cell polyomavirus in chronic lymphocytic leukemia cells. Blood 117:5776–5777.

Houben R, Shuda M, Weinkam R, Schrama D, Feng H, Chang Y, Moore PS, Becker JC. 2010. Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. J Virol 84:7064–7072.

Husseiny MI, Anastasi B, Singer J, Lacey SF. 2010. A comparative study of Merkel cell, BK and JC polyomavirus infections in renal transplant recipients and healthy subjects. J Clin Virol 49:137–140.

Imajoh M, Hashida Y, Nemoto Y, Oguri H, Maeda N, Furihata M, Fukaya T, Daibata M. 2012a. Detection of Merkel cell polyomavirus in cervical squamous cell carcinomas and adenocarcinomas from Japanese patients. Virol J 9:154.

Imajoh M, Hashida Y, Taniguchi A, Kamioka M, Daibata M. 2012b. Novel human polyomaviruses, Merkel cell polyomavirus and human polyomavirus 9, in Japanese chronic lymphocytic leukemia cases. J Hematol Oncol 5:25.

Kassem A, Technau K, Kurz AK, Pantulu D, Loning M, Kayser G, Stickeler E, Weyers W, Diaz C, Werner M, Nashan D, Zur Hausen A. 2009. Merkel cell polyomavirus sequences are frequently detected in nonmelanoma skin cancer of immunosuppressed patients. Int J Cancer 125:356–361.

Lam WY, Leung BW, Chu IM, Chan AC, Ng HK, Chan PK. 2010. Survey for the presence of BK, JC, KI, WU and Merkel cell polyomaviruses in human brain tissues. J Clin Virol 48:11–14.

Markowitz RB, Thompson HC, Mueller JF, Cohen JA, Dynan WS. 1993. Incidence of BK virus and JC virus viruria in human immunodeficiency virus-infected and -uninfected subjects. J Infect Dis 167:13–20.

McNees AL, White ZS, Zanwar P, Vilchez RA, Butel JS. 2005. Specific and quantitative detection of human polyomaviruses BKV, JCV, and SV40 by real time PCR. J Clin Virol 34:52–62.

Mehrazin M, Rahmat H, Yavari P. 2006. Epidemiology of primary intracranial tumors in Iran, 1978–2003. Asian Pac J Cancer Prev 7:283–288.

Moens U, Ludvigsen M, Van Ghelue M. 2011. Human polyomaviruses in skin diseases. Patholog Res Int 2011:123491.

Moens U, Van Ghelue M, Ehlers B. 2014. Are human polyomaviruses co-factors for cancers induced by other oncoviruses. Rev Med Virol 24:343–360.

Moore PS, Chang Y. 2010. Why do viruses cause cancer? Highlights of the first century of human tumour virology. Nat Rev Cancer 10:878–889.

Saddawi-Konefka R, Crawford JR. 2010. Chronic viral infection and primary central nervous system malignancy. J Neuroimmune Pharmacol 5:387–403.

Sadeghi F, Salehi-Vaziri M, Ghodsi SM, Alizadeh A, Bokharaei-Salim F, Saroukalaei ST, Mirbolouk M, Monavari SH, Keyvani H. 2014. Prevalence of JC polyomavirus large T antigen sequences among Iranian patients with central nervous system tumors. Arch Virol 160:61–68.

Sastre-Garau X, Peter M, Avril MF, Laude H, Couturier J, Rozenberg F, Almeida A, Boitier F, Carlotti A, Couturaud B, Dupin N. 2009. Merkel cell carcinoma of the skin: Pathological and molecular evidence for a causative role of MCV in oncogenesis. J Pathol 218:48–56.

Shollar D, Del Valle L, Khalili K, Otte J, Gordon J. 2004. JCV T-antigen interacts with the neurofibromatosis type 2 gene product in a transgenic mouse model of malignant peripheral nerve sheath tumors. Oncogene 23:5459–5467.

Shuda M, Feng H, Kwun HJ, Rosen ST, Gjoerup O, Moore PS, Chang Y. 2008. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. Proc Natl Acad Sci USA 105:16272–16277.

Shuda M, Arora R, Kwun HJ, Feng H, Sarid R, Fernandez-Figueras MT, Tolstov Y, Gjoerup O, Mansukhani MM, Swerdlow SH, Chaudhary PM, Kirkwood JM, Nalesnik MA, Kant JA, Weiss LM, Moore PS, Chang Y. 2009. Human Merkel cell polyomavirus infection I. MCV T antigen expression in Merkel cell carcinoma, lymphoid tissues and lymphoid tumors. Int J Cancer 125:1243–1249.

Siegel R, Naishadham D, Jemal A. 2013. Cancer statistics, 2013. CA Cancer J Clin 63: 11–30.

Sihto H, Kukko H, Koljonen V, Sankila R, Bohling T, Joensuu H. 2011. Merkel cell polyomavirus infection, large T antigen, retinoblastoma protein and outcome in Merkel cell carcinoma. Clin Cancer Res 17:4806–4813.

Sundsfjord A, Spein AR, Lucht E, Flaegstad T, Seternes OM, Traavik T. 1994. Detection of BK virus DNA in nasopharyngeal aspirates from children with respiratory infections but not in saliva from immunodeficient and immunocompetent adult patients. J Clin Microbiol 32:1390–1394.

Teman CJ, Tripp SR, Perkins SL, Duncavage EJ. 2011. Merkel cell polyomavirus (MCPyV) in chronic lymphocytic leukemia/small lymphocytic lymphoma. Leuk Res 35:689–692.

Tsekov I, Ferdinandov D, Bussarsky V, Hristova S, Kalvatchev Z. 2011. Prevalence of JC polyomavirus genomic sequences from the large T-antigen and non-coding control regions among Bulgarian patients with primary brain tumors. J Med Virol 83:1608–1613.

Wiemels J, Wrensch M, Claus EB. 2010. Epidemiology and etiology of meningioma. J Neurooncol 99:307–314.