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Investigation of simian virus 40 (SV40) and human JC, BK, MC, KI, and WU polyomaviruses in glioma

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Abstract

The gliomagenesis remains not fully established and their etiological factors still remain obscure. Polyomaviruses were detected and involved in several human tumors. Their potential implication in gliomas has been not yet surveyed in Africa and Arab World. Herein, we investigated the prevalence of six polyomaviruses (SV40, JCPyV, BKPyV, MCPyV, KIPyV, and WUPyV) in 112 gliomas from Tunisian patients. The DNA sequences of polyomaviruses were examined by PCR assays. Viral infection was confirmed by DNA in situ hybridization (ISH) and/or immunohistochemistry (IHC). The relationships between polyomavirus infection and tumor features were evaluated. Specific SV40 Tag, viral regulatory, and VP1 regions were identified in 12 GBM (10.7%). DNA ISH targeting the whole SV40 genome and SV40 Tag IHC confirmed the PCR findings. Five gliomas yielded JCPyV positivity by PCR and DNA ISH (2.7%). However, no BKPyV, KIPyV, and WUPyV DNA sequences were identified in all samples. MCPyV DNA was identified in 30 gliomas (26.8%). For GBM samples, MCPyV was significantly related to patient age ($p = 0.037$), tumor recurrence ($p = 0.024$), and SV40 ($p = 0.045$) infection. No further significant association was identified with the remaining tumor features ($p > 0.05$) and patient survival (Log Rank, $p > 0.05$). Our study indicates the presence of SV40, JCPyV, and MCPyV DNA in Tunisian gliomas. Further investigations are required to more elucidate the potential involvement of polyomaviruses in these destructive malignancies.

Keywords Glioma · Glioblastoma · SV40 · JCPyV · MCPyV · Infection

Introduction

Gliomas constitute the commonest malignant tumors of the brain affecting glial cells (Ostrom et al. 2018). The World Health Organization (WHO) divides gliomas into four histological grades (grade I–IV). Grade IV gliomas or glioblastomas multiforme (GBM) are the most frequent glial tumor characterized by adverse prognosis and poorer survival rates in adults (Ostrom et al. 2018; Stupp et al. 2014). The pathogenesis of gliomas remains not well recognized. Moreover, the etiological features of these aggressive tumors still remain unknown. Recent investigations explored the involvement of viral infection in gliomagenesis (McFaline-Figueroa and Wen 2017; Farias et al. 2019; Limam et al. 2019b). Several viruses have been recognized in gliomas, such as human cytomegalovirus (HCMV), Epstein-Barr virus (EBV), human papillomavirus (HPV), Kaposi sarcoma-associated herpes virus, and polyomaviruses (Limam et al. 2019b; Strojnik et al. 2017; Zavala-Vega et al. 2017; Hashida et al. 2015; Cimino et al. 2014; Vidone et al. 2014). Nevertheless, their causative function in the glioma pathogenesis remains extremely controversial (McFaline-Figueroa and Wen 2017; Farias et al. 2019). Additional surveys are required to more explore their contribution in the pathogenicity of these aggressive tumors.

The *Polyomaviridae* family includes many polyomaviruses, like simian virus 40 (SV40), human JC polyomavirus (JCPyV), BK polyomavirus (BKPyV), Merkel cell polyomavirus (MCPyV), Karolinska Institute polyomavirus (KIPyV), and Washington University

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polyomavirus (WUPyV) (DeCaprio and Garcea 2013; Toptan et al. 2016). Although they share basic features of genome structure and organization, polyomaviruses differ in tissue tropism and disease involvement (DeCaprio and Garcea 2013). Infection by polyomaviruses is commonly asymptomatic and frequent in the general population (Jiang et al. 2009; Boothpur and Brennan 2010). Nevertheless, their potential oncogenicity is attributed to the viral regulatory T antigen (Tag), which is responsible for viral replication and cellular transformation (Butel and Lednicky 1999). Viral Tag protein interacts and functionally inactivates the cellular tumor suppressor p53 and Rb proteins, leading to cell-cycle dysregulation, increased cellular proliferation, and apoptosis pathway defects (Mietz et al. 1992). The possible implication of polyomaviruses in the gliomagenesis has been investigated, but has never been proven. Previous reports described the presence of SV40, JCPyV, and BKPyV in glial tumors (Del Valle et al. 2001, 2002; Rollison et al. 2005). Nevertheless, the occurrence of new members of human polyomaviruses has been not yet examined in gliomas.

SV40 is recognized as a powerful tumor-inducing virus since it is able to transform several cell types in tissue culture, including human cells. During the last years, diverse surveys have identified the SV40 occurrence in several human tumors, including brain tumors, pleural mesothelioma, lymphomas, papillary thyroid carcinoma, and hepatocellular carcinoma (Bergsagel et al. 1992; Carbone et al. 1994; Vilchez et al. 2002; Pacini et al. 1998; Wong et al. 2003). Previous surveys from our Department described SV40 occurrence in diffuse large B cell lymphoma, breast carcinoma, and osteosarcoma in Tunisian patients (Amara et al. 2007; Hachana et al. 2009; Ziadi et al. 2012).

The BKPyV is a double-stranded DNA non-enveloped virus. It was initially recognized in the urine of a subject with renal transplantation. BKPyV induces nephropathy which is frequently related to severe cellular immunosuppression (Reploeg et al. 2001; Hirsch 2005). The JCPyV constitutes the well-known etiological factor of the progressive multifocal leukoencephalopathy in AIDS subjects. Moreover, JCPyV is related to nervous system tumors (Del Valle et al. 2001; Delbue et al. 2005). Previous reports from our Laboratory have suggested a potential implication of JCPyV in breast, colorectal, and gastric cancers (Ksiaa et al. 2010; Hachana et al. 2012; Ksiaa et al. 2015).

Two novel human polyomaviruses, KIPyV and WUPyV, were recognized in 2007 by separate researchers in the USA and Sweden (Allander et al. 2007; Gaynor et al. 2007). Both viruses were discovered independently in the respiratory secretions of patients with acute respiratory problems (Allander et al. 2007; Gaynor et al. 2007; Babakir-Mina et al. 2013). So far, KIPyV and WUPyV have not been related with specific human diseases or tumors.

The MCPyV was found integrated into Merkel cell carcinoma tissue, an unusual and aggressive histotype of skin cancer (Feng et al. 2008). Later investigations have confirmed the link between MCPyV, integration of the virus and Merkel cell carcinoma (Foulongne et al. 2010; Sastre-Garau et al. 2009). To date, MCPyV constitutes the strongest proof for polyomavirus contribution in human oncogenesis (Foulongne et al. 2010; Sastre-Garau et al. 2009). However, links between MCPyV DNA presence and other inflammatory or malignant diseases of the skin or other tissue remain unproven (Nguyen et al. 2019; Sheu et al. 2019).

The involvement of polyomaviruses in gliomas has been not yet surveyed in Africa and Arab World. To further explore the gliomagenesis, we investigated the SV40, JCPyV, BKPyV, MCPyV, KIPyV, and WUPyV infections in gliomas and their clinicopathological significance among Tunisian patients.

Materials and methods

Patients and tissue samples

One hundred twelve cases of gliomas were selected from pathological files of The Pathology Department, Farhet Hached University Hospital, Sousse (Tunisia), from 2010 to 2016. This survey was approved by the local Human Ethics Committee at our institution and it conformed to the Helsinki Declaration provisions.

All selected cases were reviewed by two pathologists, using Hematoxylin and Eosin stained sections to confirm the diagnosis. Specimens were chosen based on the accessibility of complete clinical data and the availability of formalin-fixed, paraffin-embedded (FFPE) tissues for histological, immunohistochemical, and molecular analyses. The gliomas were classified into 12 grade I gliomas, 12 grade II gliomas, six grade III gliomas, and 82 GBM (Limam et al. 2019b).

Patient's clinicopathological data, including patient age and gender, tumor localization, and the follow-up, were collected. Tumor recurrence and patient outcome were registered (Table 1).

GBM features

For GBM samples included in this study, the data of *MGMT* promoter methylation pattern as well as expression of MGMT, p53, PTEN, MDM2, and EGFR were from our recent publication (Limam et al. 2019a).

DNA extraction

DNA extraction was realized using the Qiagen QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany), following the manufacturer protocol. To verify the DNA quality, we

Table 1 Clinicopathological characteristics of glioma samples

Features	Gliomas				
	Grade I	Grade II	Grade III	Grade IV	Total
Number of cases	12 (10.7%)	12 (10.7%)	6 (5.3%)	82 (73.2%)	112 (100%)
Median age (years)	32 (20–61)	45.5 (30–64)	42 (33–53)	52 (19–75)	53 (19–75)
Age (years)					
< 50	11 (91.7%)	9 (75%)	5 (83.3%)	28 (34.1%)	53 (47.3%)
≥ 50	1 (8.3%)	3 (25%)	1 (16.7%)	54 (65.9%)	59 (52.7%)
Gender					
Male	9 (75%)	8 (66.7%)	4 (66.7%)	47 (57.3%)	68 (60.7%)
Female	3 (25%)	4 (33.3%)	2 (33.3%)	35 (42.7%)	44 (39.3%)
Type of surgery					
Gross total resection	8 (66.7%)	7 (58.3%)	4 (66.7%)	61 (74.4%)	80 (71.4%)
Partial resection	4 (33.3%)	5 (41.7%)	2 (33.3%)	21 (25.6%)	32 (28.6%)
Histological type	Pilocytic astrocytoma (100%)	Oligodendroglioma (50%) Oligoastrocytoma (50%)	Anaplastic oligoastrocytoma (66.7%) Anaplastic oligodendroglioma (33.3%)	GBM (100%)	
Tumor localization					
Temporal	3 (25%)	0	0	17 (20.7%)	20 (17.8%)
Parietal	0	0	2 (33.3%)	12 (10.7%)	14 (12.5%)
Frontal	1 (8.3%)	9 (75%)	2 (33.3%)	17 (20.7%)	29 (25.9%)
Occipital	0	0	0	1 (0.9%)	1 (0.9%)
Mixed	2 (16.7%)	1 (8.3%)	2 (33.3%)	16 (14.3%)	21 (18.7%)
Unspecified	6 (50%)	2 (16.7%)	0	19 (16.7%)	27 (24.1%)
Tumor recurrence					
Presence	3 (25%)	1 (8.3%)	2 (33.3%)	18 (21.9%)	24 (21.4%)
Absence	9 (75%)	11 (91.7%)	4 (66.7%)	64 (78%)	88(78.6%)

amplified a 268 bp sequence of the human *β-globin* gene as described recently (Limam et al. 2019a, b). Samples of high-quality DNA were used to further examine polyomaviruses occurrence.

Detection of DNA sequences of SV40, JCPyV, BKPyV, MCPyV, KIPyV, and WUPyV

All cases were screened for the SV40 DNA presence by detecting three different regions of SV40 genome, including large Tag gene, transcription regularity region, and VP1 gene as previously published by our Laboratory (Ksiaa et al. 2010; Amara et al. 2007; Hachana et al. 2009). First, SV40 DNA was detected using SVTAGP1 (nucleotide (nt) 4388–4413) and SVTAGP3 (nt 4496–4513) primers, targeting the large Tag region (Nakatsuka et al. 2003). To verify the specificity of SV40 DNA detection, we used two further primer sets amplifying other SV40 genome regions. The set of primers LA1 (nt 2251–2274) and LA2 (nt 2545–2522) targets the VP1 gene, which encodes the major capsid protein (Weggen et al. 2000). The set of primers RA1 (nt 266–245) and RA2 (nt 5195–5218) amplifies the transcription regulatory region, which distinguishes between representative SV40 strains and laboratory-adapted SV40 strains (Weggen et al. 2000).

The detection of JCPyV and BKPyV was assessed by PCR as well-documented previously by our Laboratory (Ksiaa et al. 2010, 2015; Hachana et al. 2009, 2012). JCPyV presence was revealed using PEP1 and PEP2 primers, amplifying a 173 bp sequence in the NH2-terminal region of the JCPyV large T antigen (Arthur et al. 1989). BKPyV occurrence was evaluated using BES-3 and BES-6 primers, amplifying a 151 bp sequence in the DNA binding region of the BKPyV large T antigen (Smith et al. 1998).

The detection of MCPyV, KIPyV, and WUPyV was evaluated as described previously (Antonsson et al. 2012). MCPyV and KIPyV DNA sequences were investigated by using LT-1.1 and D-LT primers, respectively, targeting the viral large T antigen (Antonsson et al. 2012). WUPyV DNA was analyzed using F-Reg primers targeting the non-coding control region (Antonsson et al. 2012). Primer sequences, annealing temperatures, and awaited product size for JCPyV, BKPyV, MCPyV, KIPyV, and WUPyV DNA detection were recorded in Table 2.

All PCR reactions were executed using 400 ng of DNA template in a final volume of 25 μl, containing 0.2 μM of each primer set, 1X PCR buffer (10 mM Tris HCl, 50 mM KCl, 1.5 mM MgCl2, pH 8.3), 0.25 mM of each dNTP, and 1 U of Taq DNA polymerase (Promega, Madison, USA) in a

Table 2 Primer sequences, annealing temperature and product size for PCR assays

Virus	Primer sequences (5′–3′)	Product size (pb)	Annealing temperature
SV40	SVTAGP1: TTAGCAATTCTGAAGGAAAG TCCTTG	126pb	53 °C
	SVTAGP3: ACCTGTTTTGCTCAGAA		
	LA1: GGGTGTTGGGCCCTTGTGCAAAGC	294pb	52 °C
	LA2: CATGTCTGGATCCCCAGGAAGCTC		
	RA1: AATGTGTGTCAGTTAGGGTGTG	242pb	52 °C
	RA2: TCCAAAAAAGCCTCCTCACTACTT		
BKPyV	BES-3-F: AATATTATGCCCAGCACACATG	151pb	57 °C
	BES-3-R: CTTTCCCTCTGATCTACACCAG		
JCPyV	PEP1-F: AGTCTTTAGGGTCTTCTACC	173pb	56 °C
	PEP2-R: GGTGCCAACCTATGGAACAG		
MCPyV	LTAg-F: AGCTCAGAAGTGACTTCTCTATGTTT GA	190pb	59 °C
	LTAg-R: ACAATGCTGGCGAGACAACT		
KIPyV	D-LT-F: CACAGGTGGTTTTCTATAAATTTTGT ACTT	113pb	60 °C
	D-LT-R: GAATGCATACATCCCACTGCTTC		
WUPyV	F-Reg-F: GCCGACAGCCGTTGGATATA	71pb	60 °C
	F-Reg-R: TTTCAGGCACAGCAAGCAAT		

Touch Thermal Cycler (Basic) LTCB-A10 (Labtron, Basingstoke, Hampshire, UK). Cycling conditions were assessed by initial denaturation at 95 °C for 5 min, followed by 35 cycles of 1 min at 95 °C, for 1 min at the specific annealing temperature and for 1 min at 72 °C, and completed by a last extension 5 min at 72 °C (Amara et al. 2007; Ksiaa et al. 2010, 2015; Hachana et al. 2009, 2012).

All PCR procedures were realized simultaneously with negative and positive controls. Positive controls were clinical samples that were virus-positive in previous reactions (Amara et al. 2007; Ksiaa et al. 2010, 2015; Hachana et al. 2009, 2012). Negative controls had no DNA template and were carried out in each set of PCR.

For DNA amplification of all polyomaviruses, PCR results were visualized on agarose gels (2%) with ethidium bromide under UV by the Gel Doc 2000 System (Bio-Rad, Marnes-la-Coquette, France). Every sample was tested four times and was considered viral DNA-positive if it displayed detectable signals at least three times. For all amplifications, approved precautions were practiced to avoid PCR contamination. Additionally, DNA extraction, PCR, and gel electrophoresis were conducted in independent rooms in the laboratory (Amara et al. 2007; Ksiaa et al. 2010, 2015; Hachana et al. 2009, 2012).

SV40 and JCPyV in situ hybridization

To recognize SV40 and JCPyV, DNA in situ hybridization (ISH) technique was conducted as reported elsewhere (Chrétien et al. 2000; Aksamit et al. 1986). We applied biotinylated probes corresponding to the whole JCPyV (Bio-Probe JC Virus) and SV40 (Bio-Probe SV40) genomes, respectively, using the manufacturer's protocol (EnzoDiagnostics, Farmingdale, NY, USA). The biotinylated probes were detected by Dako GenPoint Tyramide signal amplification system (Dako GenPoint K0620, Dako, Carpinteria, CA, USA) as stated by the manufacturer procedures. Staining was appraised by two pathologists. Two breast carcinomas, exhibiting SV40 positivity served as positive control for SV40 infection (Hachana et al. 2009). For JCPyV assessment, two gastric cancers, which were positive in previous experiments, were taken as positive controls (Ksiaa et al. 2010).

SV40 immunohistochemical staining

Immunohistochemistry was used to detect SV40 on FFPE tissue sections, using the Pab108 monoclonal antibody (Santa Cruz Biotechnology, USA) to detect the large T and the small t antigens (T/t-ag) of SV40 as previously published by our Laboratory (Hachana et al. 2009; Ziadi et al. 2012). In brief, 4-μm-thick sections of FFPE tissues were dewaxed and rehydrated. Antigen retrieval was done in citrate buffer (10 mM, pH 9) at 95 °C for 40 min. After endogenous peroxidase activity blockage with hydrogen peroxide (5%), sections were incubated with Pab108 monoclonal antibody (dilution 1:100) overnight at 4 °C. Immunoreactivity was revealed by using the Envision+ Dual Link System HRP kit (K4063, Dako, Carpinteria, CA, USA), following the manufacturer's advices. For SV40 expression, specific positive control included FFPE sections of a transgenic prostate adenocarcinoma of the mouse kindly provided by Professor P. Wikström from

The Medical Bioscience Department of Umea University (Sweden) (Hachana et al. 2009; Ziadi et al. 2012).

Statistical analysis

Statistics were assessed using the SPSS 19.0 statistical software (IBM, Armonk, NY, USA). Gliomas features were analyzed according to the presence of polyomaviruses, using chi-square test or Fisher's exact test when appropriate. For survival analysis, Kaplan-Meier method was accomplished to establish survival time distributions, according to polyomavirus status and differences in the distribution were estimated using the Log Rank test. All findings were regarded as significant when probability (p) < 0.05.

Results

Polyomavirus detection

The quality of extracted DNA was approved by amplifying human *β-globin* gene in all gliomas selected in this analysis (Fig. 1). The occurrence of SV40, JCPyV, BKPyV, MCPyV, KIPyV, and WUPyV was examined in gliomas of different histological subtypes from 112 Tunisian patients by using suitable primers (Table 3).

SV40 DNA presence was investigated by independent PCR assays, targeting distinct sequences of the viral genome. Initially, DNA samples were amplified using the SVTAGP1/3 primer set, targeting the conserved Tag N-terminal coding region. Specific SV40 Tag sequences were identified in 12 gliomas (10.7%, Fig. 1, Table 3). All of them were GBM, accounting for 14.6% of GBM samples. To confirm the viral DNA presence in GBM tissues, all samples were as well examined using two further primer set, RA1/2 and LA1/2, specific for the viral regulatory and the VP1 regions, respectively. Positive results were identified in all Tag region-positive cases (Fig. 1). Additionally, DNA ISH targeting the whole SV40 genome was practiced and confirmed the PCR findings (12/12 cases, Fig. 2b). Furthermore, SV40 Tag immunohistochemistry was conducted on all samples. All SV40-positive cases by PCR displayed positive immunostaining in tumoral cells (12/12 cases, Fig. 2c). The remaining cases, which were SV40-negative by PCR failed also to show any immunoreactivity.

Table 3 Prevalence of polyomavirus DNA sequences in gliomas

Virus	Gliomas				
	Grade I (n = 12)	Grade II (n = 12)	Grade III (n = 6)	Grade IV (n = 82)	Total (n = 112)
SV40	0	0	0	12 (14.6%)	12 (10.7%)
BKPyV	0	0	0	0	0
JCPyV	0	0	2 (33.3%)	3 (3.6%)	5 (4.5%)
MCPyV	3 (25%)	0	1 (16.7%)	26 (31.7%)	30 (26.8%)
KIPyV	0	0	0	0	0
WUPyV	0	0	0	0	0

Only five gliomas yielded JCPyV PCR products (4.5%, Fig. 1, Table 3). No viral DNA sequences were detected in the remaining gliomas (95.5%). JCPyV-positive cases included two gliomas grade III (anaplastic oligoastrocytoma and anaplastic oligodendroglioma) and three GBM. Furthermore, in all these PCR-positive tumors, DNA ISH displayed JCPyV positivity (Fig. 3).

No viral BKPyV DNA positivity was detected by PCR in all selected samples (Fig. 1, Table 3). Thus, all analyzed cases were considered BKPyV-negative. Moreover, no KIPyV and

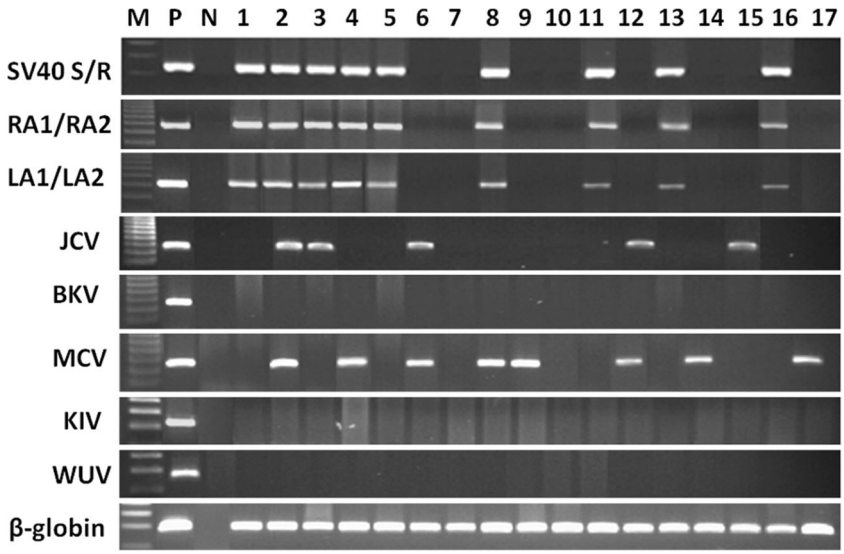

Fig. 1 Detection of polyomaviruses in gliomas. (1–17): samples of gliomas, M: molecular weight marker with 100 bp (Promega), P: positive control, N: negative control

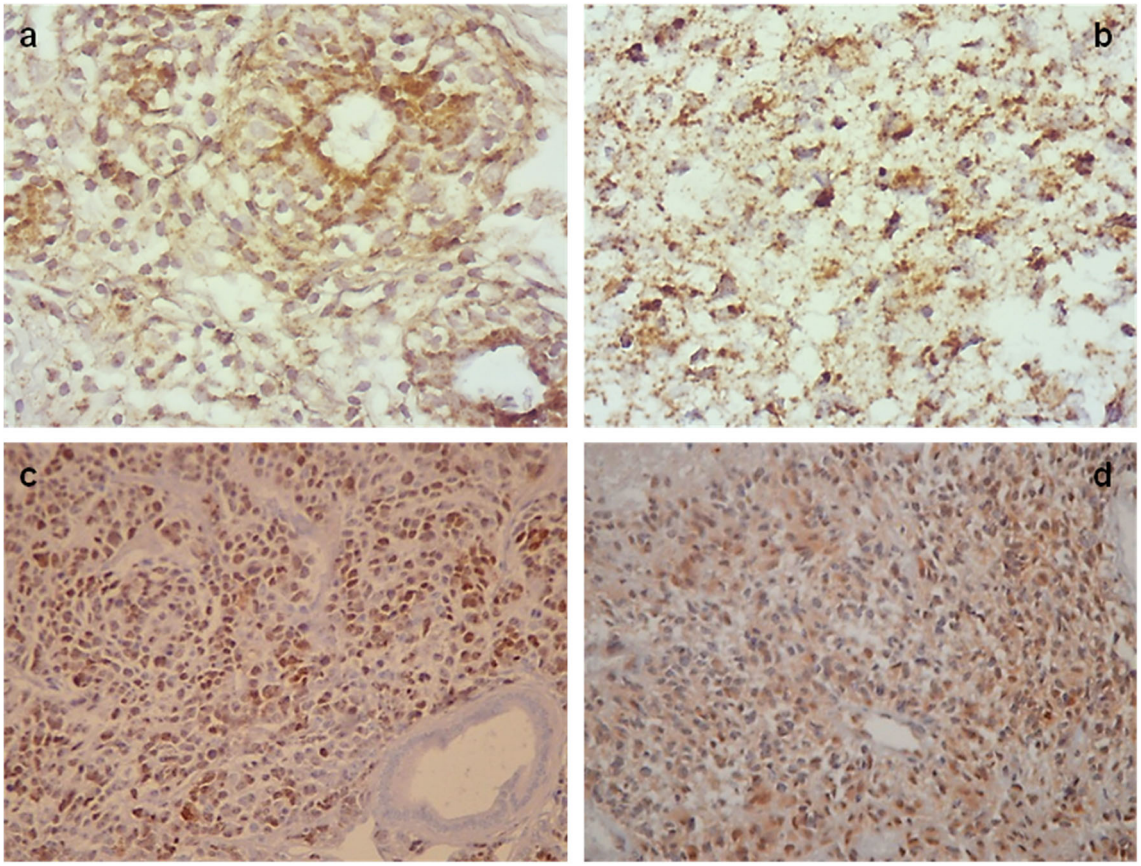

Fig. 2 Detection of SV40 by in situ hybridization and immunohistochemistry. SV40 detection in breast cancer (**a**) and GBM (**b**) by in situ hybridization. SV40 detection in transgenic prostate adenocarcinoma of the mouse (**c**) and GBM (**d**) by immunohistochemistry

WUPyV DNA sequences were identified by PCR technique in all gliomas and thereby all tumors were regarded as negative for WUPyV/KIPyV (Fig. 1, Table 3).

MCPyV DNA sequences were identified in 30 out of 112 gliomas (26.8%) by PCR (Fig. 1, Table 3). MCPyV DNA-positive tumors comprised three pilocytic astrocytomas, only one anaplastic oligodendroglioma and 26 GBM accounting for 31.7% of all GBM cases. The remaining tumor cases were regarded MCPyV-negative (72.3%).

Association of polyomaviruses with GBM features

Table 4 details the association between the presence of SV40, JCPyV, and MCPyV genomic sequences and GBM characteristics. MCPyV was significantly related to patient age ($p = 0.037$) and tumor recurrence ($p = 0.024$). No further significant association was identified between all investigated polyomaviruses and clinicopathological parameters, the expression of p53, MDM2, PTEN, EGFR, and MGMT, and the *MGMT* promoter methylation pattern ($p > 0.05$ for all, Table 4).

We also assessed the relation between the polyomaviruses (JCPyV, MCPyV, and SV40) and the EBV infection, which was found positive in our recent study of gliomas in the same Tunisian series (Limam et al. 2019a). A significant association was recognized between MCPyV and SV40 ($p = 0.045$) DNA presence. No additional significant relationship was recognized with the remaining viruses ($p > 0.05$, Table 4).

By using the Kaplan-Meier means, no significant relationship was identified between patient the recurrence free survival (RFS) and the presence of SV40 (Log Rank, $p = 0.117$), JCPyV (Log Rank, $p = 0.408$), and MCPyV (Log Rank, $p = 0.106$) genomic sequences.

Discussion

To investigate the potential role of polyomaviruses in human glial tumors, we analyzed the prevalence of six polyomaviruses in 112 gliomas diagnosed among adult patients in Tunisia. Overall, our results suggest the presence of SV40, JCPyV, and MCPyV DNA in Tunisian gliomas. Nevertheless, no DNA sequence of BKPyV, KIPyV, and WUPyV was identified in all analyzed samples.

Previously, some studies identified the SV40 DNA in GBM, while others failed to detect this viral infection. In our

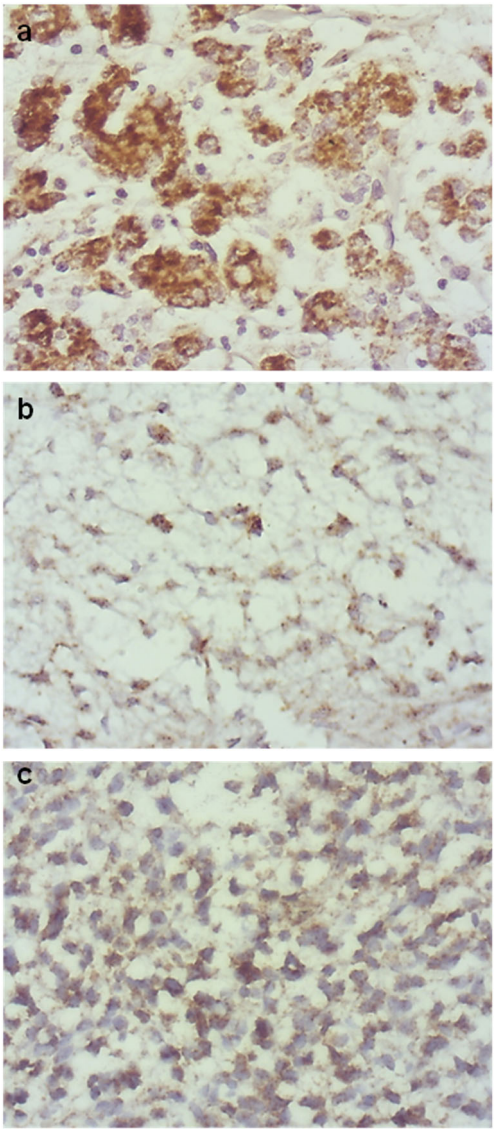

Fig. 3 Representative DNA JCPyV in situ hybridization detection. JCPyV detection in gastric cancer (**a**), glioma grade II (**b**), and GBM (**c**) by in situ hybridization

study, the amplification of SV40 specific sequences was identified in 12 GBM. IHC and ISH which targets the entire SV40 genome confirmed the SV40 presence in these GBM cases. Earlier studies which were carried out by PCR and/or IHC techniques have reported a high prevalence of SV40 in GBM (Major et al. 1985; Martini et al. 1996; Kouhata et al. 2001; Martini et al. 2002). Rollison et al. (2005) investigated the presence of SV40 (with JCPyV and BKPyV) in a large series of brain tumors, including GBM, pilocytic astrocytoma, oligodendroglioma, ependymoma, medulloblastoma, meningioma, and carcinomas/papilloma of the choroid plexus diagnosed in adults and children (Rollison et al. 2005). The results of two different laboratories, using dissimilar protocols (PCR then Southern hybridization vs. real-time quantitative PCR) showed a rare SV40 presence in these tumors (Rollison et al. 2005). However, other teams have not found any association between SV40 and human brain tumors (Engels et al. 2002; Carbone et al. 2003; Rollison et al. 2003). Using IHC, Sabatier et al. (2005) found no SV40 positivity in 82 nervous system tumors (including 20 ependymomas, 20 GBM, 12 oligodendrogliomas, three choroid plexus adenomas, two choroid plexus carcinomas, 15 meningiomas, and 10 medulloblastomas). The different approaches and methodologies adapted by the researchers or even by technical artifacts could explain the different results obtained by these studies.

Using serological methods, SV40 antibodies were identified in most samples, using SV40-like particles (Ribeiro et al. 2010). However, the final results were still influenced by some cross-reactivity since there was high protein homology between the three major polyomaviruses (SV40, BKPyV, and JCPyV) (Ribeiro et al. 2010). More recently, Mazzoni et al. (2014) utilized an indirect enzyme-linked immunosorbent technique using two synthetic peptides imitating the viral capsid protein 1–3 antigens to detect specific anti-SV40 antibodies in serum of GBM patients, as well as of normal subjects and breast cancer patients serving as control group (Mazzoni et al. 2014). The presence of antibodies against SV40 viral capsid proteins was statistically higher in serum of GBM patients when compared to control serums (Mazzoni et al. 2014). These researchers considered that SV40, or even a closely related but not yet discovered human polyomavirus, is connected with a subset of GBM and is also present in the healthy adult population, although its prevalence is lower (Mazzoni et al. 2014).

JCPyV is a neurotropic polyomavirus that infects more than 70% of the global human population during infancy (Del Valle et al. 2001). JCPyV replication in the brain of individuals with altered immune systems leads to the progressive multifocal leukoencephalopathy. In addition, JCPyV has an oncogenic power and promotes the development of various tumors of neuroectodermal origin, including medulloblastoma and glioblastoma in laboratory animals. The oncogenicity of JCPyV is ascribed to the product of the early viral gene, the Tag, which is able to functionally associate and inactivate tumor suppressor proteins, including p53 and pRb (DeCaprio and Garcea 2013; Toptan et al. 2016; Jiang et al. 2009; Boothpur and Brennan 2010). Observations from experiments on laboratory animals motivated the investigation of JCPyV DNA sequences and viral oncoprotein expression in human brain tumors. In our study, using PCR and ISH techniques, JCPyV was detected in five gliomas, including two grade III gliomas and three GBM. Previous studies have described the positive and negative relationships of JCPyV with nervous system tumors (Del Valle et al. 2001, 2002; Okamoto et al. 2005). Del Valle et al. (2001) surveyed 85 human brain tumors from Greece, the UK, and the USA, including oligodendrogliomas, pilocytic astrocytomas, oligoastrocytomas, anaplastic astrocytomas, anaplastic

Table 4 Characteristics of GBM samples, according to SV40, JCPyV, and MCPyV

Tumor features	JCPyV			MCPyV			SV40		
	− ($n = 79$)	+ ($n = 3$)	p	− ($n = 56$)	+ ($n = 26$)	p	− ($n = 70$)	+ ($n = 12$)	p
Age (years)									
< 50	29	2	0.554	17	14	***0.037***	26	5	0.758
≥ 50	50	1		39	12		44	7	
Gender									
Male	44	1	0.586	31	12	1.000	39	6	0.761
Female	35	2		25	12		31	6	
P53 expression									
Positive	1	38	1.000	15	12	0.117	23	4	1.000
Negative	2	26		30	10		33	7	
MDM2 expression									
Positive	2	53	0.245	19	7	0.611	21	5	0.512
Negative	1	24		35	19		47	7	
PTEN expression									
Positive	3	25	0.547	32	19	0.305	42	9	0.740
Negative	0	48		19	6		22	3	
EGFR expression									
Positive	1	15	0.493	44	20	0.766	55	9	0.698
Negative	2	62		10	6		13	3	
MGMT expression									
Positive	1	25	1.000	36	18	1.000	22	4	1.000
Negative	2	52		18	8		46	8	
MGMT methylation									
Unmethylated	3	45	0.548	30	18	0.618	39	9	0.309
Methylated	0	26		18	8		24	2	
Type of surgery									
GTR	3	58	1.000	40	21	0.585	54	7	0.144
Partial resection	0	19		14	5		14	5	
Tumor recurrence									
Presence	0	18	1.000	8	10	***0.024***	14	4	0.452
Absence	3	59		46	16		54	8	
EBV									
Positive	1	23	1.000	13	11	0.117	19	5	0.320
Negative	2	56		43	15		51	7	

Bold italic in Table 4 to indicate significant results

GTR gross total resection

oligodendrogliomas, and GBM for their eventual relation with JCPyV infection. By using gene amplification techniques with a set of primers recognizing the JCPyV DNA, they detected the early viral sequence in 69% of samples (Del Valle et al. 2001). More importantly, immunohistochemical analysis revealed the expression of JCPyV Tag in tumor cell nuclei in 32.9% of samples. Based on these observations, combined with earlier in vitro and in vivo data on the transformation capacity of this human neurotropic virus, these researchers considered a possible etiological role for JCPyV in human brain oncogenesis (Del Valle et al. 2001). Later, this team detected JCPyV in the brain of a patient diagnosed with GBM and multiple sclerosis (Del Valle et al. 2002). However, Okamoto et al. (2005) confirmed the JCPyV presence in 1/5 papillomas of the choroid plexus and in 5/18 ependymomas, whereas none of the 32 medulloblastomas or 7 pilocytic astrocytomas contained a JCPyV DNA sequence. Their results provided molecular support for the likely association between JCPyV DNA presence and tumorigenesis of certain choroid plexus ependymomas and papillomas and not with medulloblastomas or pilocytic astrocytomas (Okamoto et al. 2005).

A total of 30 gliomas yielded MCPyV DNA sequences, including three grade I gliomas, one grade III glioma and 26 GBM. To our knowledge, we are the first to describe the presence of MCPyV in glial tumors and especially in GBM. In addition, MCPyV DNA was significantly related to the presence of SV40 DNA in GBM samples. However, since

MCPyV is shed from the skin, it is uncertain whether these results correspond to accurate infection or only specimen contamination from the skin through surgery or sample processing (Antonsson et al. 2012). Advanced molecular investigations and larger studies are required to confirm the MCPyV DNA presence in gliomas.

In the present study, unlike SV40, JCPyV and MCPyV, no BKPyV DNA sequence was found by PCR assays in all gliomas. Only a few previous studies have investigated the BKPyV presence in brain tumors (Rollison et al. 2005; Weggen et al. 2000). Low prevalence of BKPyV has been identified in gliomas (Rollison et al. 2005; Weggen et al. 2000). Using PCR and Southern hybridization techniques, the BKPyV was detected in only 3/98 GBM by Rollison et al. (2005). Therefore, BKPyV seems to have no evident role in gliomagenesis.

In 2007, two novel polyomaviruses were revealed to infect humans: KIPyV and WUPyV (Allander et al. 2007; Gaynor et al. 2007). They have been isolated in the secretions of patients presenting respiratory indications as well as in the blood, lymphoid tissues, spleen, and stool, particularly in immunocompromised individuals (Carbone et al. 1994; Babakir-Mina et al. 2008, 2009a, c, 2013; Barzon et al. 2008). KIPyV has also been found in specific lung cancers (Babakir-Mina et al. 2009b). To our knowledge, our study constitutes the first study analyzing the presence of KIPyV and WUPyV in gliomas. Nevertheless, no KIPyV/WUPyV DNA was detected in the 112 selected tumors. Accordingly, KIPyV and WUPyV infection would not be implicated in the development of these aggressive tumors.

Finally, our study presented some limitations since it investigated only genomic sequences of polyomaviruses and not the viral protein expression. Moreover, only SV40 presence was well-characterized by amplification of three different genomic regions. Further examination of different genomic regions for JCPyV and MCPyV-positive glioma samples should be carried out to more explore our findings.

Conclusions

To the best of our awareness, this is the first evaluation of the presence of polyomaviruses in gliomas from Africa and Arab World. Our findings suggest the presence of SV40, JCPyV, and, MCPyV in gliomas from Tunisian patients. Therefore, these DNA polyomaviruses could be involved in the onset and/or progression of GBM. However, they may be just passenger viruses that multiply better in some transformed cells than in normal cells (Mazzoni et al. 2014). Advanced molecular analyses will be needed to further explore the involvement of polyomaviruses in gliomagenesis.

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Author contributions S.L. conceived the work, designed, and performed the experiments; N.M. conceived the work, analyzed data, and wrote the draft of the manuscript; A.B., M.T.Y., H.K., and M.M. provide clinical, surgical, and pathological data. S.L. and N.M. revised the manuscript for the critical scientific content; all the authors read and approved the manuscript. The study was supervised by M.M.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics statement This survey was approved by the local Human Ethics Committee at Farhet Hached University Hospital and it conformed to the provisions of Helsinki Declaration.

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