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The presence of JC virus in gastric carcinomas correlates with patient's age, intestinal histological type and aberrant methylation of tumor suppressor genes

Feryel Ksiaa, Sonia Ziadi, Moncef Mokni, Sadok Korbi and Mounir Trimeche

Department of Pathology, Farhat-Hached Hospital of Sousse, Sousse, Tunisia

JC virus (JCV) is a neurotropic polyomavirus and the causative agent of progressive multifocal leukoencephalopathy. A role for JCV in gastrointestinal malignancies has been recently suggested. This study was carried out to determine the prevalence of polyomaviruses including JCV, BKV and SV40 in gastric cancers in Tunisia and to determine the clinicopathological characteristics of virus-associated gastric carcinomas. The presence of polyomaviruses DNA sequences was surveyed in 61 cases of primary gastric carcinomas and in 53 paired non-tumor gastric mucosa by PCR. Findings were correlated to clinicopathological parameters, p53 expression and methylation status of 11 tumor-related genes. Using PCR assays, JCV T-antigen sequence was more frequently detected in gastric carcinomas than in non-tumor gastric mucosa (26 *vs* 6%, *P* = 0.03), while those of SV40 and BKV were not detected in any cases. Correlation analysis showed that JCV had higher frequency in patients older than 55 years (*P* = 0.034) and in the intestinal histological type (*P* = 0.04). With regard to methylation status, *P16* and *P14* showed significantly higher methylation frequencies in JCV-positive gastric carcinomas than in JCV-negative cases (*P* = 0.007 and *P* = 0.003, respectively). Moreover, the mean of the methylation index was significantly higher in JCV-positive than in JCV-negative cases (*P* = 0.024). In multivariate logistic regression analysis, age of patients and the methylation index are only the two independent factors associated with JCV infection. Kaplan–Meier survival analysis showed a trend toward better survival for JCV-associated gastric carcinomas patients (log-rank, *P* = 0.11). Our study suggests a role of JCV as cofactor in the pathogenesis of the intestinal type of gastric carcinomas in older persons.
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Gastric cancers belong to the group of neoplasm of public health burden and rank second as a cause of cancer-related death worldwide.[1,2] The need to understand the biology of gastric cancers has prompted the attention of many investigators. Conceptually, it is recognized that gastric carcinogenesis result from a complex combination of environmental factors, infectious agents, genetic and epigenetic alterations. Most likely, interaction of these factors would lead to the malignant transformation of the stomach.[3,4]

Correspondence: Professor M Trimeche, MD, PhD, Department of Pathology, Farhat-Hached Hospital, Sousse 4000, Tunisia. E-mail: m_trimech@yahoo.fr
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Epigenetic changes that involve aberrant DNA hypermethylation are an important way of transcriptionally silencing many genes and could have a role in the development of gastric cancer.[5,6] Hypermethylation of several gene promoters has been described in sporadic gastric carcinomas, namely in genes of the repair pathway, cell cycle regulators and those related to apoptosis and metastasis. Furthermore, concurrent promoter hypermethylation of multiple genes, which is termed descriptively as the 'CpG island methylator phenotype' (CIMP), was also described in gastric cancers.[7] Although, the epigenetic changes have been accepted as an important mechanism underlying tumor onset and progression, there are limited data related to factors inducing methylation. Recent studies have indicated the existence of a

mechanistic linkage between DNA methylation and infectious agents.[8,9] It is noteworthy that in gastric carcinomas, several studies have suggested that *Helicobacter pylori*, acting through inflammatory mediators, may have a key role in the development of such molecular alterations.[10] Similarly, it has been reported that Epstein–Barr virus may have an important role in suppression of gene expression and may contribute, in conjunction with other factors to *de novo* methylation.[11]

The human neurotropic JC virus (JCV) is a member of the polyomaviruses family, which includes the human BK virus (BKV) and Simian Virus 40 (SV40). JCV is the established etiologic agent of the fatal demyelinating disease progressive multifocal leukoencephalopathy in AIDS patients and is associated with tumors of the central nervous system.[12] Several recent studies have suggested a potential role of JCV in colorectal carcinomas[13,14] and gastric cancers.[15,16] Reports regarding the other polyomaviruses have shown that BKV induces nephropathy, disease usually developed under conditions of severe cellular immunosuppression, while the presence of SV40 was associated with several types of human neoplasms including pleural mesotheliomas, osteosarcomas and lymphomas.[17–19]

The oncogenic potential of polyomaviruses is mediated by the viral regulatory T-antigen that can dysregulate control of the cell cycle by sequestering and inactivating the cellular tumor suppressor proteins p53 and members of the pRb family.[20,21] However, more recent findings have potentially expanded the range of cell targets and have suggested more broad implications for the oncogenic potential of T-antigen. Accordingly, there are current studies that have indicated the existence of an association between methylator phenotype and JCV infection in colorectal carcinomas.[22,23] There is, however, virtually no published information on the methylation changes during the pathogenesis of JCV-related gastric lesions.

In this study, we suggested to determine the prevalence of polyomaviruses JCV, BKV and SV40 in a series of gastric carcinomas from Tunisian patients. We correlated the findings with clinicopathological parameters including p53 accumulation, patients' outcome and methylation status of 11 tumor-related genes, thought to have a role in gastric carcinogenesis.

Table 1 Demographic data and clinicopathological characteristics of 61 patients with gastric cancer in Tunisia

Characteristics	*Number of patients (%)*
Age (years)	
Median 62; range (27–88)	
Gender	
Male	35 (57)
Female	26 (43)
Site of malignancy[a]	
Corpus	25 (43)
Antre	33 (57)
Tumor histology (Lauren)	
Intestinal	24 (39)
Diffuse	37 (61)
Helicobacter pylori infection[b]	
Present	36 (59)
Absent	25 (41)
Depth of invasion[c]	
Early (pT1-pT2)	11 (18)
Advanced (pT3-pT4)	50 (82)
Lymph node involvement	
Positive	28 (46)
Negative	33 (54)

[a]Data are missing in three cases.
[b]*Helicobacter pylori* infection was assessed by histological evaluation.
[c]According to the UICC, TNM classification of malignant tumors, fifth edition, 1997.

Materials and methods

Clinical Samples

A total of 61 gastric cancer samples, from Tunisian patients who had undergone tumor resection, were enrolled in this study. For most of these tumors, paired normal tissues were also obtained. The clinicopathological data of the patients were reviewed and recorded. None of the patients have received preoperative anticancer therapy. The tumors were staged at the time of surgery using the standard criteria for TNM staging with the unified international gastric cancer staging classification.[24] Histological assessment was performed according to the Lauren criteria.[25] The clinical outcomes of the patients were surveyed by a review of their medical records. The demographic and clinical details of the patients are shown in Table 1. As a control group, we have included in this study 23 gastric mucosal samples from Tunisian patients without gastric cancer. This group consisted of 15 men and 8 women, ranging in age from 15 to 82 years. All patients underwent upper gastrointestinal endoscopy for the medical examination.

DNA Extraction and β-Globin Amplification

To achieve adequate tumor DNA for analysis, we picked a representative blocks of formalin-fixed, paraffin-embedded tissue samples from primary tumors and paired non-malignant lesions. The DNA was removed from the tissues in a microtube containing lysis buffer (50 mM Tris-HCl at pH 8.5; 1 mM EDTA; 0.5% Tween 20) and digested with proteinase K. After incubation overnight with shaking at 56 °C, the tubes were boiled for 7 min to

inactivate the enzyme.[26] A volume of 2 µl of these extracts were used for PCR amplification. It is noteworthy that extreme caution was exercised to prevent any contamination, including a dedicated microtome, autoclaved blade-holder and one-time handled blade.

In a separate room, the extracted genomic DNA was first checked by PCR for human β-globin sequences to assess that each sample contained DNA of suitable quality for use in later PCR.[27] Samples of high-quality DNA were submitted to analysis for the presence of polyomaviruses.

Detection of Polyomaviruses Sequences

To examine the presence of polyomaviruses, PCR amplification was performed on DNA extracted from tumor samples and paired non-tumor gastric mucosa using Pep1 and Pep2 primers, which amplify sequences in the N-terminal region of the T-antigen of JCV. In a separate amplifications, two additional sets of primers were used targeting the T-antigen sequence of BKV and SV40. Primer sequences, annealing temperatures and expected products size for JCV, BKV and SV40 are listed in Table 2. The PCR amplification was performed with the presence of 400 ng of DNA template, in a reaction medium (25 µl) that contain 1x PCR buffer (50 mM Tris-HCl, pH 8.5; 1 mM EDTA); 2.5 mM $MgCl_2$; 0.25 mM dNTPs mix, 20 pmol of each primer set and 0.75 unit of Taq DNA polymerase (Promega, Madison, WI, USA). A PTC 200 DNA engine thermal cycler (MJ Research, Watertown, MA, USA) was used exclusively for this analysis with denaturation at 92 °C for 5 min, followed by 40 cycles (1 min at 92°C, 1 min at appropriate annealing temperature and 1 min at 72 °C). The extension time of the last cycle was increased to 10 min. The positive controls for polyomavirus PCR reactions were plasmid DNA samples containing cloned SV40 (pSVSph21-N), JCV (pBRJC-MAD-1), or BKV (pBRBKV-Dunlop) genomes (kindly provided by Dr Regis A Vilchez, Baylor College of Medicine, Houston, TX, USA). Negative control (no DNA template) was run in parallel and was always the final samples in the series to verify that no contamination had occurred during the analysis. The PCR products were subjected to electrophoresis on a 2% agarose gel containing ethidium bromide and visualized under UV illumination using the Gel Doc 2000 System (Bio-Rad, Marnes-la-Coquette, France). PCR experiments for each case were repeated at least three times.

Methylation-Specific PCR Assays

A panel of 11 tumor suppressor and -related genes thought to have a role in the pathogenesis of gastric cancer and involved in signal transduction (*RASSF1A* and *APC*), repair and protection of DNA (*hMLH1*, *MGMT* and *GSTP1*), cell cycle regulation and apoptosis (*P14*, *P16* and *DAPK*), cell differentiation and metastasis (*SHP1*, *RAR-β2* and *TIMP3*) were analyzed. After DNA bisulfite modification as described by Herman *et al*,[31] promoter methylation analysis by methylation-specific PCR was conducted for each gene as described in our previous study.[32] PCR products were directly loaded onto 2% agarose gel containing ethidium bromide and visualized under UV illumination. Samples were scored as methylated when there was a clearly visible band on the gel with the methylated primers. The results were reported without knowledge of the polyomaviruses status.

Immunohistochemical Analysis

Immunohistochemistry was performed using a polymer-based method: EnVision + labeled Polymer (DakoCytomation, Glostrup, Denmark). Formalin-fixed, paraffin-embedded tissue sections (4 µm) were placed on silane-coated slides. The sections were placed in an oven at 60 °C to melt the paraffin and then were deparaffinized in xylene, hydrated through gradient alcohol series up to water. Next, slides were submerged for 10 min in 3% hydrogen peroxide to block endogenous peroxidase activity. Antigen retrieval was carried out in a water bath by heating the sections in 0.01 M citrate buffer (pH 6) at 98 °C for 20 min. Afterward, the sections were incubated with anti-p53 primary antibody (mouse monoclonal, clone DO-7; DakoCytomation; 1:100) followed by an incubation with EnVision + labeled Polymer, these both steps were performed for

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

Virus	*Oligonucleotide sequences (5′→3′)*	*Annealing temperature*	*Amplicon length (bp)*	*References*
JCV	Forward: 5′-AGTCTTTAGGGTCTTCTACC-3′ Reverse: 5′-GGTGCCAACCTATGGAACAG-3′	54 °C	173	Arthur *et al*[28]
SV40	Forward: 5′-TTAGCAATTCTGAAGGAAAGTCCTTG-3′ Reverse: 5′-ACCTGTTTTGCTCAGAA-3′	54 °C	126	Nakatsuka *et al*[29]
BKV	Forward: 5′-AATATTATGCCCAGCACACATG-3′ Reverse: 5′-CTTTCCCTCTGATCTACACCAG-3′	55 °C	151	Smith *et al*[30]

30 min at room temperature. Sections were gently rinsed in Tris-buffered saline (pH 7.4) between the incubation steps. Finally, sections were visualized using 3,3′-diaminobenzidine as a chromogen, counterstained with Mayer's hematoxylin and cover slipped with Permount. Only cases with more than 10% of tumor cell nuclei showing staining were considered to over-express the p53 gene product.

Statistical Analysis

Clinicopathological parameters, immunohistochemical findings and frequencies of methylation were analyzed with regard to polyomaviruses status using χ^2 test or Fisher's exact test when appropriate. To compare the methylation extent of the genes examined, we determined the methylation index in each case, which is the sum of all methylated promoters divided by the total number of genes analyzed in the subset, and then we calculated the mean for the different groups. The comparison of the mean of methylation index between the two groups was made using the Mann–Whitney non-parametric *U*-test. To assess independent relations of polyomaviruses with number of variables, a multivariate logistic regression analysis was performed. For survival analysis, Kaplan–Meier analysis was performed to assess survival time distributions according to polyomaviruses status and differences in the distribution were evaluated using log-rank test. All the data were analyzed with SPSS 13.0 statistics software (Chicago, IL, USA). All *P*-values presented are two-tailed and the statistical significant threshold was set at 0.05.

Results

Polyomaviruses Detection and Clinicopathological Correlations

Results from multiple PCR experiments, have revealed the presence of JCV T-antigen sequences in 16 (26%) of 61 gastric carcinomas and in three (6%) of 53 paired non-tumor gastric mucosa. The difference between the detection rates was statistically significant ($P = 0.03$). In gastric mucosal samples from patients without gastric carcinoma, JCV T-antigen sequences were detected in two cases (9%). None of the samples were positive for SV40 or BKV. Several controls were included in each set of PCR denoting for a correct procedural experiment and the negative control, which involved the use of a water template was negative and excluded the likelihood of laboratory contamination. Figure 1 illustrates representative data depicting results from PCR experiments.

Correlation analysis was also made, the clinicopathological characteristics of JCV-associated gastric carcinomas were summarized in Table 3. We did not observe a significant difference between the two groups, JCV-positive *vs* JCV-negative gastric carcinomas, with regard to gender, tumor location, tumor invasion and lymph node involvement. Although the presence of JCV was significantly associated with patients older than 55 years ($P = 0.034$) and intestinal histological type of gastric carcinomas ($P = 0.04$). No significant association was found between JCV infection and p53 accumulation ($P = 0.27$). *Helicobacter pylori* co-infection was found in 63% of JCV-positive cases, without reliable significant relationship.

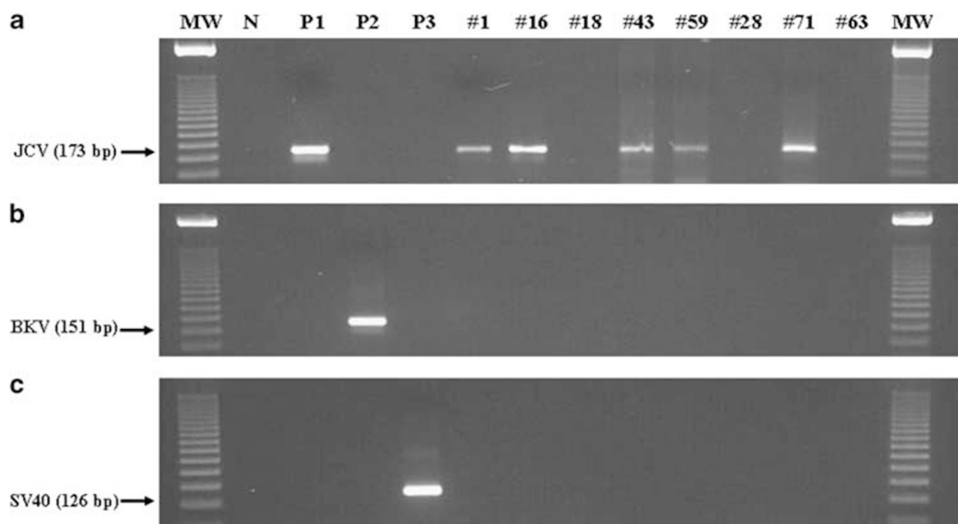

Figure 1 (**a**) Representative PCR amplifications for the detection of JCV T-antigen sequences in gastric carcinomas; #1, #16, #43, #59 and #71 represent JCV-positive cases and #18, #28 and #63 represent JCV-negative cases. (**b**) and (**c**) PCR amplifications for the detection of BKV and SV40 T-antigen; none of the samples were positives for BKV or SV40, only positive controls showed positive signal. MW, molecular weight (50-pb DNA ladder); N, negative control (no DNA template); P1, positive control for JCV (plasmid pBRJC-MAD-1, containing cloned JCV genome); P2, positive control for BKV (plasmid pBRBKV-Dunlop, containing cloned BKV genome); P3, positive control for SV40 (plasmid pSVSph21-N, containing cloned SV40 genome). The sizes of the amplified regions are shown next to each of the blots.

Table 3 Clinicopathological features of JCV-positive and JCV-negative gastric carcinomas

Variables	*JCV-positive cases*	*JCV-negative cases*	P-*value*[a]
Gender			
Male	10	25	0.62
Female	6	20	
Age group(years)			
≤55	1	15	**0.034**
>55	15	30	
Tumor location[b]			
Corpus	6	19	0.59
Antre	10	23	
Tumor histology (Lauren)			
Intestinal	10	15	**0.04**
Diffuse	6	30	
Helicobacter pylori infection[d]			
Present	10	26	0.74
Absent	6	19	
Tumor invasion[c]			
Early (pT1-pT2)	4	7	0.45
Advanced (pT3-pT4)	12	38	
Lymph node involvement			
Positive	7	21	0.84
Negative	9	24	
P53 status[e]			
Positive	7	13	0.27
Negative	9	32	

[a]Bold numbers indicates significant correlation ($P<0.05$).
[b]Data are missing in three cases.
[c]According to the UICC, TNM classification of malignant tumors, fifth edition, 1997.
[d]*Helicobacter pylori* infection was assessed by histological evaluation.
[e]Evaluated by immunohistochemistry and considered positive if >10% of tumor cells showed nuclear staining.

Survival analysis, using the Kaplan–Meier method, was conducted to assess the influence of the presence of JCV on the survival of patients with gastric cancers. We found a trend toward better survival rate for patients with JCV infection compared with patients without infection, but the difference was not statistically significant (log-rank, $P=0.11$; Figure 2).

Correlation Between the Presence of JCV Sequences and Promoter Hypermethylation of Tumor-Related Genes

Methylation-specific PCR analysis showed a methylation frequencies ranging from 10% for *hMLH1* to 61% for *RASSF1A*. Figure 3 illustrates the main results regarding the association between JCV status and epigenetic markers. High methylation frequency was generally observed in JCV-positive gastric carcinomas compared with JCV-negative cases. Significant increase in methylation frequencies were observed for the *P16* ($P=0.007$) and *P14* ($P=0.003$) genes in JCV-positive gastric carcinomas compared with JCV-negative ones. A trend toward statistical significance was found between *TIMP3* promoter hypermethylation and JCV infection ($P=0.07$). The methylation index was also calculated and ranges from 0 to 0.81 with an average of 0.29. The mean of the methylation index in JCV-positive patients' group was significantly higher than that in the JCV-negative group (0.4 ± 0.24 *vs* 0.25 ± 0.14; $P=0.024$) (Figure 3b). Concurrent hypermethylation, defined as aberrant methylation in more than three gene promoters, was more frequently observed in JCV-positive than in JCV-negative cases ($P=0.003$).

Multivariate Analysis

We performed multivariate logistic regression analysis, to examine, which variables were independently associated with JCV infection (Table 4). Results show that patient's age and the mean of methylation index were the only two independent parameters significantly associated with the presence of JCV in gastric carcinomas.

Discussion

Mounting evidence indicates that several common viruses contribute to a variety of malignancies worldwide.[33,34] Among infection-related neoplasms, cancers of the stomach, liver and cervix uteri detain the highest incidence figures. It is generally agreed that *Helicobacter pylori* infection is the major etiological factor in gastric cancers. Conversely to viral involvement, notably with EBV infection that may define subsets of gastric malignancies. For the human polyomaviruses including JCV the association with gastric cancers is still emerging.

Taking cues from the potential involvement of JCV in a subset of gastro-intestinal tumors[35,36] in this study we determined the prevalence of polyomaviruses JCV, BKV and SV40 in Tunisian patients with gastric carcinomas. Findings were analyzed in relation to clinical, pathologic, molecular features of gastric cancers and patient survival. In this study, none of the samples were positives for SV40 or BKV, while for JCV T-antigen sequences were found in 26% of gastric carcinomas. It is noteworthy that the detection rate of JCV in the tumor cases was significantly higher than that in paired non-tumor gastric mucosa (26 *vs* 6%, $P=0.03$), suggesting a role of JCV in the development of gastric carcinomas. However, the detection rate of JCV as a total (26%) was lower than that reported in recent studies in gastric carcinomas (57–86%).[15,16] One possible explanation for these discrepant results is likely caused by difference in the DNA quality, as

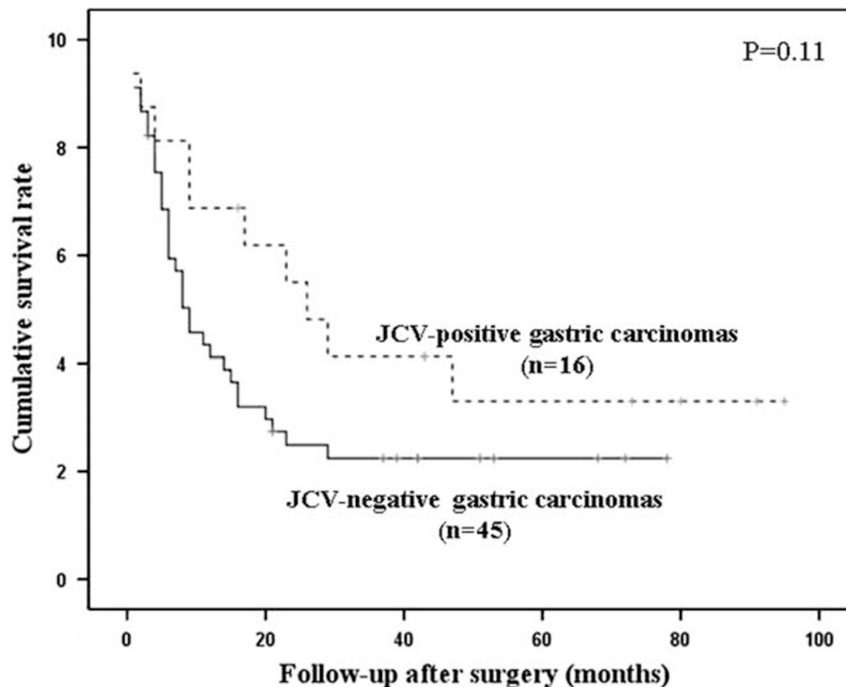

Figure 2 Kaplan–Meier survival curves showing survival differences between the two groups: JCV-positive and JCV-negative gastric carcinomas.

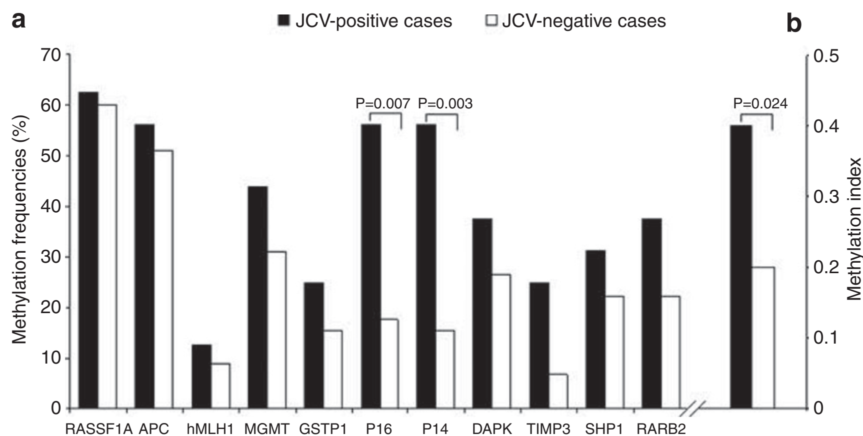

Figure 3 In (**a**) the paired bars depict methylation frequencies for individual tumor suppressor genes in JCV-positive gastric carcinomas ($n = 16$; solid bars) *vs* JCV-negative gastric carcinomas ($n = 45$; open bars). A significant increase in methylation frequencies was observed for the *P16* and *P14* genes in JCV-positive gastric carcinomas compared with JCV-negative cases. Frequencies of methylation between the two groups were compared using χ^2 test or Fisher's exact test when appropriate. In (**b**) the paired bars represent the mean of the methylation index with regard to JCV status. The methylation index was significantly greater in JCV-positive gastric carcinomas compared with JCV-negative cases. *P*-value is calculated using the Mann–Whitney nonparametric *U*-test.

suggested by Murai *et al*[16] the detection rate of JCV sequences is severely reduced in formalin-fixed and paraffin-embedded tissues compared with fresh tissues. Another plausible explanation for this inconsistency is that JCV-associated gastric carcinomas may show a variation in geographic distribution because of ethnic diversity, genetic predisposition and/or environmental factors. The low frequency of JCV T-antigen sequences detected in non-neoplastic gastric mucosa from Tunisian patients in comparison with those reported earlier[15,16] argues for a geographic variation in virus distribution. Overall, our results are in agreement with previous findings that report the presence of JCV DNA sequences in the mucosa of the gastrointestinal tract and in gastric cancers.

In the literature, no data were available regarding the clinicopathological features of JCV-associated gastric carcinomas. In this study, a significant association was observed between the presence of

Table 4 Multivariate analysis was carried out on all variables that were found to be significant with the presence of JCV on univariate analysis using the multivariate logistic regression models

Variables associated with JCV status	*JCV-positive cases*	*JCV-negative cases*	*Multivariate OR (95% CI)*	P-*value*[a]
Age group (years)				
≤55	1	15	0.100 (0.010–0.960)	**0.046**
>55	15	30		
Tumor histology (Lauren)				
Intestinal	10	15	2.566 (0.670–9.826)	0.169
Diffuse	6	30		
Mean of methylation index				
<0.3	6	35	0.174 (0.045–0.680)	**0.012**
≥0.3	10	10		

OR, odds ratio; CI, confidence interval.
[a]Bold number indicates significant correlation ($P<0.05$).

JCV and patients older than 55 years ($P=0.034$). Seemingly, this observation implies that impairment or decrease in the functioning of the immune system because of old age will allow active replication of the virus and development of malignancies. Similar finding has been reported for Epstein–Barr virus-associated gastric carcinomas.[37] Furthermore, in this study, we found that JCV is more commonly detected in intestinal histological type of gastric carcinomas ($P=0.04$). This finding seems in harmony with the known description in literature. In deed, similar to human colorectal cancer, gastric carcinogenesis is a multistep process with morphological progression, in which an intestinal metaplasia–dysplasia-invasive carcinoma sequence exists in both kinds of cancers. Moreover, it is well established that JCV is a reasonable candidate implicated in colorectal carcinogenesis because of its frequent presence in the tumor. Along with those observations and this study, the detection of JCV sequences in the intestinal histological type of gastric cancers provides further support for the possibility that the virus is harbored in the gastrointestinal tract and may be implicated in pathways leading to the development or progression of some cancers of the gastrointestinal tract.

One of the biological implications of the polyomaviruses infection would be the accompanying interaction with some well-characterized tumor suppressor gene, including p53. In this study, p53 over-expression was detected in 20 (33%) cases. However, we did not observe a relationship as previously reported in colorectal cancers,[23] between the presence of JCV and p53 accumulation ($P=0.277$). This discrepant result may be attributed to the differences in the sample sizes ($n=766$ in reference[23] *vs* $n=61$ in this study).

In several studies, many authors have indicated the existence of a mechanistic linkage between DNA methylation and infection agents.[11,22,38–41] This observation has prompted us to suggest the analysis of the relation between JCV infection and the heritable loss related to promoter hypermethylation. To our knowledge, no study has assessed the relationship between JCV-associated gastric carcinomas and the methylation of tumor-related genes. In this study, correlation analysis with regard to JCV status was made and results show generally a high methylation frequency in JCV-positive gastric carcinomas compared with JCV-negative gastric carcinomas.

A particular focus was addressed to *P16* and *P14* genes. The *P16* is a tumor suppressor gene located on chromosome 9p21. *P16* is expressed from a complex locus, with an alternative promoter and alternative reading frame encoding *P14*, which has also been shown to be a tumor suppressor gene. *P16* and *P14* are involved in two major cell cycle regulatory pathways, referred to as the p53 and Rb pathways, respectively and are frequently inactivated in many human malignancies including gastric cancers. There is substantial evidence indicating that viral infection can participate in the dysregulation of the cell cycle. Several studies have shown that methylation of the *P16* gene was intimately associated with Epstein–Barr virus infection in gastric carcinomas.[42,43] Similarly, Goel *et al*[22] have reported an association between the presence of JCV in colorectal carcinomas and hypermethylation of several tumor-related genes including *P16*. Consistent with the aforementioned findings, in this study we found that hypermethylation of *P16* and *P14* were significantly correlated with JCV presence ($P=0.007$, $P=0.003$, respectively). The discern relationship between the presence of JCV and promoter hypermethylation of cell cycle regulatory genes plead for a potential involvement of JCV in pathways leading to the development and/or progression of gastric cancer. Furthermore, we noted that within the JCV-infection group, the hypermethyled genes showed a wide distribution and was significantly higher than depicted in non infection group. This result is in keeping with that

reported by many other studies regarding a viral involvement in the molecular mechanisms of methylator phenotype.

In this study, survival analysis, showed no significant difference in survival rate with regard to JCV status, albeit a better survival for patients with JCV-positive gastric carcinomas was suggested (see Figure 2). This result may be attributed to the relatively small sample size, which makes observing survival differences challenging. Moreover, we have reported previously that patients belonging to the high-methylated group had a better overall survival than patient's group with low-methylated genes or lacking promoter hypermethylation in gastric carcinomas.[32] Altogether, these observations let us suggest that the epigenetic alterations and virus infection are of less aggressive behavior and particularly of better prognostic marker than other genetics alterations.

Overall, although the involvement of JCV in the carcinogenesis of the stomach is not yet clear, the detection of viral DNA and its association with aberrant methylation of multiple tumor suppressor genes, raising the possibility that this virus may have a mechanistic role in the development and/or progression of gastric cancer.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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