Oncogenic T-Antigen of JC Virus Is Present Frequently in Human Gastric Cancers

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Sponsored by the National Institutes of Health (grant RO1-CA 98572) and through funds provided by the Baylor Research Institute to C. Richard Boland.

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Received December 19, 2005; revision received March 9, 2006; accepted March 28, 2006.

BACKGROUND. JC virus (JCV) is a polyomavirus that commonly infects humans and is the causative agent of progressive multifocal leukoencephalopathy in immune-compromised patients. An association between JCV and human cancers long has been suspected, because this virus induces brain tumors in several animal models. The oncogenic potential of JCV is mediated by a transforming protein, the T-antigen (T-Ag), which is a multifunctional protein that transforms cells through interactions with various growth-regulatory genes, including p53 and pRb, and by stabilizing β-catenin. Previously, the laboratory at the authors' institution demonstrated that JCV is present frequently in the human gastrointestinal tract and may play a role in colorectal carcinogenesis. However, to date, no studies have determined whether JCV sequences are present specifically in gastric cancers. The current study was designed to investigate whether JCV sequences and expression are found in human gastric cancers.

METHODS. DNA was extracted from 23 paraffin embedded and 14 frozen gastric cancer specimens. For the detection of JCV gene sequences, polymerase chain reaction amplifications were performed using gene-specific primers for T-Ag, VP-1 (a JCV capsid gene), and the viral regulatory region (or transcriptional control region). Immunohistochemical staining was performed with an anti-T-Ag monoclonal antibody to detect protein expression.

RESULTS. Twenty-one of 37 gastric cancers (57%) harbored JCV T-Ag sequences, and 13 of 37 gastric cancers (30%) contained VP-1 sequences. T-Ag sequences also were found in adjacent nonneoplastic mucosa. In addition, JCV regulatory region sequences were present frequently in gastric cancers and adjacent nonneoplastic mucosa. T-Ag protein expression was found in 9 of 23 gastric cancers (39%), whereas no expression was observed in any of the nonneoplastic tissues.

CONCLUSIONS. To the authors' knowledge, this is the first demonstration of the presence of JCV T-Ag expression in human gastric cancers. These findings suggest a possible role for this polyomavirus in gastric carcinogenesis. ***Cancer* 2006; 107:481–8.** *© 2006 American Cancer Society.*

KEYWORDS: JC virus, polyomavirus, T-antigen, gastric cancer, chromosomal instability.

Although the overall incidence of gastric cancer has decreased steadily in the last few decades, it remains the second most common cause of cancer-related deaths worldwide, with >600,000 deaths anually.[1] Analogous to other malignancies, gastric carcinogenesis also is regarded as a multistep and multifactorial process. Gastric neoplasms are characterized by the successive accumulation of mutations in genes that control epithelial cell growth and differentiation.[2,3] The development of genomic instability is an important event in the multistep progression of gastric carcinogenesis. Two apparently independent pathways of genomic instability have been identified.[4,5] The

DOI 10.1002/cncr.22028
Published online 22 June 2006 in Wiley InterScience (www.interscience.wiley.com).

first pathway is characterized by tumors that have widespread "microsatellite instability" (MSI), which is the consequence of a defective DNA mismatch-repair (MMR) system that occurs either because of germ-line mutations in MMR genes or because of methylation-induced silencing of hMLH1.[6] Typically, MSI is identified in approximately 15% of sporadic gastric cancers.[7,8] It is believed that inactivation of hMLH1 through promoter hypermethylation is the cause of the MSI phenotype in a majority of sporadic gastric cancers.[9]

The second and more common pathway of genomic instability is characterized by the sequential inactivation of tumor suppressor genes and activation of oncogenes. Tumors that evolve through this pathway display chromosome instability, which is measured through loss of heterozygosity events, chromosomal amplifications, and translocations.[10] Evidence for chromosomal instability has been demonstrated in >50% of gastric cancers and is typified by the presence of loss of heterozygosity and rearrangements that involve various chromosomal arms.[11,12] Such chromosomal aberrations are essential to the malignant transformation of a cell, because this process can create oncogenes and delete tumor suppressor genes.[13] Although our current understanding of the mechanism for MSI is quite clear, there is little agreement on the mechanistic basis for the chromosomal instability observed in human cancers. In this context, it has been proposed that the carcinogenic potential of the "transforming antigen" (T-Ag), which is encoded by all polyomaviruses, may be sufficient by itself for the malignant transformation of human embryonic cells.[14] The polyomavirus family includes the simian virus 40 (SV40), JC virus (JCV), and BK virus (BKV). All encode a version of T-Ag, which is a multifunctional protein capable of transforming cells both in vitro and in vivo by interacting with p53 and the cell cycle regulator pRb, leading to their destruction, and allowing unregulated growth of the cancer cells.[15–17]

We previously provided evidence for the presence of JCV in the normal gastrointestinal tract and colon cancers in humans.[18–21] More recently, we demonstrated that JCV induced chromosomal instability in a colon cancer cell line that expressed wild-type adenomatous polyposis coli (APC), p53, and β-catenin proteins.[22] JCV is a 5.12-kb, double-stranded, circular, negatively supercoiled DNA virus. JCV genomes consist of early and late coding regions that are separated by a bidirectional, noncoding regulatory region. This regulatory region, also known as the transcriptional control region (TCR), contains the promoter and enhancer elements for early and late transcription. The genes encoded by the late coding region are 3 viral capsids (VP-1, VP-2, and VP-3) and an agnoprotein. The large-T (T-Ag) and small-t (t-Ag) antigens are encoded by the early coding region of the viral genome.

JCV infects humans worldwide, and >80% of the adult population carries antibodies against the virus.[23] JCV has been considered an innocent and passive passenger in humans; however, it can cause progressive multifocal leukoencephalopathy in patients with compromised immune status.[24] It has been hypothesized that JCV is oncogenic primarily because it encodes the T-Ag protein. JCV can cause aneuploid tumors when it is injected into the brains of rodents.[25] Furthermore, there is evidence for the presence of JCV and T-Ag in high-grade human brain tumors.[26]

Our observations that JCV sequences are present frequently in human colon cancers recently were supported by several other independent laboratories.[27–30] It has been suggested that, in addition to the association of T-Ag with p53 and pRb, this oncogenic protein also may interact with β-catenin, causing its stabilization, which dysregulates the WNT signaling pathway in gastrointestinal cancers.[27] Collectively, these data suggest a possible role of JCV in gastrointestinal carcinogenesis. The current study is an extension of our previous work, and the objective was to investigate whether JCV sequences also are present in human gastric cancers, which, by inference, may help account for some degree of chromosomal instability observed in these neoplasms.

MATERIALS AND METHODS

Gastric Tissue Specimens

For this study, 37 sporadic gastric cancer tissues were investigated, including 23 paraffin embedded tissue blocks from the archives of Baylor University Medical Center (Dallas, TX) and 14 fresh-frozen tissues from Yonsei University (Seoul, South Korea). The diagnosis for each of the samples was gastric adenocarcinoma and was confirmed by expert pathologists from both institutions. All patients were immunocompetent, and none showed signs or symptoms of progressive multifocal leukoencephalopathy. This study was approved by the Institutional Review Boards of both institutions.

DNA Extraction

Genomic DNA was extracted from paraffin-embedded tissue sections or from frozen tissues by using the QIAamp DNA mini kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions with some modifications. Extreme caution was exercised to perform all preparatory polymerase chain reaction (PCR) steps, including DNA extraction, in a

TABLE 1
Primer Sequences for Amplification and Detection of JC Virus Genomes

Primer name/Position*	Primer sequence
T-Ag gene	
154 F: 4381–4408	5′-ATGTATTCCACCAGGATTCCCATTCATC-3′
154 R: 4511–4534	5′-AGTTCTTGGAGACACCCCCTACAG-3′
VP-1 gene	
F: 1828–1848	5′-TGTGCACTCTAATGGGCAAGC-3′
R: 2019–2039	5′-CTAGGTACGCCTTGTGCTCTG-3′
TCR gene	
F: 4986–5006	5′-TACTTCTGAGTAAGCTTGGAG-3′
R: 238–258	5′-AAAACAGCTCTGGCTCGCAA-3′

T-Ag indicates T-antigen; F, forward; R, reverse; TCR, transcriptional control region.
*Based on JC virus sequence Genebank accession no. NC_001699.

separate room to avoid contamination issues with PCR-amplified products.

Detection of JCV Genomic Sequences

PCR amplifications for JCV T-Ag, VP-1, and TCR were performed by using the specific primer sequences listed in Table 1, which shows that the primers T-Ag 154 forward (F) and T-Ag 154 reverse (R) amplified a 154-base pair (bp) sequence encoding the NH_2-terminal region of JCV T-Ag. Similarly, the primers VP-1F and VP-1R were used to generate a 212-bp PCR product specific for the VP-1 gene, which encodes for 1 of the viral capsid proteins. Each PCR reaction consisted of a 25-μL reaction mixture that contained 0.2 μg of genomic DNA, 1 $\times$ HotStarTaq master mix (Qiagen Inc.), and 0.75 mM of each primer (Invitrogen Life Technologies Inc., Carlsbad, CA). The PCR reactions were carried out by using a PTC 200 DNA Engine System (MJ Research Inc., Watertown, MA). The PCR reactions were performed in a separate room in the laboratory, as described above; and, after amplification, the PCR products were subjected to agarose gel electrophoresis in another part of the laboratory. All PCR results subsequently were confirmed by sequencing the products using an ABI Prism Big Dye Terminator version 1.1 Cycle Sequencing Kit on an ABI Prism 3100 Avant Genetic analyzer (Applied Biosystems, Foster City, CA). The sequencing data obtained were aligned with published JCV sequences to ensure that the sequences were of JCV origin only and did not match either BKV sequences or SV40 sequences.

Analysis of JCV Regulatory Region Sequences

Based on the regulatory region sequences, JCV can be classified into 2 forms: the archetype and the tandem repeat variants. Among the tandem repeat variants of JCV, the most studied strain of JCV is Mad-1[31] (Fig. 1).

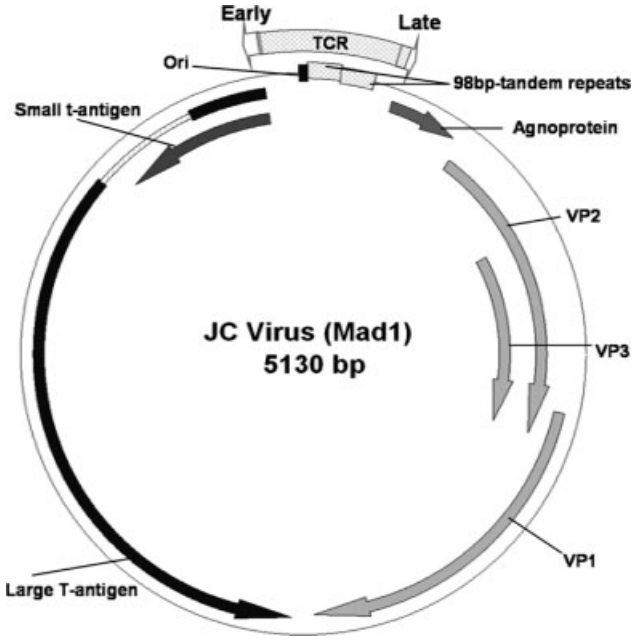

FIGURE 1. This is a schematic representation of the Mad-1 JC virus (JCV) genome. Based on the regulatory region sequences, JCV can be classified into 2 forms, the archetype and the tandem repeat variant. Among tandem repeat variants, the Mad-1 strain of JCV is the causative agent of progressive multifocal leukoencephalopathy. JCV (Mad-1) is a 5.13-Kb, double-stranded, circular, negatively supercoiled DNA virus. The noncoding regulatory region or the transcriptional control region (TCR) of Mad-1 JCV is characterized by the absence of 23-base pair (bp) and 66-bp sequences that are present otherwise in the archetype along with the presence of 98-bp tandem repeats. The early and late coding regions are separated by the bidirectional TCR, and transcription in both directions initiates at the origin of replication (ori). The genes encoded by the late coding regions are 3 viral capsids (VP-1, VP-2, VP-3) and agnoprotein. The large T-antigen (T-Ag) and small t-Ag are encoded by the early coding region of the viral genome.

The noncoding regulatory region or TCR of Mad-1 JCV is characterized by the absence of 23-bp and 66-bp sequences that otherwise are present in the archetype along with the presence of 98-bp tandem repeats. More important, in our previous study, we found only Mad-1 or rearranged forms of Mad-1 TCR sequences, and we never found evidence for the archetype strain of JCV in gastrointestinal cancers.[20] Rearranged forms of Mad-1 are characterized by a deletion or addition of a 98-bp sequence that results in Δ98 or Mad-1A sequences, respectively.[20] For the current study, we used PCR primers that were outside of the these regions of rearrangement, which allowed us to amplify DNA from multiple different JCV genotypes that yielded different sized PCR products. The primer sequences for the amplification of TCR sequences are shown in Table 1. PCR amplification was carried out on 0.2 μg of template DNA with a total reaction volume of

25 μL. PCR reactions were comprised of 1 × HotStarTaq master mix (Qiagen Inc.) and 0.5 mM of each primer (Invitrogen Life Technologies Inc.). Subsequent to PCR, sequencing of the regulatory region was performed to confirm further the presence of JCV and to exclude the other 2 polyomaviruses (BKV and SV40), which are distinct in their regulatory regions. All PCR products were resolved on a 1.0% low-melting-point agarose gel (Invitrogen Life Technologies Inc.) and were purified by using a QIAamp gel extraction and purification kit (Qiagen Inc.). Purified PCR products thereafter were cloned into a pCR®4-TOPO® vector by using a TOPO TA cloning kit (Invitrogen Life Technologies Inc.). Both strands of plasmid DNA subsequently were sequenced by using an ABI Prism Big Dye Terminator version 1.1 Cycle Sequencing Kit (Applied Biosystems) on an ABI Prism 3100 Avant-Genetic analyzer (Applied Biosystems). Along with each PCR amplification of unknown TCR sequences from clinical tissues, we amplified positive controls for various rearranged forms of TCR using plasmid DNA samples that were obtained from cloned, full-length JCV TCRs (Mad-1, Δ98, and Mad-1A). The classification of JCV subtypes from clinical tissues was performed according to the scheme proposed by Jensen and Major.[32]

Immunohistochemical Staining for T-Ag in Gastric Cancer Tissues

Immunohistochemical staining was performed only on the paraffin embedded gastric cancer tissues. The tissue sections used measured 4 μm in thickness and were mounted on positively charged slides. Tissue sections were deparaffinized with xylene and rehydrated through gradient alcohol series. The sections subsequently were immersed in 0.01 M citrate buffer (pH 6.0) and were heated intermittently in a microwave oven for a total of 20 minutes for nonenzymatic antigen retrieval. Dual endogenous enzyme block solution (Dako Cytomation Inc., Carpinteria, CA) was applied to tissue sections for 10 minutes to block the endogenous peroxidase. The primary antibody used to detect specific T-Ag protein expression was a mouse monoclonal antibody against SV40 large T-Ag that cross reacts with JCV T-Ag (clone pAb416; 1:100 dilution; Oncogene Research Products, San Diego, CA). Incubation of the primary antibody was performed overnight and was followed by incubation in Dako En Vision-labeled polymer (Dako Cytomation Inc.) for 30 minutes. Staining was developed by reaction with diaminobenzide chromogen for 5 to 10 minutes and then counterstaining for 5 minutes with hematoxylin. The brown chromogen complexes were indicative of T-Ag-specific expression in the gastric cancer tissues.

RESULTS

Detection of JCV DNA Sequences in Gastric Cancer Tissues

In the current study, we observed that 21 of 37 patients (57%) harbored JCV T-Ag sequences in the neoplastic tissues and that 11 of 37 nonneoplastic gastric tissues (30%) also harbored T-Ag sequences. In addition, the viral capsid VP-1 sequences were detected in 9 of 37 gastric cancer tissues (24%) and in 7 of 37 nonneoplastic mucosae (19%) (Table 2). Each PCR amplification was accompanied by amplifying a positive control for JCV using the genomic DNA from JCI cells (kindly provided by Dr. Kazuo Nagashima, Hokkaido University, Sapporro, Japan), which persistently is infected with JCV.[33] The negative control, which involved the use of a water template, was negative and excluded the likelihood of laboratory contamination. Figure 2 shows a panel of representative PCR amplifications of JCV sequences from gastric cancer tissues and nonneoplastic tissues. Because JCV T-Ag demonstates >70% homology with SV40 and BKV T-Ag at the nucleotide level, our PCR primers were designed specifically to amplify JCV T-Ag sequences only. However, each of these PCR amplicons was confirmed further through DNA sequencing; and, in each instance, the presence of JCV was validated.

Analysis of the JCV TCR

To confirm further the identity of the JCV sequences that were observed in gastric cancer tissues and in adjacent nonneoplastic tissues, all samples that were amplified for T-Ag also were PCR amplified for the detection of regulatory region, or TCR, sequences. Again, after PCR amplification of these genomic sequences, the sequences were confirmed by using an ABI Avant automated sequencer. The TCR sequences that occur in the 98-bp tandem repeats of Mad-1 were used as identification signatures, because these differ substantially among JCV and the related SV40 and BKV. Sixteen of 37 gastric cancer samples (43%) and 13 of 37 nonneoplastic tissue samples (35%) demonstrated the presence of JCV Mad-1 and/or its rearranged TCR forms, but the archetype strain was not observed in any sample (Table 2, Fig. 3).

It is noteworthy that several samples (9 gastric cancers and 5 nonneoplastic mucosae) harbored multiple regulatory strains of JCV in a single tissue specimen. These findings indicate the presence of different strains of JCV, although the predominant sequence rearrangements of the regulatory region were identified as either the Δ98 or the Mad-1 variant, similar to what we observed in our previous study of colorectal

TABLE 2
The Prevalence of JC Virus DNA Sequences and T-Antigen Protein Expression in Gastric Cancer

	T-Ag		VP-1		TCR		
No.	N	T	N	T	N	T	IHC for T-Ag
1*	−	+	−	−	−	−	−
2*	−	−	−	−	−	−	−
3†	−	−	−	−	I-R	−	ND
4†	−	−	−	−	−	−	ND
5*	−	+	−	+	−	I-R	+
6†	+	−	+	−	I-R	−	ND
7*	+	+	+	+	I-R	I-R‡	−
8*	−	+	−	+	−	−	+
9*	−	−	−	−	−	−	−
10*	−	+	−	−	I-R‡	I-R	+
11†	+	+	−	−	I-R, I-S	I-R‡, I-S	ND
12†	−	+	−	−	−	I-R, I-S	ND
13†	+	+	−	−	I-R	I-R‡	ND
14*	−	+	−	+	−	−	−
15†	−	−	−	−	I-R	I-R, I-S	ND
16*	−	−	−	−	−	−	−
17*	−	−	−	−	−	−	−
18†	+	+	+	−	I-R, I-S	I-R, I-S	ND
19*	−	−	−	−	−	−	−
20*	−	−	−	−	−	−	−
21*	−	+	−	−	−	I-R	+
22†	−	+	−	−	−	−	ND
23*	−	−	−	−	−	−	−
24*	−	+	−	−	−	I-R‡, I-S	+
25*	−	−	−	−	−	−	−
26†	−	−	−	−	−	I-R	ND
27*	+	+	+	+	I-R, I-S	I-S	+
28†	+	+	+	+	I-R	I-R‡	ND
29*	+	+	+	+	I-R, I-S	−	+
30*	+	+	+	+	I-S	I-R	−
31*	+	+	−	−	−	−	+
32†	−	−	−	−	−	−	ND
33*	−	+	−	−	−	−	−
34*	−	−	−	−	−	−	−
35†	−	−	−	−	−	−	ND
36*	+	+	−	−	−	I-R‡	+
37†	−	+	−	+	I-S	I-S	ND

T-Ag indicates T antigen; TCR, transcriptional control region; IHC, immunohistochemistry; N, adjacent nonneoplastic tissues; T, cancer tissues; I-R, type-I repeat; ND, not done; I-S, type-1 single.
* Paraffin-embedded tissues.
† Frozen tissues.
‡ Mad-1 and Mad-1A TCRs coexisted.

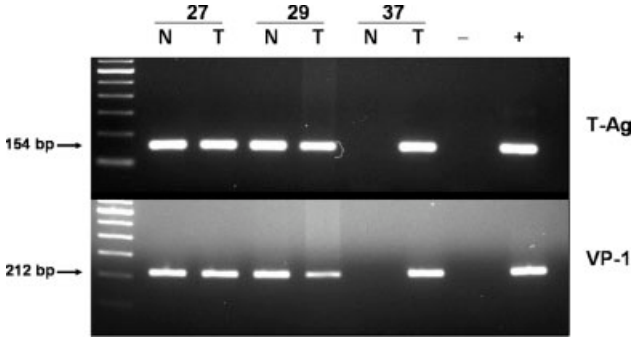

FIGURE 2. Polymerase chain reaction (PCR) amplification of T-antigen (T-Ag) and VP-1 sequences of JC virus (JCV) in gastric cancer and nonneoplastic tissues. Top panel: The presence of 154-base-pair (bp) JCV T-Ag sequences in a panel of representative samples showed that 21 of 37 tumors (57%) harbored T-Ag sequences, and these sequences were present in 11 of 37 adjacent nonneoplastic tissues (30%). Bottom panel: Results from the PCR amplification of an amplicon from VP-1, a viral capsid protein, show that VP-1 sequences were positive in 9 of 37 gastric cancers (24%) and in 7 of 37 nonneoplastic tissues (19%). N indicates adjacent nonneoplastic mucosa; T, gastric tumor; −, negative control using water template; +, positive control from JCI cells.

Expression of Oncogenic T-Ag Protein Is Confined to Cancer Cells

In this study, we observed JCV T-Ag expression frequently in gastric cancer samples but never in nonneoplastic tissue samples. Figure 4 and Table 2 show the immunohistochemical data obtained from the gastric cancers and nonneoplastic paraffin embedded tissue sections that were stained for JCV T-Ag. Not surprisingly, not every gastric cancer that was amplified for JCV T-Ag sequences demonstrated T-Ag protein expression. Overall, 9 of 23 (39%) cancer tissues demonstrated positive T-Ag expression in the nuclei of malignant cells. None of the tissue specimens that were negative for T-Ag DNA sequences expressed JCV T-Ag protein in the immunohistochemical analysis ($P < .01$; Pearson chi-square test). T-Ag expression was observed specifically in gastric cancer tissues only and never was seen in the adjacent nonneoplastic tissues, which is consistent with an active role for this oncogenic protein in gastric carcinogenesis.

DISCUSSION

The results of this study demonstrate the presence of JCV in human gastric cancer tissues. More important, this work provides evidence that T-Ag expression is a tumor-specific phenomenon. These observations are in agreement with our previous findings, which also have been supported by data from other laboratories in reporting the presence of JCV DNA sequences in

cancers.[20] There was no evidence of the archetypal TCR of JCV in the gastric cancers. According to the classification criteria stipulated by Jensen and Major, the Mad-1 and Mad-1A TCRs are classified as Type I-Repeat (I-R), and Δ98 is classified in the I-Single (I-S) category.[32] These data were another verification of the identification of JCV in the gastric cancers, because the TCRs are unique in JCV, BKV, and SV40.

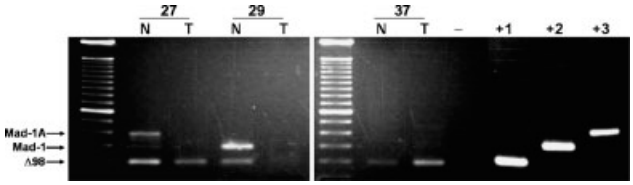

FIGURE 3. The results from polymerase chain reaction (PCR) amplification of the regulatory region of JC virus (JCV) in gastric tissues are shown from representative gastric tumor specimens (T) and corresponding nonneoplastic tissues (N). Full-length Δ98, Mad-1, and Mad-1A (+1, +2, and +3, respectively) cloned into plasmids also were amplified with same primer pairs for sizing the PCR products from the clinical tissues. The PCR amplifications subsequently were confirmed by sequencing, and the majority of the samples showed Δ98 and rearranged forms of the Mad-1 transcriptional control region. Archetypal JCV sequences were not found in any of the gastric cancers or adjacent nonneoplastic tissues.

the mucosa of the gastrointestinal tract and in colon cancers.[18–20,27,30,34,35]

It has been proposed that viruses may play an important role as causative agents of cancer, but this has been more difficult to demonstrate in humans than in animal models. Evidence that supports the role of viruses in human cancers has been emerging at a constant pace, and associations have been made between the presence of hepatitis B and C viruses and hepatocellular carcinoma, the presence of SV40 and mesothelioma, and the presence of Epstein–Barr virus in several malignancies.[36–38] Furthermore, there is mounting evidence that implicates SV40 and JCV in certain types of human cancers.[39] One of the reasons for suspecting an oncogenic role for these viral agents in human cancers may be attributed to the presence of potent transforming genes in the putative oncogenic viruses. The most important of those is the T-Ag, which is encoded by the 3 polyomaviruses: JCV, SV40, and BKV. T-Ag is a multifunctional protein with the ability to bind and inactivate pRb and p53. It has been suggested that, with these functions, T-Ag can disrupt chromosomal integrity and inactivate cell cycle check points that would limit clonal expansion of such damaged cells.[16,17]

Several investigators previously reported that the presence of JCV is associated strongly with aneuploid brain tumors in animal models and in humans.[25,40] In addition, elevated antibody titers to JCV have been linked with aneuploid lymphocytes that were termed "rogue cells" by Neel et al.[41] Previous reports from our laboratory and from others have shown that JCV T-Ag DNA sequences were present in 80% to 90% of normal colonic tissues and colorectal cancers.[18,19,27] T-Ag expression was never detected in normal colon but was present exclusively in ≈63% of colorectal cancers, supporting the oncogenic role of T-Ag in these neoplasms.[27] Recently, we also demonstrated that JCV induced chromosomal instability in a diploid colon cancer cell line with wild-type APC, p53, and β-catenin.[22] Analogous to human colorectal cancer, gastric carcinogenesis commonly is associated with increased aneuploidy and chromosomal instability. Based on these observations, the detection of JCV sequences in gastric cancers and the tumor-specific expression of T-Ag in neoplastic tissues ($P < .01$; Pearson chi-square test) support our previous observations in the colon and suggest a role for this polyomavirus in gastric cancer.[18,19]

The mechanisms of transmission of JC viral infection are not clear; however, the presence of JCV in raw sewage has suggested that the ingestion of contaminated water or food may represent a possible portal of entry for JCV into the human body.[42,43] Those findings are all the more interesting in light of a recent report of the detection of JCV T-Ag sequences and protein expression in esophageal carcinomas.[34] Along with those findings and our previous studies, the current report of the presence of JCV in gastric cancers provides further support for the possibility that the virus is harbored in the gastrointestinal tract and may participate in carcinogenesis throughout the gut.

It has been observed that JCV genomes isolated and sequenced directly from human tissues have a highly variable, noncoding regulatory region, most often referred to as the TCR. This regulatory region can be divided into 7 distinct segments, starting with the origin of replication (ori), followed by 6 regions denoted alphabetically as regions a, b, c, d, e, and f.[44] Among these, sequences represented by regions a, c, e, and f are indicative of viral activity or functionality, whereas the interspersed regions b and d inhibit efficient transcription and replication.[44] Duplication of any single region or multiple regions in the a to e boxes in tandem patterns are considered to enhance viral activity.[45] Specific TCR sequences affect the level of viral transcription and replication; consequently, 2 types of JCV strains have been identified based on TCR structure.[44] First among these is the archetype, which is detected predominantly in urine and sewage. The second type is the rearranged form of JCV, called Mad-1, which is the principal form detected in patients with progressive multifocal leukoencephalopathy.[31] Recently, Jensen and Major proposed a new scheme for classifying JCV according to the structural variation of the TCRs.[32] Those investigators classified JCV strains into different "quadrants" based on the DNA sequence of the promoter. I-S forms include sequence domains a, c, and e but lack domains b and d. I-R forms all have 98-bp tan-

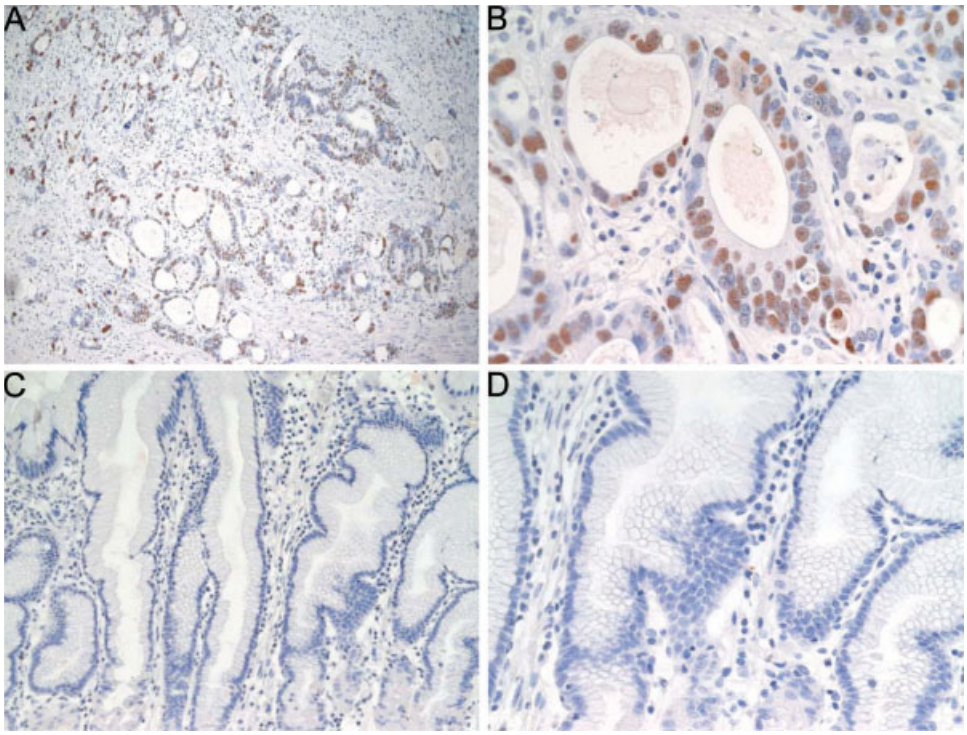

FIGURE 4. These photomicrographs show immunohistochemical staining for JC virus (JCV) T antigen (T-Ag) in gastric cancers. (A) A representative paraffin embedded gastric cancer tissue specimen demonstrates T-Ag-specific staining (shown in brown). (B) This higher magnification of the tissue specimen shown in A clearly demonstrates that expression of JCV T-Ag was localized only to the nuclear compartment of the cancer cells. (C,D) These images represent T-Ag expression from corresponding adjacent nonneoplastic gastric tissues. There was no evidence of any immunoreactivity for T-Ag in these nonneoplastic gastric tissue specimens. Original magnification ×100 (A); ×200 (C); ×400 (B,D).

dem repeats, and this includes Mad-1 and other strains. II-S forms include archetype strains. II-R structures include duplication of the tandem repeats present in II-S forms but with some variations in the b and d segments. According to this classification, Mad-1 belongs to type I-R. We previously reported that only Mad-1 or its rearranged forms could be found in nonneoplastic mucosa of the gastrointestinal tract and in malignant epithelial cells of colon cancers.[20] Similar to our previous observation in the colon, in the current study, we observed that Mad-1 or type I-R was the most frequent form of JCV strain present in gastric cancers, and we found no evidence for II-S or the archetypal TCR. The sequence variability of JCV TCR between and within specimens may suggest that individuals are infected by different strains or that there is an evolution of the diverse viral sequences through multiple rounds of viral replication. In this study, we did not find consistent evidence for the presence of JCV T-Ag, VP-1, and TCR sequences in every sample that expressed JCV T-Ag protein. This partly may be because we do not know whether JCV infection in the gastrointestinal tract is "lytic," which would suggest a productive viral infection, or whether JCV merely acts a "passenger." Further studies will be required to address this issue and may help explain why we detected TCR sequences in only some of the T-Ag-positive gastric cancers investigated in the current study.

In conclusion, the current results demonstrated that the presence of JCV is frequent in human gastric cancers and that the expression of the oncogenic T-Ag is present in a tumor-specific manner. Clearly, the mechanistic role of JCV in gastric cancer cannot be ascertained from the current study, and future studies will be needed to address these issues. Nonetheless, these data clearly support an association between JCV polyomavirus and gastric cancers and provide compelling reasons to investigate whether the presence of JCV correlates with increased genomic instability in these neoplasms.

REFERENCES

1. Parkin DM, Bray F, Ferlay J, Pisani P. Estimating the world cancer burden: Globocan 2000. *Int J Cancer.* 2001;94:153–156.
2. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res.* 1992;52:6735–6740.

3. Hiyama T, Yokozaki H, Kitadai Y, et al. In situ mRNA hybridization technique for analysis of human telomerase RNA in gastric precancerous and cancerous lesions. *Jpn J Cancer Res.* 1998;89:1187–1194.
4. Tahara E. Molecular mechanism of stomach carcinogenesis. *J Cancer Res Clin Oncol.* 1993;119:265–272.
5. Loeb LA. Microsatellite instability: marker of a mutator phenotype in cancer. *Cancer Res.* 1994;54:5059–5063.
6. Liu B, Parsons R, Papadopoulos N, et al. Analysis of mismatch repair genes in hereditary non–polyposis colorectal cancer patients. *Nat Med.* 1996;2:169–174.
7. Semba S, Yokozaki H, Yamamoto S, Yasui W, Tahara E. Microsatellite instability in precancerous lesions and adenocarcinomas of the stomach. *Cancer.* 1996;77:1620–1627.
8. Miyoshi E, Haruma K, Hiyama T, et al. Microsatellite instability is a genetic marker for the development of multiple gastric cancers. *Int J Cancer.* 2001;95:350–353.
9. Toyota M, Ahuja N, Suzuki H, et al. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Res.* 1999;59:5438–5442.
10. Lengauer C, Kinzler KW, Vogelstein B. Genetic instabilities in human cancers. *Nature.* 1998;396:643–649.
11. Seruca R, Castedo S, Correia C, et al. Cytogenetic findings in eleven gastric carcinomas. *Cancer Genet Cytogenet.* 1993;68:42–48.
12. Yustein AS, Harper JC, Petroni GR, Cummings OW, Moskaluk CA, Powell SM. Allelotype of gastric adenocarcinoma. *Cancer Res.* 1999;59:1437–1441.
13. Vogelstein B, Fearon ER, Kern SE, et al. Allelotype of colorectal carcinomas. *Science.* 1989;244:207–211.
14. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. Creation of human tumour cells with defined genetic elements. *Nature.* 1999;400:464–468.
15. Dorries K. New aspects in the pathogenesis of polyomavirus-induced disease. *Adv Virus Res.* 1997;48:205–261.
16. Dyson N, Bernards R, Friend SH, et al. Large T antigens of many polyomaviruses are able to form complexes with the retinoblastoma protein. *J Virol.* 1990;64:1353–1356.
17. Bollag B, Chuke WF, Frisque RJ. Hybrid genomes of the polyomaviruses JC virus, BK virus, and simian virus 40: identification of sequences important for efficient transformation. *J Virol.* 1989;63:863–872.
18. Laghi L, Randolph AE, Chauhan DP, et al. JC virus DNA is present in the mucosa of the human colon and in colorectal cancers. *Proc Natl Acad Sci USA.* 1999;96:7484–7489.
19. Ricciardiello L, Laghi L, Ramamirtham P, et al. JC virus DNA sequences are frequently present in the human upper and lower gastrointestinal tract. *Gastroenterology.* 2000;119:1228–1235.
20. Ricciardiello L, Chang DK, Laghi L, Goel A, Chang CL, Boland CR. Mad-1 is the exclusive JC virus strain present in the human colon, and its transcriptional control region has a deleted 98-base-pair sequence in colon cancer tissues. *J Virol.* 2001;75:1996–2001.
21. Niv Y, Goel A, Boland CR. JC virus and colorectal cancer: a possible trigger in the chromosomal instability pathways. *Curr Opin Gastroenterol.* 2005;21:85–89.
22. Ricciardiello L, Baglioni M, Giovannini C, et al. Induction of chromosomal instability in colonic cells by the human polyomavirus JC virus. *Cancer Res.* 2003;63:7256–7262.
23. Major EO, Amemiya K, Tornatore CS, Houff SA, Berger JR. Pathogenesis and molecular biology of progressive multifocal leukoencephalopathy, the JC virus-induced demyelinating disease of the human brain. *Clin Microbiol Rev.* 1992;5:49–73.
24. Padgett BL, Walker DL, ZuRhein GM, Eckroade RJ, Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet.* 1971;1:1257–1260.
25. Walker DL, Padgett BL, ZuRhein GM, Albert AE, Marsh RF. Human papovavirus (JC): induction of brain tumors in hamsters. *Science.* 1973;181:674–676.
26. Rencic A, Gordon J, Otte J, et al. Detection of JC virus DNA sequence and expression of the viral oncoprotein, tumor antigen, in brain of immunocompetent patient with oligoastrocytoma. *Proc Natl Acad Sci USA.* 1996;93:7352–7357.
27. Enam S, Del Valle L, Lara C, et al. Association of human polyomavirus JCV with colon cancer: evidence for interaction of viral T-antigen and beta-catenin. *Cancer Res.* 2002;62:7093–7101.
28. Duran A, Nerurkar V. Association of polyomavirus JC with colon cancer. *Ethn Dis.* 2005;15:S4–S9.
29. Theodoropoulos G, Panoussopoulos D, Papaconstantinou I, et al. Assessment of JC polyoma virus in colon neoplasms. *Dis Colon Rectum.* 2005;48:86–91.
30. Casini B, Borgese L, Del Nonno F, et al. Presence and incidence of DNA sequences of human polyomaviruses BKV and JCV in colorectal tumor tissues. *Anticancer Res.* 2005;25:1079–1085.
31. Frisque RJ, Bream GL, Cannella MT. Human polyomavirus JC virus genome. *J Virol.* 1984;51:458–469.
32. Jensen PN, Major EO. A classification scheme for human polyomavirus JCV variants based on the nucleotide sequence of the noncoding regulatory region. *J Neurovirol.* 2001;7:280–287.
33. Nukuzuma S, Yogo Y, Guo J, et al. Establishment and characterization of a carrier cell culture producing high titres of polyoma JC virus. *J Med Virol.* 1995;47:370–377.
34. Del Valle L, White MK, Enam S, et al. Detection of JC virus DNA sequences and expression of viral T antigen and agnoprotein in esophageal carcinoma. *Cancer.* 2005;103:516–527.
35. Hori R, Murai Y, Tsuneyama K, et al. Detection of JC virus DNA sequences in colorectal cancers in Japan. *Virchows Arch.* 2005;447:723–730.
36. Monto A, Wright TL. The epidemiology and prevention of hepatocellular carcinoma. *Semin Oncol.* 2001;28:441–449.
37. Rizzo P, Bocchetta M, Powers A, et al. SV40 and the pathogenesis of mesothelioma. *Semin Cancer Biol.* 2001;11:63–71.
38. Knecht H, Berger C, Rothenberger S, Odermatt BF, Brousset P. The role of Epstein–Barr virus in neoplastic transformation. *Oncology.* 2001;60:289–302.
39. Butel JS. Viral carcinogenesis: revelation of molecular mechanisms and etiology of human disease. *Carcinogenesis.* 2000;21:405–426.
40. Rencic A, Gordon J, Otte J, et al. Detection of JC virus DNA sequence and expression of the viral oncoprotein, tumor antigen, in brain of immunocompetent patient with oligoastrocytoma. *Proc Natl Acad Sci USA.* 1996;93:7352–7357.
41. Neel JV, Major EO, Awa AA, et al. Hypothesis: "rogue cell"-type chromosomal damage in lymphocytes is associated with infection with the JC human polyoma virus and has implications for oncopenesis. *Proc Natl Acad Sci USA.* 1996;93:2690–2695.
42. Bofill-Mas S, Formiga-Cruz M, Clemente-Casares P, Calafell F, Girones R. Potential transmission of human polyomaviruses through the gastrointestinal tract after exposure to virions or viral DNA. *J Virol.* 2001;75:10290–10299.
43. Bofill-Mas S, Clemente-Casares P, Major EO, Curfman B, Girones R. Analysis of the excreted JC virus strains and their potential oral transmission. *J Neurovirol.* 2003;9:498–507.
44. Ault GS. Activity of JC virus archetype and PML-type regulatory regions in glial cells. *J Gen Virol.* 1997;78(Pt 1):163–169.
45. Daniel AM, Swenson JJ, Mayreddy RP, Khalili K, Frisque RJ. Sequences within the early and late promoters of archetype JC virus restrict viral DNA replication and infectivity. *Virology.* 1996;216:90–101.