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ORIGINAL ARTICLE

Assessment and biological significance of JC polyomavirus in colorectal cancer in Tunisia

Feryel Ksiaa, Asma Allous, Sonia Ziadi, Moncef Mokni, Mounir Trimeche

Department of Pathology, Centre Hôpital Universitaire Farhat Hached, Sousse, Tunisia

Summary

Purpose: *Several reports have indicated the presence of JC polyomavirus (JCV) in many human tumors, including colorectal cancers (CRCs). The presence of JCV infection in CRC patients has not been investigated in African countries.*

Methods: *We examined the prevalence and the biological significance of JCV in Tunisian CRC patients. The presence of JCV was assessed by polymerase chain reaction (PCR) in a series of 105 CRCs and 89 paired non-tumor colonic mucosa samples from Tunisian patients. Results were correlated with the clinicopathological features and immunohistochemical expression of β-catenin, p53, and the proliferation marker Ki-67.*

Results: *JCV DNA was detected in 58.1% (61/105) of CRC and in only 14.6% (13/89) of paired non tumor colonic mucosa samples (p=0.03). The presence of JCV was significantly correlated with tumor differentiation (p=0.03). Moreover, JCV presence was significantly correlated with nuclear accumulation of β-catenin (p=0.008) and p53 accumulation (p=0.0001). Multivariate logistic regression analysis showed that tumor differentiation, β-catenin and p53 accumulation were independent parameters significantly associated with the presence of JCV in CRC (p=0.04; p=0.05; p=0.001, respectively).*

Conclusion: *We support a role of JCV in colorectal carcinogenesis in Tunisian patients, especially of well differentiated morphology.*

Key words: *β-catenin, colorectal cancer, JC polyomavirus, Ki-67, p53*

Introduction

CRC is one of the most common causes of cancer related deaths in the world, especially in Western countries [1]. There is increased evidence that CRC can develop through different pathways. The more common mechanism is characterised by large genomic rearrangements, found in about 85% of sporadic CRCs, which is called chromosomal instability (CIN) pathway. The second pathway, concerning 35% of sporadic CRCs, is caused by epigenetic inactivation of tumor suppressor genes [2].

Viruses have long been proposed to play an etiologic role in human cancers. Overall, it is estimated that 20% of all cancers are linked to infectious agents. Accordingly, it has been reported that JCV is frequently present throughout the normal human gastrointestinal tract [3,4] in gastric [5-7] and CRCs [8-12].

The human JCV belongs to the Polyomaviridae family, which also includes, the human BK virus (BKV) and the simian virus 40 (SV40). JCV is a non-enveloped virus with double-stranded DNA that forms mini chromosomes with cellular histones [13]. JCV and BKV are ubiquitous human viruses that infect 80% of the population worldwide [14]. These two viruses are thought to infect indi-

Correspondence to: Feryel Ksiaa, PhD. Department of Pathology, Farhat-Hached Hospital, Sousse 4000, Tunisia. Tel: +216 21 098 784, E-mail: feryel_ksia@yahoo.fr
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viduals in early childhood and establish life-long latency in the kidney.

JCV encodes a transforming gene, T antigen, which is believed to mediate the oncogenic potential of the virus. The JCV T antigen is a multifunctional protein and is able to bind and inactivate the tumor suppressor proteins p53 and pRb, leading to dysregulation of the cell cycle and permitting replication of cells with damaged chromosomes [13]. More importantly in the context of colorectal carcinogenesis, the JCV T antigen interacts with β-catenin, which can dysregulate the Wnt signaling pathway [15]. Moreover, JCV can bind to p53 and β-catenin and induce chromosomal instability in the colon cancer cell line RKO [16].

To the best of our knowledge, no previous study has investigated the presence of JCV in CRC in an African country. In the current investigation, we first determined the prevalence of JCV in a series of CRC and paired non-tumor colonic mucosa. We correlated our findings with the demographic and clinicopathological data. Secondly, we examined the immunohistochemical deregulation of some molecular pathways well characterized in CRC with regard to JCV infection. These included p53, an important tumor suppressor protein, β-catenin, proteins that serve key roles in the Wnt signaling pathway, and Ki-67 index used to measure proliferative activity of cancer cells. The current study was undertaken to improve our understanding of colorectal tumorigenesis in patients living in Tunisia.

Methods

Tumor characteristics

Archival tumor specimens in the Department of Pathology from patients treated for CRC at the Farhat-Hached Hospital, Tunisia, from 2005 to 2008 were included in this prospective study. The study material consisted of 105 colorectal tumors and 89 paired non tumor colonic mucosa samples. None of the patients had received preoperative neoadjuvant chemoradiation therapy. Written informed consent was obtained from all patients.

Data relating to cancer site, depth of invasion and nodal spread were obtained from pathological records. Tumors in the cecum, ascending colon and transverse colon were classified as right-sided and those in the descending colon and sigmoid were left-sided. Tumors were typed as adenocarcinomas or mucinous carcinomas according to the criteria of the World Health Organization [17]. Tumor differentiation (well, moderate or poor) was determined following the World Health Organization guidelines [17].

DNA isolation and β-globin amplification

Hematoxylin-eosin (H&E)-stained slides were reviewed to confirm the diagnosis. Formalin-fixed, paraffin-embedded tissue blocks were selected to include carcinoma and/or paired non-tumor colonic mucosa. Then 5 μm-thick sections from the selected blocks were collected into an Eppendorf tube containing lysis buffer. DNA was extracted as described in our previous study [7]. It is noteworthy that extreme caution was exercised to prevent any contamination; we used a dedicated microtome for this study, and a new microtome blade for each block.

In a separate room, the extracted tumor and normal DNA was first checked for 268-bp sequences of human β-globin to assess that each sample contained DNA of suitable quality for use in later PCR [18]. Samples of high-quality DNA were investigated for JCV detection.

JCV detection

The JCV was searched in CRC samples and paired non-tumor mucosa. Viral DNA detection was carried out by PCR using the primer sets PEP1/PEP2, which amplify a 173 bp sequence in the NH2-terminal region of the JCV T antigen [19]. Primer sequences, annealing temperatures and expected products size for JCV are listed in Table 1.

The PCR amplification was performed in the presence of 200 ng of DNA template, in a reaction mixture (25 μl) containing 1× PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each primer and 1 U of Taq DNA polymerase (Promega, Madison, USA).

PCR reactions were carried out on PTC 200™ DNA engine thermal cycler (MJ Research, Watertown, USA). The PCR conditions were as follows: first step of denaturation at 95°C for 10 min, followed by 35 cycles consisting of denaturation at 95°C for 1 min, specific annealing temperature for 1 min (Table 1), and extension at 72°C for 1 min. The reaction was finished with a 7 min extension step at 72°C.

All sets of PCR were conducted in parallel with negative and positive controls. Negative controls were reactions without adding DNA template. Positive controls were plasmid DNA samples containing cloned JCV (pBRJC-MAD-1) genome (kindly provided by Dr. Regis A. Vilchez, Baylor College of Medicine, Houston, Texas, USA). The negative controls were always the final samples in a series to verify that no contamination had occurred during the analysis.

PCR products were analyzed by electrophoresis on 2% agarose gels containing ethidium bromide. The electrophoretic pattern was photographed under ultraviolet light with the Gel Doc 2000 System (Bio-Rad, Marnes-la-Coquette, France). PCR experiments for each case were repeated in triplicate.

Immunohistochemical staining

Immunohistochemical analysis was performed on

Table 1. Primer sequences, annealing temperature and product size for polyomaviruses PCR assays

Target	*Oligonucleotide sequences (5'-3')*	*Annealing temperature*	*Product size*
β-Globin	Sense: CAACTTCATCCACGTTCACC	56°C	268 pb
	Anti-sense: GAAGAGCCAAGGACAGGTAC		
JCV	Sense: AGTCTTTAGGGTCTTCTACC	54°C	173 pb
	Anti-sense: GGTGCCAACCTATGGAACAG		

Table 2. Details of the antibodies used for immunohistochemical analysis in our study

Antigen	*Source*	*Dilution*	*Buffer*
Ki-67	DakoCytomation	1/40	Citrate pH 6.0
β-catenin	DakoCytomation	1/200	Citrate pH 6.0
p53	DakoCytomation	1/100	Citrate pH 6.0

4-μm tissue sections using a panel of commercially available antibodies listed in Table 2.

Immunostaining was conducted using a polymer-based method (EnVision-labeled Polymer; DakoCytomation, Glostrup, Denmark), following the manufacturers' instructions with slight modifications. Briefly, sections were deparaffinized with xylene for 2×10 min and rehydrated using a graded ethanol series, then treated with 3% H_2O_2 for 15 min to inhibit endogenous peroxidase followed by antigen retrieval under variable conditions specified in Table 2 with occasional interruption to avoid tissue degradation by excessive heat. After that, sections were incubated with the primary antibody for 1 hr at room temperature, followed by further incubation with EnVision-labeled polymer (DakoCytomation). The staining was developed by reaction with 3,3-diaminobenzidine substrate chromogen solution. The samples were then counterstained with hematoxylin, rinsed with ethanol, dried and visualized by light microscopy. Tissue samples to which no primary antibody had been added were used as negative controls. The immunostaining patterns (membranous, cytoplasmic or nuclear) were independently analyzed by two observers. For β-catenin and p53, a tumor was considered positive if more than 10% of the tumor cells exhibited nuclear immunoreactivity. In the case of Ki-67, high-level expression was defined if more than 30% of the tumor cells showed nuclear staining. Adjacent non-tumor epithelium was used as a negative internal control for β-catenin, p53 and Ki-67. The slides were read by pathologists unaware of the polyomaviruses status.

Statistics

Clinicopathological data and immunohistochemical findings were analyzed with regard to polyomaviruses status using x^2 test or Fisher's exact test as appropriate. To assess independent relations of polyomaviruses with number of variables, multivariate logistic regression analysis was performed with the Cox proportional hazards model. Statistical analyses were performed using a SPSS 13.0 statistical software program (SPSS Inc., Chicago, Ill, USA). All p values were two-tailed and the statistical significant threshold was set at 0.05.

Results

JCV Detection

The integrity of the extracted DNA was evaluated by performing β-globin DNA PCR. All samples showed adequate DNA quality (Figure 1a). Each case was analyzed for the presence of JCV.

JCV DNA has been searched by PCR in CRC and in non-tumor colonic mucosa samples. Figure 1 illustrates representative data depicting results from PCR experiments. JCV DNA has been detected in 58.1% (61/105) of CRC (Figure 1b) and in 14.6% (13/89) of paired non-tumor colonic mucosa samples (Figure 1c). The difference between the detection rates in tumor and paired non-tumor colonic mucosa was statistically significant (p=0.03).

Clinicopathological correlations

Comparison of the clinicopathological data with regard to JCV status in CRC is summarized in Table 3. The mean age of patients was 62 years (range 20-95). There were no statistically significant associations between the presence of JCV DNA and patient's age, gender, histological type, tumor location, depth of invasion and lymph node metastasis. However, tumor differentiation correlated significantly with the presence of JCV in CRC (p=0.03; Table 3). Indeed, JCV was found more

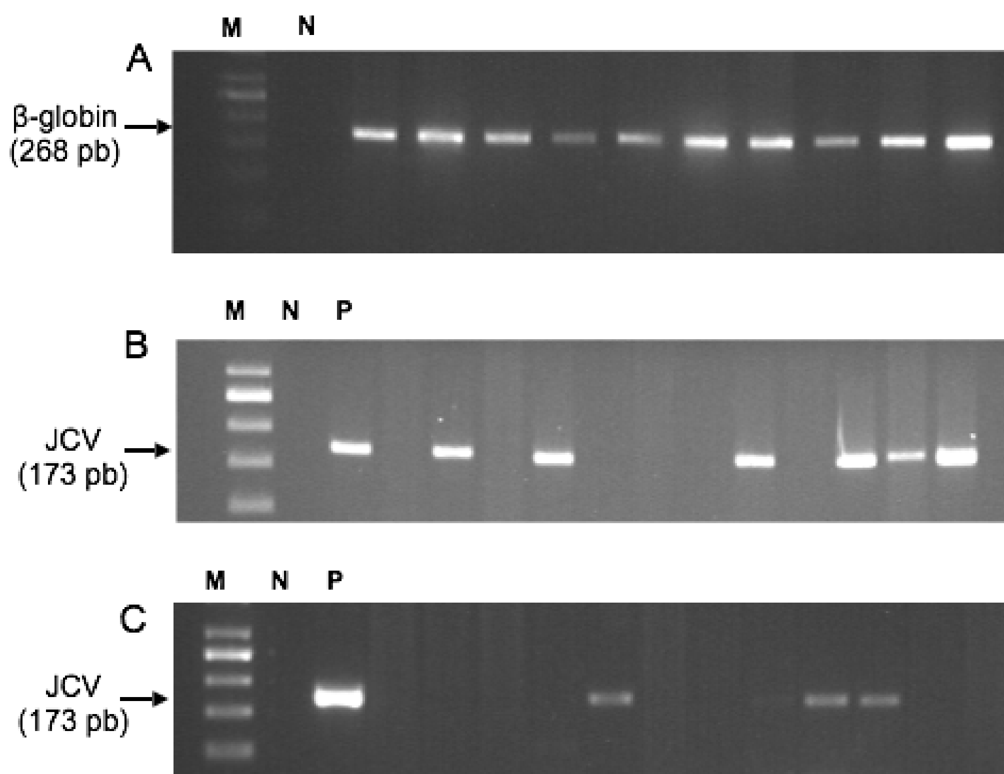

Figure 1. (A) β-globin was positive in all cases of CRC; **(B)** Detection of JCV T antigen sequences in CRC; representative examples of positive and negative JCV cases; **(C)** Detection of JCV T antigen sequences in non-tumor colonic mucosa; representative examples of positive and negative JCV cases. M: molecular weight (50 pb DNA ladder), N: negative control (no DNA template), P: positive control for JCV (plasmid pBRJC-MAD-1,containing cloned JCV genome). The sizes of the amplified regions are shown next to each of the blots.

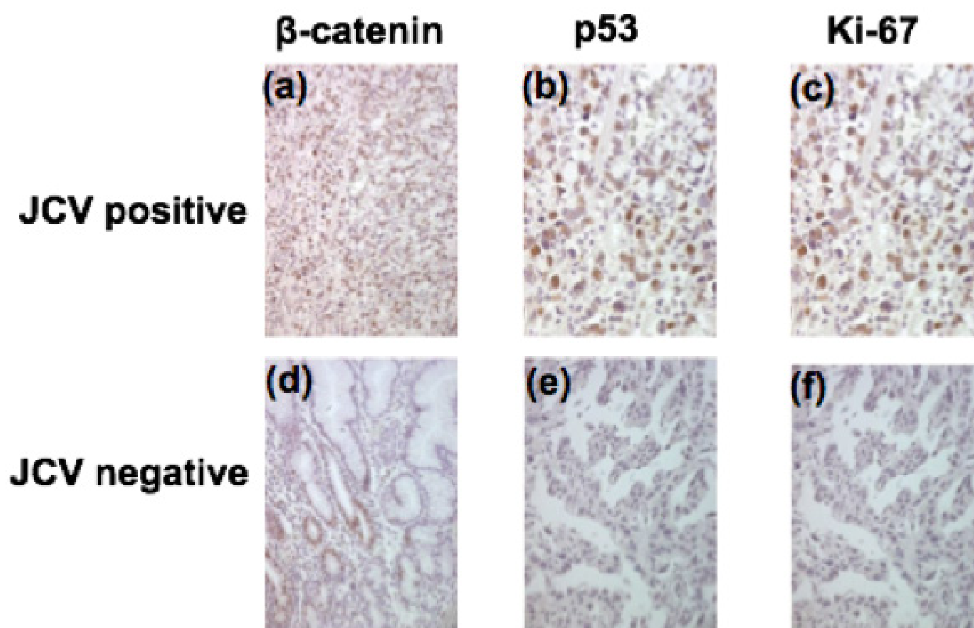

Figure 2. Immunohistochemical staining of CRC cases. Representative examples of the protein expression of b-catenin (a,d), p53 (b,e) and Ki-67 (c,f) in JC positive (a,b and c) and JC negative (d,e and f) CRC (original magnification x200).

Table 3. Clinicopathological features of JCV positive and JCV negative colorectal carcinomas

Variables	*Total*	*JCV positive cases*	*JCV negative cases*	*p value*
Number of cases	105	61	44	
Gender				
Male	60	37	23	0.39
Female	45	24	21	
Age group (years)*				
≤ 60	37	17	20	0.17
> 60	62	40	22	
Tumor location				
Right colon	37	20	17	0.33
Left colon	49	32	17	
Rectum	19	9	10	
Tumor histology**				
Adenocarcinoma	97	56	41	1
Mucinous carcinoma	8	5	3	
Tumor differentiation§				
Well	66	44	22	0.03
Moderate	28	14	14	
Poor	11	3	8	
Tumor invasion				
pT1 / pT2	8	4	4	0.71
pT3 /pT4	97	57	40	
Lymph node metastasis§				
Positive	42	20	22	0.4
Negative	60	39	21	

*Data is missing for 6 patients, **According to WHO classification, §Data is missing for 3 patients

Table 4. Association between immunohistochemical staining patterns of Ki-67, β-catenin, and p53 and JCV status in colorectal carcinomas

Variables	*Total*	*JCV positive cases*	*JCV negative cases*	*p value*
Number of cases	105	61	44	
Ki-67*				
High	62	38	24	0.42
Low	43	23	20	
β-catenin**				
Positive	42	31	11	0.008
Negative	63	30	33	
p53**				
Positive	55	41	14	0.0001
Negative	50	20	30	

*Cases were considered with high expression if >30% of tumor cells showed nuclear staining, **Cases were considered positive if >10% of tumor cells showed nuclear staining

frequently in tumors of well differentiated morphology (in 44 of 66 cases, 66.7%), in contrast to tumors of poor differentiated type (in 3 of 11 cases, 27.3%) which did not harbor JCV.

Immunohistochemical findings and correlation analysis

Immunohistochemical analysis was performed in all cases and interpreted independently by the experiment pathologists (MT,SZ). Representative stainings for β-catenin, p53 and Ki-67 expressions are shown in Figure 2.

β-catenin staining was detected in 40% (42/105) of CRC cases. These tumors typically

Table 5. Multivariate logistic regression analysis was carried out on all variables that were found significantly correlated with the presence of JCV on univariate analysis

Variables associated with JCV status	*JCV positive cases*	*JCV negative cases*	*Multivariate OR (95% CI)*	*p value*
Tumor differentiation				
Well/Moderate	58	36	0.2 (0.44-0.96)	0.04
Poor	3	8		
β-catenin				
Positive	31	11	2.47 (0.99-6.14)	0.05
Negative	30	33		
p53				
Positive	41	14	4.55 (1.87-11.04)	0.001
Negative	20	30		

OR: odds ratio, CI: confidence interval

exhibited strong nuclear immunoreactivity for β-catenin (Figure 2a).

Strong nuclear staining for p53 in tumor cells was detected in 52.4% (55/105) of CRC cases. In the remaining cases, the staining was either focally positive or completely negative (Figure 2b). In non-tumor colorectal mucosa there was complete absence of nuclear expression for p53. Ki-67 was detected in 59% (62/105) of CRC cases (Figure 2c).

Immunohistochemical findings were also correlated with the presence of JCV in CRC and are summarized in Table 4. The presence of JCV was significantly associated with β-catenin and p53 ($p=0.008$; $p=0.0001$, respectively). However, we did not observe a significant difference between the two groups with regard to Ki-67 expression (JCV positive vs JCV negative CRC).

Multivariate analysis

We performed multivariate logistic regression analysis to assess which variables were independently associated with JCV infection (Table 5). The results showed that tumor differentiation, β-catenin and p53 accumulation were independent parameters significantly associated with the presence of JCV in CRC ($p=0.04$; $p=0.05$; $p=0.001$, respectively).

Discussion

CRC develops through a stepwise progression from normal colonic mucosa to adenomas and to adenocarcinomas of the colorectum. The tumor progression is accompanied by multiple genetic and epigenetic alterations. Most CRCs are sporadic in their origin and are associated with different risk factors.

Infectious agents contribute to a significant fraction of human malignancies including CRC. Several studies have reported the presence of JCV and have even suggested its involvement in colorectal carcinogenesis through multiple mechanisms of genetic and epigenetic instability [20,21].

To the best of our knowledge, this study is the first analysis of JCV DNA in CRC from North African patients. In the present study, JCV DNA was detected in about 58% of human CRCs, which was in intermediate rate as compared with previous studies. It is noteworthy that the detection rate of JCV in the tumor cases was significantly higher than that in paired non-tumor colon mucosa (58.1 vs 13.4%, $p<0.05$), suggesting a role of JCV in the development of CRC.

JCV is a ubiquitous virus, well adapted to humans. JCV infection is subclinical, but in immunocompromised individuals may be associated with neural disease as progressive multifocal leukoencephalopathy (PML) and some brain tumors [22]. JCV DNA sequences have also been found in colon and rectum cancers. Indeed, all but 4 of the published studies of JCV in CRC report positive findings in tumors and far fewer positives in controls.

The work of Laghi et al. was the first analysis examining JCV in colorectal neoplasm [3]. The authors have found JCV DNA sequences in both the mucosa of normal human colons and CRC, but the viral copy number was significantly higher in the cancer cells than in normal colon tissues. Accordingly, Laghi et al. have suggested that JCV can be a potential risk factor for CRC [3]. Subsequent analyses have confirmed the presence of JCV sequences in CRC at varying frequencies, ranging from 26 to 89% of JCV positive cases. In American patients, JCV DNA was frequently demonstrated

in as much as 80% of CRC tissues [23,24]. Similar results have been reported by Italian authors [9], who found JCV DNA in 89% of CRC tissues. Two Asian studies have identified JCV in 26.1 and 84.6%, respectively of CRC tissues [8,11]. In Chinese CRC patients, JCV DNA was detected in 49.6% of the cases [25]. In contrast, 4 studies have shown that no detectable JCV DNA was present in colorectal tissues indicating no association between JCV and colon neoplasia [26-29].

Several reasons account for the lack of agreement, concerning the detection rates of JCV sequences in CRC throughout studies, which include potential contamination during sample collection, process in DNA extraction or technical procedure. Indeed, several techniques have been used for the detection of JCV sequences, for instance nested PCR, quantitative real-time PCR, southern blot, in situ hybridization and immunohistochemistry. This technical disparity in both sensitivity and targets makes the comparison among the results of relevant studies complicated or difficult. Overall, the positive findings with all methodologies strengthen for the presence of JCV in some CRCs.

As a second purpose for this investigation, we have examined the pathobiological features of JCV-related CRC from Tunisian patients. With regard to clinicopathological data, we did not find correlation between the presence of JCV and demographic or pathological data such as patient age and gender, clinical stage and site of tumor, a fact which was consistent with previous studies [8,10,25]. Nosho et al. [30] have demonstrated that JCV T antigen expression was inversely associated with proximal location, high grade, and mucinous component. In the present study, we have found that JCV status was significantly correlated with tumor differentiation (p=0.03). Indeed, we have found that JCV was more frequently detected in tumors of well differentiated morphology (in 44 of 66 cases; 66.7%), in contrast to tumor of poor differentiated type (in 3 of 11 cases; 27.3%), which did not harbor JCV. This observation seems obvious if we take into account that colon cancers that arise in the context of hereditary nonpolyposis colon cancer (HNPCC) are often poorly differentiated [31-33].

One of the biological implications of the polyomaviruses infection would be the accompanying interaction with some well-characterized tumor suppressor genes, including p53. In our study, p53 accumulation was detected in 55/105 (50%) of the cases, which is in keeping with the current knowledge of the high frequency of p53 alteration in sporadic CRC. As expected, a significant correlation was found between p53 accumulation and the presence of JCV in CRC (p=0.0001). This observation supports a role for JCV in the inactivation of p53 in human CRC. Similar results have been previously documented in CRC [30]. The dysregulation of the p53 pathways can induce unchecked cellular replication, and lead to chromosomal instability, which is common in sporadic CRC.

Another target of JCV is β-catenin. Gan and Khalili [15] have found that JCV T antigen increases the stability of β-catenin, leading to its nuclear accumulation. In the nucleus, β-catenin acts as a trans-activating factor for the transcription factor Tcf-4 that regulates the transcription of a number of downstream target genes that are believed to be the mediators of neoplastic transformation [34]. Moreover, Bhattacharyya et al. [35] have reported that JCV T antigen and β-catenin synergistically activate Rac1, an event that can trigger several oncogenic factors [35]. In our study, nuclear immunostaining for β-catenin was found in 40% (42/105) of the CRC cases. Moreover, we have found that JCV positive CRCs display more frequently β-catenin accumulation in tumor than that in negative cases (65 vs 40%; p=0.008). Our findings are in accordance with those reported by other investigators who examined β-catenin and JCV in CRC [30]. Together with our findings, we presume that the dual presence of JCV and β-catenin accumulation is a crucial step in the activation of the Wnt signaling pathway that leads to tumorigenesis.

Ki-67 antigen is expressed by cells in the G1, G2, S and M phase of the cell cycle and is commonly used as a marker of proliferative activity. In the current study, Ki-67 was observed in 59% (61/105) of the CRC cases. However, analyses have not shown a significant correlation between the presence of JCV DNA and the expression of Ki-67 in CRC.

In multivariate analysis, we have found that only p53 expression is independently associated with JCV DNA, but not β-catenin or Ki-67. Our data support the hypothesis that JCV DNA may affect the p53 expression rather than β-catenin in CRC.

In summary, the data presented in this report extend previous observations in CRC. JCV DNA was detected in a significantly higher percentage in CRCs compared to paired non-tumor colon mucosa, suggesting thus its role in colorectal carcinogenesis. By examining some well-established different tumorigenic pathways for CRC, we demonstrated that JCV may affect p53 expression and β-catenin in Tunisian patients with CRC.

References

1. Parkin DM, Bray F, Ferlay J et al. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74-108.
2. Winawer SJ. The multidisciplinary management of gastrointestinal cancer. Colorectal cancer screening. Best Pract Res Clin Gastroenterol 2007;21:1031-1048.
3. 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.
4. Boland CR, Luciani MG, Gasche C et al. Infection, inflammation, and gastrointestinal cancer. Gut 2005;54:1321-1331.
5. Shin SK, Li MS, Fuerst F et al. Oncogenic T-antigen of JC virus is present frequently in human gastric cancers. Cancer 2006;107:481-488.
6. Murai Y, Zheng HC, Abdel Aziz HO et al. High JC virus load in gastric cancer and adjacent non-cancerous mucosa. Cancer Sc 2007;98:25-31.
7. Ksiâa F, Ziadi S, Mokni M et al. The presence of JC virus in gastric carcinomas correlates with patient's age, intestinal histological type and aberrant methylation of tumor suppressor genes. Mod Pathol 2010;23:522-530.
8. 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.
9. 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.
10. Theodoropoulos G, Panoussopoulos D, Papaconstantinou I et al. Assessment of JC polyoma virus in colon neoplasms. Dis Colon Rectum 2005;48:86-91.
11. Lin PY, Fung CY, Chang FP et al. Prevalence and genotype identification of human JC virus in colon cancer in Taiwan. J Med Virology 2008;80:1828-1834.
12. Vilkin A, Ronen Z, Levi Z et al. Presence of JC virus DNA in the tumor tissue and normal mucosa of patients with sporadic colorectal cancer (CRC) or with positive family history and Bethesda criteria. Dig Dis Sci 2012;57:79-84.
13. Khalili K, Del Valle L, Otte J et al. Human neurotropic polyomavirus, JCV, and its role in carcinogenesis. Oncogene 2003;22:5181-5191.
14. Stolt A, Sasnauskas K, Koskela P et al. Seroepidemiology of the human polyomaviruses. J Gen Virol 2003;84:1499-1504.
15. Gan DD, Khalili K. Interaction between JCV large T-antigen and beta-catenin. Oncogene 2004;23:483-490.
16. Gan DD, Reiss K, Carrill T et al. Involvement of Wnt signaling pathway in murine medulloblastoma induced by human neurotropic JC virus. Oncogene 2001;20:4864-4870.
17. Morson BC, Sobin LH. Histological typing of intestinal tumors. International Histological Classification of Tumors. World Health Organization; 1976.
18. Saiki RK, Scharf S, Faloona F. Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 1985;20:1350-1354.
19. Arthur RR, Dagostin S, Shah KV. Detection of BK virus and JC virus in urine and brain tissue by the polymerase chain reaction. J Clin Microbiol 1989;27:1174-1179.
20. Goel A, Li MS, Nagasaka T et al. Association of JC virus T-antigen expression with the methylator phenotype in sporadic colorectal cancers. Gastroenterology 2006;130:1950-1961.
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. White MK, Gordon J, Reiss K et al. Human polyomaviruses and brain tumors. Brain Res Brain Res Rev 2005;50:69-85.
23. 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.
24. Jung WT, Li MS, Goel A. JC virus T-antigen expression in sporadic adenomatous polyps of the colon. Cancer 2008;112:1028-1036.
25. Mou X, Chen L, Liu F et al. Prevalence of JC virus in Chinese patients with colorectal cancer. PLoS On 20127:e35900.
26. Hernández Losa J, Fernandez-Soria V, Parada C et al. JC virus and human colon carcinoma: an intriguing and inconclusive association. Gastroenterology 2003;124:268-269.
27. Newcomb PA, Bush AC, Stoner GL. No evidence of an association of JC virus and colon neoplasia. Cancer Epidemiol Biomarkers Prev 2004;13:662-666.
28. Giuliani L, Ronci C, Bonifacio D et al. Detection of oncogenic DNA viruses in colorectal cancer. Anticancer Res 2008;28:1405-1410.
29. Chiaravalli AM, Longhi E, Vigetti D et al. Gastrointestinal cancers reactive for the PAb416 antibody against JCV/SV40 T-Ag lack JCV DNA sequences while showing a distinctive pathologic profile. J Clin Pathol 2013;66:44-49.
30. Nosho K, Shima K, Kure S et al. JC virus T-antigen in colorectal cancer is associated with p53 expression and chromosomal instability, independent of CpG island methylator phenotype. Neoplasia 2009;11:87-95.
31. Alexander J, Watanabe T, Wu TT et al. Hamilton SR: Histopathological identification of colon cancer with microsatellite instability. Am J Pathol 2001;158:527-535.
32. Jass JR. Classification of colorectal cancer based on correlation of clinical, morphological and molecular features. Histopathology 2007;50:113-130.
33. Kim H, Jen J, Vogelstein B. Clinical and pathological characteristics of sporadic colorectal carcinomas with DNA replication errors in microsatellite sequences. Am J Pathol 1994;145:148-161.
34. Gonçalves V, Matos P, Jordan P. The betacatenin/TCF4 pathway modifies alternative splicing through modulation of SRp20 expression. RNA 2008;14:2538-2549.
35. Bhattacharyya R, Noch EK, Khalili K. A novel role of Rac1 GTPase in JCV T-antigen-mediated beta-catenin stabilization. Oncogene 2007;26:7628-7636.