Systematic analysis of human oncogenic viruses in colon cancer revealed EBV latency in lymphoid infiltrates

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Abstract

Background: Environmental factors may play a role in colon cancer. In this view, several studies investigated tumor samples for the presence of various viral DNA with conflicting results.

Findings: We undertook a systematic DNA analysis of 44 consecutive, prospectively collected primary tumor samples by real time and qualitative PCR for viruses of known or potential oncogenic role in humans, including polyomavirus (JCV, BKV, Merkel cell polyomavirus), HPV, HTLV, HHV-8 and EBV. Negative controls consisted of surgical resection margins. No evidence of genomic DNA fragments from tested virus were detected, except for EBV, which was found in a significant portion of tumors (23/44, 52%). Real-time PCR showed that EBV DNA was present at a highly variable content (median 258 copies in 10⁵ cells, range 15–4837). Presence of EBV DNA had a trend to be associated with high lymphocyte infiltration (p = 0.06, χ² test), and in situ hybridization with EBER1-2 probes revealed latency in a fraction of these lymphoid cells, with just a few scattered plasma cells positive for BZLF-1, an immediate early protein expressed during lytic replication. LMP-1 expression was undetectable by immunohistochemistry.

Conclusions: These results argue against a significant involvement of the tested oncogenic viruses in established colon cancer.

Keywords: Colon cancer, Oncogenic viruses, EBV

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samples from various pathologies, including tumor samples, and plasmid DNA. Amplification methods consisted of real-time (EBV, JCV, BKV, HTLV and HHV-8) [8-11] and qualitative PCR (MCPyV, HPV) [12,13]. Given the very controversial evidence concerning JCV, which was found from 0% to nearly 80% of the tumor samples tested in the literature [6,14-18], JCV was additionally investigated by the specific qualitative PCR described in positive reports [18] and that employed primers spanning a different portion of the large T antigen. Sensitivity for JCV real-time PCR assay was 1 viral copy in 10^5 cells. Experiments showed that no genomic DNA fragments from the tested viruses were detectable, with the notable exception of EBV that was positive in a consistent portion of cases (23/44 samples, 52%). Tumors associated with EBV positivity had EBV negative surgical resection margins. EBV DNA content was highly variable in tumors (median 258 viral copies in 10^5 cells, range 15–4837), and EBV had a trend to be observed in tumors displaying high lymphoid infiltration (p = 0.06, χ² test). In situ hybridization analyses for the detection of EBER1-2 RNAs (PNA Probe/FITC and ISH detection kit, Dako, Denmark) demonstrated virus latency in a variable fraction of infiltrating non-neoplastic lymphoid cells, which could reach 20% in a few cases (Figure 1), but
not in tumor cells. On the same line, immunohistochemistry to EBNA-1 nuclear protein (Fitzgerald Industries International – USA, clone M5042521), an antigen that is expressed during both latent and lytic phases, failed to show positive nuclei in neoplastic cells. Immunohistochemistry for LMP-1, a membrane associated protein involved in activation, was also negative, while lytic cycle was detected via expression of the immediate early protein BZLF1 (ZEBrA antigen, LSBio, clone LS-C102904) in a few scattered plasma cells (Figure 2). These findings essentially confirm latency of EBV in lymphoid infiltrates. The presence of EBV was not associated with the other tumor or clinical parameters studied including age, stage, tumor localization, or the presence of necrosis.

In conclusion, we performed a PCR-based systematic analysis for potential oncogenic viruses in clinically established colon cancer and EBV was the only one detected. The viral infection was restricted to latency in the lymphoid infiltrate, in line with the few reports that used in situ hybridization with EBER probes [19,20], while we noted an association with high lymphocyte infiltration, a well-recognized favorable prognostic parameter. EBV positivity in lymphoid infiltrates may occasionally be extensive (Figure 1), much higher than expected on the numbers of circulating EBV positive memory B-lymphocytes in normal individuals, and it might be of interest to study this phenomenon in specifically designed studies. In summary, the present analysis does not support a significant involvement of the tested viruses in manifest colon cancer, and suggests that new approaches [21] capable of detecting known and unknown non-human sequences should be investigated to study the role of infectious agents in colon cancer.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
VP and FB designed and coordinated the study and wrote the manuscript; MR collected data on patients and contributed to elaboration and interpretation of results; LF and SP performed PCR experiments; ED and RR carried out in situ hybridization and immunohistochemistry analyses; OL performed histological evaluation; AV performed primary tumor collection, histological evaluation and contributed to study design and interpretation of results, reviewed the manuscript; SB collected data on patients and critically reviewed the manuscript; MP critically reviewed the manuscript; PP contributed to study coordination and critically reviewed the manuscript. All authors read and approved the final manuscript.

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References
1. Bergonzini V, Salata C, Calistri A, Parolin C, Palù G: View and review on viral oncology research, Infect Agent Cancer 2010, 5:11.
2. de Martel C, Ferlay J, Franceschi S, Vignat J, Bray F, Forman D, Plummer M: Global burden of cancers attributable to infections in 2008: a review and synthetic analysis, Lancet Oncol 2012, 13(6):607–615.
3. Perfetti V, Ricotti M, Buonaguro F, Tirelli U, Pedrazzoli P: An overview of viral oncology in Italy - report from the Pavia meeting on solid tumors. Infect Agent Cancer 2012, 7:23.
4. Grady WM, Careythers JM: Genomic and epigenetic instability in colorectal cancer pathogenesis, Gastroenterology 2008, 134(5):1079–1090.
5. van Engeland M, Derks S, Smits KM, Meijer GA, Herman JG: Colorectal cancer epigenetics: complex simplicity, J Clin Oncol 2011, 29(10):1382–1391.
6. Hasan N, Pollack A, Cho I: Infectious causes of colorectal cancer, Infect Dis Clin North Am 2010, 24(4):1019–1039.
7. Travaglione S, Fabbri A, Fiorentini C: The Rho-activating CNF1 toxin from pathogenic E. coli: a risk factor for human cancer development? Infect Agent Cancer 2008, 3:22.
8. Baldanti F, Gatti M, Furione M, Paduccion S, Tirelli C, Comoli P, Merli P, Locatelli F: Kinetics of Epstein-Barr virus DNA load in different blood compartments of pediatric recipients of T-cell-depleted HLA-haploidentical stem cell transplantation. J Clin Microbiol 2008, 46(11):3672–3677.
9. Moens B, López G, Adau V, González E, Keremans L, Clark D, Verdonck K, Gotuzzo E, Vanham G, Cassar O, Gessain A, Vandamme AM, van Dooren S: Development and validation of a multiplex real-time PCR assay for simultaneous genotyping and human T-lymphotropic virus type 1, 2, and 3 proviral load determination. J Clin Microbiol 2009, 47(11):3682–3691.
10. Watzinger F, Suda M, Preunier S, Baumgartinger E, Eberle K, Baskova L, Niesters HG, lawnitska A, lion T: Real-time quantitative PCR assays for detection and monitoring of pathogenic human viruses in immunosuppressed pediatric patients. J Clin Microbiol 2004, 42(1):5189–5198.
11. Saundh BK, Tibble S, Baker R, Sasnauskas K, Harris M, Hale A: Different patterns of BK and JC polyomavirus reactivation following renal transplantation. J Clin Pathol 2010, 63(8):714–718.
12. Pancaldi C, Corazzari V, Maniero S, Mazzone E, Comar M, Martini F, Tognon M: Merkel cell polyomavirus DNA sequences in the buffy coats of healthy blood donors. Blood 2011, 117(26):7099–7101.
13. Qu W, Jiang G, Cruz Y, Chang CJ, Ho GY, Klein RS, Burk RD: PCR detection of human papillomavirus: comparison between MY09/MY11 and GP+9/GP+) primer systems. J Clin Microbiol 1997, 35(8):1304–1310.
14. Newcomb PA, Bush AC, Stoner GL, Lampe JW, Potter JD, Bigler J: No evidence of an association of JC virus and colon neoplasia. Cancer Epidemiol Biomarkers Prev 2004, 13(4):662–666.
15. Mitelli V, Trevisan M, Squarzon L, Biasol MA, Rugge M, Mitelli C, Palù G, Barzon L: Investigation on the presence of polyomavirus, herpesvirus, and papillomavirus sequences in colorectal neoplasms and their association with cancer. Int J Cancer 2004, 114(10):2501–2503.
16. Theodoropoulos G, Panousiopoulos D, Papanicolaou C, Gazaris M, Perdiki M, Brinas J, Lazaris AC: Assessment of JC polyoma virus in colon neoplasms. Dis Colon Rectum 2005, 48(1):86–91.
17. Chiaravalli AM, Longhi E, Vigetti D, De Stefano FI, Deleonibus S, Capella C, Solcia E, Paravincini C: Gastrointestinal cancers reactive for the PAb416 antibody against JC virus/ SV40 T-Ag lack JCV DNA sequences while showing a distinctive pathologic profile. J Clin Pathol 2010, 66:44–49.
18. Laghi L, Randolf AH, Chauhan DP, Marra G, Major EO, Neel JV, Boland CR: JC virus DNA is present in the mucosa of the human colon and in colorectal cancers. Proc Natl Acad Sci U S A 1999, 96(13):7484–7489.
19. Liu HY, Ding YQ, Li X, Yao KT: Investigation of Epstein-Barr virus in Chinese colorectal tumors. World J Gastroenterol 2003, 9(11):2464–2468.
20. Wong NA, Herbst H, Herrmann K, Kirchner T, Krajewski AS, Moorghen M, Niedobitek F, Rooney N, Shepherd NA, Niedobitek G. Epstein-Barr virus infection in colorectal neoplasms associated with inflammatory bowel disease: detection of the virus in lymphomas but not in adenocarcinomas. *J Pathol* 2003, 201(2):312–318.

21. Barzon L, Lavezzo E, Costanzi G, Franchin E, Toppo S, Palù G. Next-generation sequencing technologies in diagnostic virology. *J Clin Virol* 2013, 58(2):346–350.

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