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Merkel cell polyomavirus is not detected in prostate cancers, surrounding stroma, or benign prostate controls

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Abstract

Background—Merkel cell polyomavirus (MCV or MCPyV) is a recently-discovered human polyomavirus that is implicated in the pathogenesis of Merkel cell carcinoma (MCC). Although the transmission route for MCV is not yet known, other polyomaviruses, such as BKV, cause non-malignant pathology in the urinary tract. Like MCC, prostate cancer predominantly affects the elderly. Furthermore, prostate cancers and premalignant precursors exhibit chronic inflammation, which suggests a possible infectious involvement. We therefore examined whether MCV might participate in the pathogenesis of prostate cancer.

Objective—To determine the presence of MCV RNA in prostate cancer and surrounding stroma or normal prostate tissue.

Study Design—RNA was extracted from 28 patient-matched cancerous and 28 benign prostate epithelial samples, and 6 additional cancer-adjacent stromal samples. All tissues were laser-capture micro-dissected. DNA and RNA from a sequence-verified MCV-containing MCC tumor served as a positive control. Quantitative reverse-transcription PCR was used to assess the presence or absence of MCV T antigen transcript.

Results—No MCV T antigen was detected in prostate carcinomas, patient-matched benign samples, or tumor-adjacent stroma, with appropriate sensitivity of the assay demonstrated by an MCC tumor.

Conclusions—MCV infection appears unlikely to be a significant factor in prostate carcinogenesis and there is no evidence of the prostate serving as a reservoir for MCV.

Keywords

Merkel cell; prostate cancer; polyomavirus; MCV; MCPyV

1. Background

Prostate cancer is the most common cancer affecting men living in North America and Europe. Commonly associated with a chronic inflammatory condition called proliferative inflammatory atrophy, prostate cancer has been suspected of having an infectious contribution to its etiology¹. Two viruses have been recently associated with prostate cancer, but a causal role for these viruses has not been established. The XMRV gammaretrovirus is a virus that is more commonly found in the stroma of cancerous prostates than in normal controls, however, it is

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Conflict of interest

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not often detectable in prostatic epithelium^{2–4}. A recent study suggests that epithelial BK polyomavirus infection may serve as a cofactor in the development of some prostate cancers⁵.

Merkel cell carcinoma (MCC) is an increasingly common skin cancer of the neuroendocrine Merkel cell. Like prostate cancer, MCC predominately affects the elderly and the median age at diagnosis is similar: 75 years for prostate cancer and 69–74 years for MCC^{6–8}. Recently, a new polyomavirus, Merkel cell polyomavirus (MCV or MCPyV), was discovered in MCC tumors⁹. This virus is strongly associated with MCC^{9–11} and appears to contribute to causation.

Like others in the *Polyomaviridae* family, MCV is expected to mediate tumorigenesis via expression of the oncogenic tumor (T) antigens. In particular, T antigen expression is thought to be important for MCC tumor development because genome-stabilizing mutations in the MCV T antigen are found in MCC tumors¹². These mutations differ between MCC tumors, but all produce a truncated, replication-deficient T antigen, that suggests selective pressures have been placed upon the virus by the host cells. The route of MCV transmission has not yet been established, but some other polyomaviruses, like BK, are transmitted via the urinary tract. If MCV is transmitted similarly, it is conceivable that prostate cancers would be exposed to this cancer-associated virus.

2. Objectives

In order to determine whether MCV might contribute to the carcinogenesis of prostate cancer, we analyzed 28 cancerous epithelia, 28 patient-matched non-cancerous epithelia, and 6 additional stromal samples from prostate tumors for the presence or absence of MCV T antigen RNA.

3. Study design

3.1 Tissue Sampling and RNA Isolation

All materials were acquired and used in conformity with the Institutional Review Board-approved protocols at the University of Washington and the Fred Hutchison Cancer Research Center. Prostate tissues were collected as previously described¹³. In brief, 28 radical prostatectomy samples were selected from patients who did not receive treatment prior to surgery. Stromal cells were collected from tissue samples acquired via ultrasound guided needle biopsy from six patients with localized prostate cancer. All tissues were snap-frozen in liquid nitrogen and stored at –80C prior to use to prevent RNA degradation. Prostate stromal tissue, benign and cancerous epithelium were captured via laser-capture microdissection (LCM) as described¹³. Approximately 5000 cells (benign and cancerous epithelia) and 3000 cells (stromal) were collected per sample. Total RNA was collected using the Picopure RNA Isolation kit (Arcturus) according to the manufacturer's instruction. Extracted RNA underwent two rounds of linear amplification using the MessageAMP aRNA kit (Ambion).

A Merkel cell carcinoma tumor previously described to contain ample MCV DNA was used as a positive control¹⁰. Briefly, a piece of discarded tumor histologically verified to be >90% tumor cells was placed in RNAlater (Ambion) <5 minutes after excision. RNA and DNA were extracted simultaneously using the AllPrep DNA/RNA mini kit (Qiagen).

3.2 Reverse Transcription and Quantitative Real-Time PCR (qRT-PCR)

Prostate cDNA was synthesized from 1ug of amplified RNA using 2ug of random hexamers for reverse-priming and 1ug (200 units) of SuperScriptII (Invitrogen). Quantitative PCR were done in triplicate, using ~5ng of cDNA, 0.75 umol/L of each primer, and 5ul of 2x SYBR Green PCR Master Mix (Applied Biosystems) in a 10ul reaction volume. Reactions were

analyzed on an Applied Biosystems 7900HT sequence detector using the following cycling conditions: 50 degrees for 2 minutes, 95 degrees for 10 minutes, followed by 40 cycles of 95 degrees for 15 seconds and 60 degrees for 1 minute. A water negative control did not produce significant reaction products and a human male lymphocyte pooled DNA control did not produce T antigen products. All non-water samples produced a positive signal with the control gene (RPL13a). Positive control reactions for the MCV T antigen with MCV positive MCC cDNA and genomic DNA produced a significant signal. The sequences of the primers used in this study are as follows^{10,14}: 5'-CCTGGAGGAGAAGAGGAAAGAGA-3' (Rpl13a forward), 5'-TTGAGGACCTCTGTGTATTTGTCA-3' (Rpl13a reverse), 5'-GCAAGCTTTTGGAGATTGCT-3' (MCV T antigen forward), 5'-TCCAAAGGGTGTTCATTCC-3' (MCV T antigen reverse). MCV primers have no significant similarity to the BKV genome (BLAST; <http://blast.ncbi.nlm.nih.gov/>).

4. Results

In this study, cDNA from a total of 28 cancerous prostate epithelia, 28 patient-matched benign epithelia, and six tumor-adjacent stromal samples were tested for MCV infection by qRT-PCR for MCV T antigen RNA. Genomic DNA and cDNA from an MCV-positive Merkel cell carcinoma were used as T antigen-positive controls. PCR for ribosomal protein L13a was measured to confirm successful PCR and was positive in all samples. The MCV T antigen was detected only in the MCV-positive MCC. There was no significant measurement of MCV T antigen PCR product in the negative controls or the cDNA samples from benign prostate epithelium, cancerous prostate epithelium, or prostate stroma.

5. Conclusions

Given the similar age distribution of MCC patients and prostate cancer patients, association of prostate cancer with inflammation, and natural history of a related virus involving the prostate (BKV), we investigated whether MCV might also contribute to prostate cancer. We were unable to detect MCV T antigen expression in the epithelia of prostate tumors or in normal prostatic epithelia from the same patients. XMRV, a gammaretrovirus associated with prostate cancer, is found not in the cancerous epithelia but instead in surrounding stroma². To test whether MCV might exhibit a similar localization, we tested stroma from 6 additional prostate tumors, and did not detect viral RNA in these tissues. Although we cannot rule out a potential role for MCV in prostate tumor initiation, these findings strongly suggest MCV does not play an ongoing role in prostate cancer maintenance and that the prostate is not a significant reservoir of ongoing infection in non-immune suppressed individuals.

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