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RAPID COMMUNICATION

No evidence for a role of xenotropic murine leukaemia virus-related virus and BK virus in prostate cancer of German patients

Baki Akgül · David Pfister · Ruth Knüchel · Axel Heidenreich · Ulrike Wieland · Herbert Pfister

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Abstract Prostate cancer is one of the most prevalent types of cancer in men. Controversial data exist concerning the role of BKPyV and the xenotropic murine leukaemia virus-related gammaretrovirus (XMRV) in prostate cancer development. We therefore assessed the association between prostate cancer and viral infections. We could detect BKPyV in only 1 out of 85 prostate cancer samples, whereas none of the tissue samples showed evidence for XMRV positivity. Lack of detection of BKPyV and XMRV in prostate cancer tissues suggests that these viruses do not play a role in the pathogenesis of this type of cancer.

Keywords Prostate cancer · Viral infection · BKPyV · XMRV

Introduction

Prostate cancer is one of the most prevalent types of cancer in men. In Europe, prostate cancer is responsible for approximately 87,000 deaths per year [1]. Sporadic (non-familial) prostate cancer is the most common form of prostate cancer (80–90%), and its incidence increases with age. Well-known factors contributing to the risk of prostate cancer are androgens and environmental and genetic factors [2]. Epidemiological studies designed to describe viral infections as risk factor for prostate cancer development are still inconclusive [3–5].

BKPyV, a member of the polyomavirus family, is widely distributed in the human population. Following primary infection, the virus disseminates and establishes a persistent infection, predominantly in the kidney and urinary tract. In immunosuppressed patients, BKPyV can become reactivated and cause haemorrhagic cystitis and polyomavirus-associated nephropathy [6]. Since BKPyV possesses potentially oncogenic properties, tumours of the genitourinary tract have been proposed as candidates for an association with BKPyV. In the literature, there are reports on a positive correlation of BKPyV DNA with prostate carcinomas [7–9]. A previous US study on 30 prostate cancer biopsies, however, did not provide evidence for a pathogenic role of BKPyV in prostate cancer development [10]. In line with this report, a seroepidemiologic study conducted on 31 patients did also not support a relationship between BKPyV infection and an increased risk of prostate cancer [11].

More recently, a new gammaretrovirus named xenotropic murine leukaemia virus-related virus (XMRV) was claimed to be present in samples of human prostate tissue. In contrast to the original report detecting XMRV in stromal cells of prostate tumours [12], the study by Schlaberg et al. [13] reported expression of XMRV in the malignant cells. However, since then, several studies have reported the absence or extremely low prevalence of XMRV in prostate cancer. In previous German and US studies, XMRV could also not be detected in non-familial prostate cancer samples [14–16]. These conflicting epidemiological data have questioned whether XMRV plays a role in prostate cancer development.

B. Akgül · U. Wieland · H. Pfister (✉)
Institute of Virology, National Reference Center for Papillomaviruses and Polyomaviruses, University of Cologne, Fürst-Pückler-Str. 56, 50935 Cologne, Germany
e-mail: herbert.pfister@uk-koeln.de

D. Pfister · A. Heidenreich
Department of Urology, Oncological Urology, Pediatric Urology and Renal Transplantation, RWTH University Aachen, Pauwelsstr. 30, 52074 Aachen, Germany

R. Knüchel
Institute of Pathology, RWTH University Aachen, Pauwelsstr. 30, 52074 Aachen, Germany

To further investigate a possible relationship between prostate cancer and virus infection, we analysed 85 samples from prostate cancers for the presence of BKPyV and XMRV by quantitative PCR (qPCR).

Materials and methods

Patients and samples

The radical prostatectomy specimens of 85 consecutive patients from North Rhine Westphalia, Germany, with biopsy-proven prostate cancer treated between January 2008 and May 2009 were reviewed by the pathologist. All patients had received at least a six-core biopsy and were transferred to our institution for further treatment. Mean patient age was 63.9 (42–74) years. Mean PSA value was 13.6 (2.1–133.5) ng/ml. According to the d'Amico criteria, 36 patients had a good, 34 patients an intermediate and 15 patients a poor prognosis, respectively. All patients underwent a standardized retropubic prostatectomy. According to the risk stratification, an extended pelvic lymphadenectomy was performed. All prostatectomy specimens were sent on ice to the department of pathology. In the final histopathologic specimen, there was an upgrading in the Gleason Score in 37 patients and a downstaging in 7 patients. In almost 50% of the patients, a locally advanced tumour stage was verified. The exact distribution of the tumour stages can be seen in Fig. 1.

Cellular DNA extraction from prostate tissue

Total cellular DNA was extracted from 50 µm formalin-fixed, paraffin-embedded tissue sections using the QIAamp DNA Mini Kit (Qiagen, Hilden) following the manufacturer's instructions. Amplification of a 268-bp fragment from the beta-globin gene with primers PC04 and GH20 was used to check for the quality of isolated DNA samples [17].

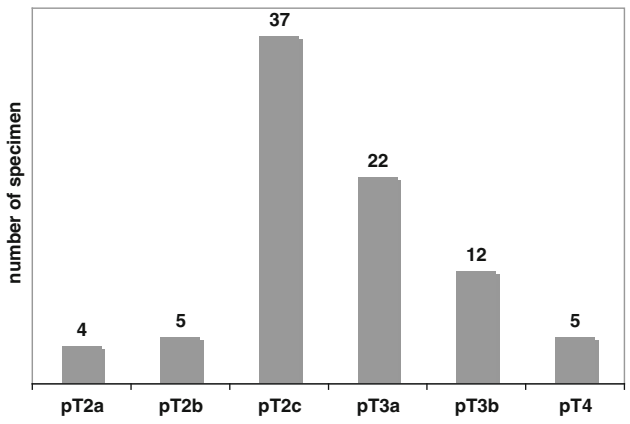

Fig. 1 pT-stage in radical prostatectomy specimens

Quantitative PCR amplification of proviral XMRV and BKPyV DNA

Serial dilutions of the full-length molecular clone XMRV-VP62, cloned into pcDNA3.1, were prepared in 200 ng of human placenta DNA (500,000–5 copies/reaction) and used as standards in q-PCR. XMRV-VP62 was obtained through the AIDS Research and Reference Program, Division of AIDS, NIAID, NIH; XMRV VP62 cDNA was from Drs. Robert H. Silverman and Beihua Dong [12, 18] and used as positive control and for creating a standard curve for qPCR. qPCRs for the detection of proviral XMRV DNA were performed using the Light Cycler System (Roche, Mannheim, Germany) in a total volume of 20 µl containing 1.25 units Platinum Taq Polymerase and the associated buffer, 4 mM $MgCl_2$, 1.6 µl of a 1:1,000 dilution of SybrGreen, 5% DMSO, 500 ng/µl non-acetylated bovine serum albumin, 0.2 mM dNTP each and 0.5 µM forward and backward primer each. Primers used for the detection of proviral XMRV DNA were XMRV4552F (5′-CGAGAGGCAGCCATGAAGG-3′) and XMRV4673R (5′-CCCAGTTCCCGTAGTCTTTTGAG-3′) as described previously [13]. The thermocycling conditions were 95°C for 10 s, followed by 45 cycles of 95°C for 10 s and 60°C for 20 s and 72°C for 30 s.

Detection of BKPyV DNA was performed using the BKPyV PCR Kit 1.0 (Astra, Hamburg, Germany) according to the manufacturer's recommendations and additional dilutions of the standard to 1 copy/PCR.

Results and discussion

Specific qPCR assays were standardized for BKPyV and XMRV using positive controls (Fig. 2). BKPyV was only detected in 1 out of 85 tissue samples. The positive tumour was classified stage 2c, which was the most frequently observed tumour stage among the analysed samples. The viral load was only 1 BKPyV DNA copy/5,360 cells. XMRV was not detected in any of the prostatic tissues examined.

The findings of the present study provide further evidence against a causative role of BKPyV in the development of prostate carcinoma since BKPyV DNA was identified in only 1/85 carcinoma cases. Similarly, a more recent Mexican study analysing 55 prostate cancer biopsies came also to the same conclusion [4].

Our negative data on XMRV are in line with the more recent studies from the Netherlands [19], Mexico [4], Japan

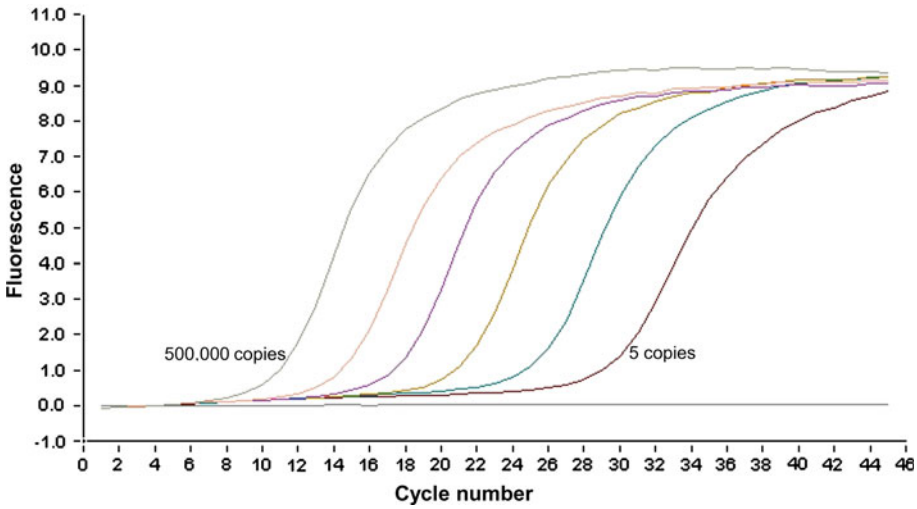

Fig. 2 Sensitivity of the qPCR for detection of XMRV DNA. Detection of 5–500,000 copies of the XMRV molecular clone in 200 ng of human placenta DNA

[20] and the USA [21, 22]. Very recently, it could be shown that XMRV was generated in the past by recombination of two proviruses during tumour passaging in mice. These data therefore suggested that the positive association of XMRV with human diseases is most likely due to laboratory contamination of human samples [23].

The data presented herein argue that BKPyV and XMRV have no aetiological significance in the pathogenesis of prostate cancer.

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