

Polyomavirus BK in the pathogenesis of bladder cancer

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ABSTRACT

Polyomaviruses are able to drive malignant transformation in rodent models, and have been implicated in the aetiology of a variety of human malignancies. However, the reports on this association in humans are strongly conflicting. Here we describe a renal transplant (RT) recipient with ureteral stenosis against the background of polyomavirus BK (BKV) activity. Six and a half years after transplantation, this patient developed metastasised bladder cancer. Prior to the diagnosis of cancer, atypical cells were detected in the urine that were denoted as 'decoy cells': virally infected epithelial cells that are frequently seen in the urine of RT recipients with BKV (re)activation, which may morphologically resemble malignant cells. Intriguingly, the primary urothelial carcinoma, as well as the mesenterial and two intestinal metastases, stained positive with antibodies against polyomavirus virus large T antigen protein, whereas the adjacent healthy tissue did not. This case suggests a role for BKV in the pathogenesis of bladder cancer, at least in the context of immunodeficiency.

KEYWORDS

Polyomavirus BK, bladder cancer, kidney transplantation, transforming viruses

INTRODUCTION

Polyomavirus BK (BKV) latently infects 70-100% of the human population but has not been associated with disease in immunocompetent individuals.¹ The most important site of latency is thought to be the urogenital

tract. In immunocompromised renal transplant (RT) recipients, BKV frequently (re)activates, and may cause nephropathy (BKVN) and ureteral stenosis.² It has remained a topic of ongoing debate whether polyomaviruses are involved in the pathogenesis of human malignancies. Here, we report on the case of an RT recipient with active BKV replication who developed a metastasised bladder carcinoma. Both the primary tumour and the intestinal metastases stained positive with antibody against the Simian vacuolating virus 40 (SV40) large T antigen protein (LTA_g), which cross-reacts with BKV- and polyomavirus JC (JCV)-LTA_g.

CASE

A 56-year-old man of Caucasian ethnicity with end-stage renal disease due to polycystic kidney disease received a renal transplant from a living unrelated donor. Induction therapy consisted of anti-CD25 monoclonal antibody. Initial immunosuppressive treatment consisted of tacrolimus, mycophenolate mofetil and prednisolone, which was tapered to tacrolimus and prednisolone one year after transplantation. Four years after transplantation, he presented with high fever and pain over the transplant region. There was a drop in the Modification of Diet in Renal Disease (MDRD) score from 42 to 23 ml/min/1.73 m². Ultrasound examination and antegrade pyelography revealed hydronephrosis of the allograft due to a distal ureteral stenosis. Urine and blood cultures were positive for *Escherichia coli*. Plasma BKV viral load was high, mounting to 1.16 x 10⁵ copies/ml as measured by large T antigen protein-PCR. In his urine, microscopic haematuria and cells with intranuclear inclusions and a high degree of

mitosis were seen. These cells were denoted as decoy cells consistent with BKV infection but urothelial malignancy could not be excluded (figure 1). A nephrostomy catheter was inserted, immunosuppressive therapy was tapered and antibiotic treatment was initiated. After an initial decline to undetectable levels, the viral load rose again to 1.69×10^4 copies/ml. Meanwhile, the patient developed two episodes of deep venous thrombosis of the calf veins and one arterial thrombosis of the iliac artery. The last two events occurred while using oral anticoagulation. Microscopic haematuria and decoy cells persisted, and cystoscopy showed an irregular papillary aspect of the posterior wall of the bladder. Biopsies revealed a high-grade urothelial carcinoma invading the bladder muscle and perivesicular fat. During surgery, extensive intraperitoneal and retroperitoneal metastases to the mesentery and intestines became apparent. Ultimately, two years after detection of BKV infection, the patient died due to tumour progression. Remarkably, the primary tumour and the intestinal metastases but not the adjacent healthy tissue stained positive for SV40 LTAg (figure 2).

DISCUSSION

The patient described here presented with overt BKV replication four years after starting immunosuppressive medication. He was diagnosed with ureteral stenosis, a well-known complication of overt BKV replication.² Because there are no therapeutic agents that directly target BKV, immunosuppressive therapy was tapered to improve the patient's antiviral immune response.³ The urinary decoy cells and microscopic haematuria were first considered to be compatible with BKV replication. Decoy

Figure 1. Decoy cells in urine cytology preparation. Urine cytology specimen from our patient showing cells with enlarged nuclei with homogenised chromatin and possible nuclear ground-glass inclusions. Papanicolaou stain, 400 x original magnification

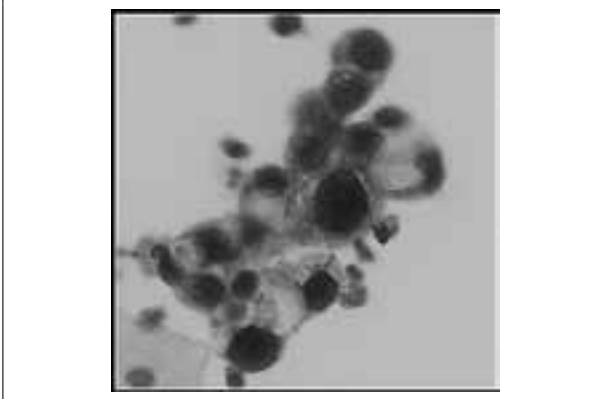
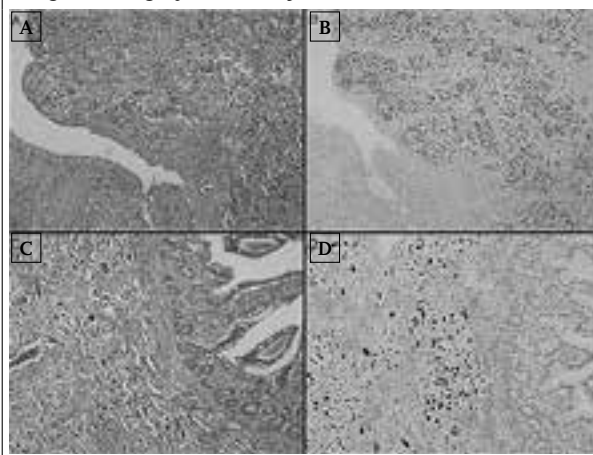


Figure 2. Representative photographs of urothelial cell carcinoma of the bladder and small bowel metastasis of the patient described here. (A) The high-grade urothelial cell carcinoma shows a predominant nested growth pattern with infiltration in the lamina propria. (B) Immunohistochemical staining with an antibody against the SV40 T-antigen shows diffuse strong positive nuclear staining of the high-grade urothelial cell carcinoma. (C) Metastasis of the urothelial cell carcinoma in the small bowel wall shows extensive sarcomatoid dedifferentiation. (D) The SV40 T-antigen shows the same staining pattern in the small bowel metastasis as in the primary tumour. Original magnification x 50



cells are defined as enlarged nuclei with viral inclusions, owing to virus-induced DNA replication, and may indeed resemble malignant cells. Nevertheless, the finding of decoy cells is highly unspecific and they can be found in up to 42% of RT recipients.⁴ Persistent haematuria and recurring thrombotic events strengthened the suspicion of malignancy, which was indeed found to be the case.

The transforming potential of BKV and other polyomaviruses has long been recognised in rodent models. However, human cells are less prone to undergo transformation *in vitro* and conflicting literature on an association between polyomaviruses and various malignancies has only fuelled the debate.^{2,5} Polyomaviruses possess several tools that promote malignant transformation of the host cell, amongst which a set of viral proteins produced early in the viral replication cycle, the T antigen proteins (TAgs).² Large TAgs can inhibit tumour suppressor protein p53 as well as retinoblastoma proteins.^{6,7} This drives a cell towards replication, providing the virus with increased host transcription factors. Also the viral agnoprotein was shown to inhibit double-stranded DNA repair by negatively affecting the expression of Ku70 and Ku80 proteins.⁸ Another mechanism may be the 'permissiveness' of cells to infection. BKV is thought not to complete its full replication cycle in non-permissive cells,

resulting in the transcription of only TAg proteins from the early region of the BKV genome.² Lastly, the BKV genome rearranges its non-coding control region (NCCR), altering the number and effectiveness of transcription factor binding sites and thereby also virulence and transforming potential. Such NCCR mutants have indeed been linked to certain urogenital malignancies.⁹

In this case, both the primary tumour as well as the distal intestinal metastases stained positive for LTag, whereas the non-neoplastic tissue stained negative for LTag. Perhaps, owing to tropism of BKV for urothelium, urothelial metastases are more susceptible to BKV infection in the setting of systemic BKV activity. Nevertheless, the adjacent non-neoplastic urothelium in the bladder was negative for LTag staining (*figure 2B*). Publications on the association between BKV and bladder cancer are rare.^{10,11} Larger series on an association of BKV or other polyomaviruses with bladder cancer vary greatly in their outcome and remain inconclusive. These differences in outcome may very well be explained by the different methods of detection used, ranging from BKV-LTAG, NCCR, VP1 PCR to SV40 LTag antibody staining on paraffin sections, and Papanicolaou staining on urine cytology specimens.^{9,12-15}

CONCLUSION

Reports on an association between BKV and bladder cancer are rare, and the association between polyomaviruses and human malignancy is a topic of ongoing debate. Here, we convincingly show the presence of the viral transforming LTag protein in the primary urothelial carcinoma and in the intestinal metastases, whereas the adjacent non-neoplastic tissue stained negative. Also since the intestines are normally not considered to be a niche for BKV infection, this case adds evidence for an implication of BKV in the pathogenesis of bladder cancer, a relation that may very well be more apparent in the context of immunodeficiency.

REFERENCES

1. Shah KV, Daniel RW, Warszawski RM. High prevalence of antibodies to BK virus, an SV40-related papovavirus, in residents of Maryland. *J Infect Dis.* 1973;128:784-7.
2. van Aalderen MC, Heutinck KM, Huisman C, Ten Berge IJ. BK virus infection in transplant recipients: Clinical manifestations, treatment options and the immune response. *Neth J Med.* 2012;70:172-83.
3. Schaub S, Hirsch HH, Dickenmann M, et al. Reducing immunosuppression preserves allograft function in presumptive and definitive polyomavirus-associated nephropathy. *Am J Transplant.* 2010;10:2615-23.
4. Huang G, Chen LZ, Qiu J, et al. Prospective study of polyomavirus BK replication and nephropathy in renal transplant recipients in China: a single-center analysis of incidence, reduction in immunosuppression and clinical course. *Clin Transplant.* 2010;24:599-609.
5. Abend JR, Jiang M, Imperiale MJ. BK virus and human cancer: innocent until proven guilty. *Semin Cancer Biol.* 2009;19:252-60.
6. 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;64:1353-6.
7. Lilyestrom W, Klein MG, Zhang R, Joachimiak A, Chen XS. Crystal structure of SV40 large T-antigen bound to p53: interplay between a viral oncoprotein and a cellular tumor suppressor. *Genes Dev.* 2006;20:2373-82.
8. Darbinyan A, Siddiqui KM, Slonina D, et al. Role of JC virus agnoprotein in DNA repair. *J Virol.* 2004;78:8593-600.
9. Monini P, Rotola A, Di Luca D, et al. DNA rearrangements impairing BK virus productive infection in urinary tract tumors. *Virology.* 1995;214:273-9.
10. Geetha D, Tong BC, Racusen L, Markowitz JS, Westra WH. Bladder carcinoma in a transplant recipient: evidence to implicate the BK human polyomavirus as a causal transforming agent. *Transplantation.* 2002;73:1933-6.
11. Galed-Placed I, Valbuena-Ruvira L. Decoy cells and malignant cells coexisting in the urine from a transplant recipient with BK virus nephropathy and bladder adenocarcinoma. *Diagn Cytopathol.* 2011;39:933-7.
12. Fioriti D, Pietropaolo V, Dal FS, Laurenti C, Chiarini F, Degener AM. Urothelial bladder carcinoma and viral infections: different association with human polyomaviruses and papillomaviruses. *Int J Immunopathol Pharmacol.* 2003;16:283-8.
13. Rollison DE, Sexton WJ, Rodriguez AR, Kang LC, Daniel R, Shah KV. Lack of BK virus DNA sequences in most transitional-cell carcinomas of the bladder. *Int J Cancer.* 2007;120:1248-51.
14. Roberts IS, Besarani D, Mason P, Turner G, Friend PJ, Newton R. Polyoma virus infection and urothelial carcinoma of the bladder following renal transplantation. *Br J Cancer.* 2008;99:1383-6.
15. Weinreb DB, Desman GT, Amolat-Apiado MJ, Burstein DE, Godbold JH, Jr., Johnson EM. Polyoma virus infection is a prominent risk factor for bladder carcinoma in immunocompetent individuals. *Diagn Cytopathol.* 2006;34:201-3.