Annals of Diagnostic Pathology 40 (2019) 77–80

Contents lists available at ScienceDirect

Annals of Diagnostic Pathology

journal homepage: www.elsevier.com/locate/anndiagpath

Original Contribution

BK polyomavirus and urothelial carcinoma: Experience at a tertiary care centre in India with review of literature

Kalpana Kumari[a], Immanuel Pradeep[a], Aanchal Kakkar[a], Amit Kumar Dinda[a], Amlesh Seth[b], B. Nayak[b], Geetika Singh[a,*]

[a] Department of Pathology, All India Institute of Medical Sciences, New Delhi, India
[b] Department of Urology, All India Institute of Medical Sciences, New Delhi, India

ARTICLE INFO

Keywords:
Polyomavirus
Chromogenic *in situ* hybridization
Urothelial carcinoma
Immunocompetent
SV40 T-Antigen

ABSTRACT

Introduction: BK polyomavirus is ubiquitous and remains dormant in the urothelial tract, reactivating and replicating in the immunocompromised state especially in the setting of post-renal transplantation where it is believed to be directly oncogenic based on recent reports. Its oncogenic role in the immunocompetent host is controversial. This study aimed to investigate the association of BK polyomavirus in Urothelial Carcinoma.

Material and methods: Patients with suspected urothelial carcinoma (UC) admitted under Department of Urology over a period of one year were recruited and transuretheral bladder tumor (TURBT) resection was performed, along with sampling of cystoscopically normal-appearing urothelium away from the tumor. In addition, cystectomy specimens with UC were included, with sampling of grossly normal-appearing urothelium away from the tumor. Immunohistochemistry (IHC) for SV40 T-Antigen and chromogenic *in situ* hybridization (CISH) using BK polyomavirus specific probe was performed on the paired samples (tumor and normal).

Results: Twenty-three TURBT and 14 cystectomy specimens were assessed. None of the cases showed evidence of BK polyomavirus infection in tumor or in surrounding mucosa by IHC. CISH performed in ten cases were also found to be negative. In comparison, one post-renal transplant urothelial carcinoma in our experience showed diffuse SV40 staining.

Conclusions: This study suggests that BK polyomavirus infection is not associated with urothelial malignancy in the immunocompetent setting unlike in the immunocompromised setting where it should always be investigated for.

1. Introduction

BK polyomavirus (BKV) is a member of the polyoma subgroup of papovaviruses and affects kidney, lung, liver, eye and brain causing diseases which can be primary, latent and reactivated [1,2]. It causes asymptomatic primary infection in 50–90% of general population during early childhood where it remains dormant primarily in renal tubular epithelial cells and urothelial cell layers [3]. Virus reactivation occurs in 10% of immunocompetent individuals; however, replication is effectively suppressed [4]. Recent studies have demonstrated that in settings of immunosuppression, BK polyomavirus undergoes replication and acts as a potential transforming virus [5,6]. Viral reactivation has been described in one-third of renal transplant patients in the first three months post-transplantation in the form of BKV viruria and/or BKV viremia [6,7]. Significant BKV viremia (> 4 log copies/mL) is associated with the development of polyomavirus nephropathy (PVN) in approximately 40% of renal transplant recipients routinely detected by immunohistochemistry for SV40 T-Antigen on the renal biopsy [6,7]. Increasing number of case reports have provided evidence that BKV is linked to urothelial carcinoma (UC) occurring in solid organ transplant recipients, particularly post-renal transplant [8-14]. These studies demonstrated that UC arising in the setting of renal transplant strongly expressed SV40 T-Antigen, supporting the role of BKV in the etiopathogenesis [8-14]. However, its role in oncogenesis in immunocompetent individuals with genitourinary cancer is still controversial and remains to be established. While a few initial studies suggested that BKV plays a role in pathogenesis of bladder UC (Table 1) [15-17], subsequent studies demonstrated that BKV DNA is present in equal frequency in neoplastic as well as normal mucosa and did not support the involvement of BKV in immunocompetent individuals (Table 1) [18-20]. With this background, we aimed to investigate the association of BKV with UC in immunocompetent patients.

* Corresponding author at: Room no. 1048, First Floor, Teaching Block, Department of Pathology, All India Institute of Medical Sciences, New Delhi 110029, India.
E-mail address: ggmed22@gmail.com (G. Singh).

https://doi.org/10.1016/j.anndiagpath.2019.04.006

Table 1
Review of available literature: urothelial carcinoma of bladder in immunocompetent host.

Authors/year	Number of cases	Method of detection	Polyomavirus infection detection	Large T Ag expression
Studies not in favor of association of BKV in UC in the immunocompetent settings				
Current study	23 TURBT 14 cystectomy & paired normal urothelium	IHC & CISH	Absent	Negative
Rollison et al, 2007	76 UC with paired normal urothelium (46)	Q PCR & IHC	Low copy number BKV in both tumor and control	Negative
Polesel et al, 2012	114 UC, 140 control	Urine samples for DNA	BKV DNA in both tumor & control	ND
Knoll et al, 2003	55 UC, 83 RCC with paired normal urothelium	Q PCR	Present in equal frequency in tumor and control	No integration
Studies in favor of association of BKV in UC in the immunocompetent settings				
Weinreb et al, 2006	133 IC host of which 21 had BC	Urine cytology	Decoy cells	ND
Fioriti et al, 2003	32 UC and 20 autopsy bladder tissue	PCR	High % of BKV in UC	ND
Monini et al, 1995	89 UC	PCR	URO1 present in both control and neoplastic tissue	ND

Abbreviations: IC, immunocompetent; BC, bladder cancer; UC, urothelial carcinoma; RCC, renal cell carcinoma.

2. Materials and methods

A prospective study of one year duration was undertaken with ethical clearance (No. F.8-148/A-148/2012/RS). All consecutive patients with suspected urothelial carcinoma (UC) admitted under the Department of Urology were recruited. After obtaining patient consent, transurethral resection of bladder tumor (TURBT) was performed, along with sampling of cystoscopically normal appearing urothelium away from the tumor. In addition, cystectomy samples with UC were included, with sampling of grossly normal appearing urothelium away from the tumor. For the purpose of this study immunocompetent hosts were defined as those with no significant history of diabetes, major illness including retroviral, chemotherapy, radiotherapy or recipients of transplant. We also included a single case of pelvic UC arising in redundant graft of the recipient of a second kidney (previously published) for comparison [8]. The patient underwent redundant graft nephrectomy after a course of chemotherapy.

2.1. Histological assessment

On routine hematoxylin and eosin (H&E) stained sections, the tumor were evaluated by two pathologists and graded based on the 2016 WHO/ISUP classification of UC. Presence or absence of lamina propria and muscle invasion was noted. Sections from the cystoscopically normal appearing urothelium were assessed for presence of dysplasia and cystitis.

2.2. Immunohistochemistry for BKV

Five-micron-thick sections cut from formalin-fixed, paraffin-embedded (FFPE) tissue blocks were immunolabeled with SV40-T Antigen (1:50, clone MRQ-4, Cell Marque, Sigma Aldrich, USA) in all cases along with paired normal mucosa samples, as described previously [17]. In brief, sections were deparaffinized and rehydrated in descending grades of alcohol. Antigen retrieval was performed by boiling in citrate buffer (0.01 mol/L) at pH 6.0 in a microwave oven for 30 min. To diminish endogenous peroxide activity, slides were treated with 4% hydrogen peroxide in methanol for 30 min. After rinsing briefly with TRIS buffer, sections were overlaid with appropriately diluted primary antibody and incubated overnight at 4 °C in a humid chamber. Sections were washed in TRIS buffer, treated with the biotin-labeled secondary antibody for 60 min at room temperature, and then washed in TRIS buffer. Peroxidase-conjugated streptavidin was applied, and the sections were incubated at room temperature for 30 min. Sections were rinsed, stained with the chromogen diaminobenzidine for 5 to 10 min, washed, counterstained with hematoxylin, and then mounted. The antibody stains all human polyoma viruses, including BKV. Sections from post-transplant renal biopsy with Polyomavirus nephropathy were used as positive control.

2.3. BK polyomavirus detection by chromogenic in situ hybridization

Chromogenic *in-situ* hybridization (CISH) using a BK polyomavirus specific probe (BKV virus bioprobe; ENZO, Farmingdale, NY) was standardized on post-transplant renal biopsy material with polyomavirus nephropathy. It was then performed in 10 cases with paired normal mucosal tissue. Four-micron-thick sections were cut from paraffin-embedded tissue block and put on charged slides for *in-situ* hybridization using ENZO ultrasensitive enhanced horseradish peroxidase-DAB *In situ detection* system (Enzo life Sciences, Switzerland). Deparaffinization and rehydration were performed in xylene and decreasing solution of ethanol. Proteinase K working solution was added and sections incubated at 37 °C for 15 min. 10 μL of biotin-labeled probe was added. DNA was denatured for 10 min at 95 °C and hybridization continued for 1 h at 37 °C. After wash buffer, post-hybridization reagent was added and incubated for 10 min. Antibody blocking buffer was added and incubated for 10 min, followed by rabbit anti-biotin and bio-goat anti-rabbit IgG and incubated for 20 min, respectively. Detection reagent was then added to the slide, followed by staining and counterstaining. Brown nuclear deposits in the tubular epithelial cells of all intensities were considered positive.

3. Results

Twenty-three TURBT specimens and 14 cystectomy specimens with UC with paired normal mucosal samples, all from immunocompetent individuals, were assessed, along with a single case of UC in a renal graft recipient presenting with UC in first redundant graft. Among the immunocompetent hosts, a male preponderance was noted (Male = 33; Female = 4) and mean age at diagnosis was 53.8 years (age range: 30 to 85 years). Cases included were high grade UC (76%, n = 29), low grade UC (18%, n = 7) and papillary urothelial neoplasm of low malignant potential (3%, n = 1).

A case of graft recipient included is a 57-year-old female who underwent her first live related renal transplant in 2005 with a complicated post-transplant course including crypotcoccal meningitis and disseminated herpes zoster which ended in graft failure in 2013. She underwent her second live authorized renal transplant in 2015, was on standard triple drug immunosuppression and had an uneventful course. In the third year post-transplant she presented with generalized weakness, loss of appetite and right flank heaviness. On CT the transplant kidney in the right iliac fossa appeared diffusely infiltrated by a heterogeneously hypoenhancing mass with enlarged conglomerate right external iliac lymph nodes. Plasma BKV titres were 2.7×10^3 copies/mL and urine titres were 3.6×10^5 copies/mL. A trucut biopsy was performed from the mass lesion which showed features of high grade UC with focal squamous differentiation and areas of necrosis, SV40 T-Antigen positive. Thereafter a course of chemotherapy she underwent right redundant allograft nephrectomy. On gross examination showed a lobulated solid tumor measuring 6.5 × 5.5 × 4 cm

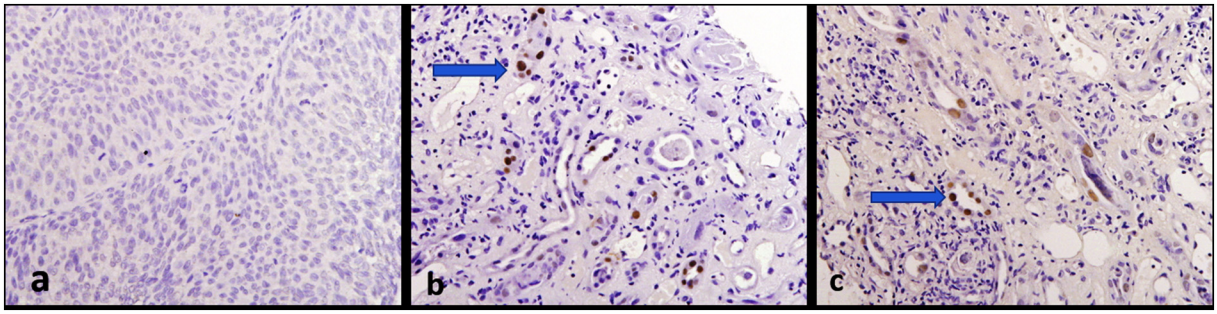

Fig. 1. High grade papillary urothelial carcinoma with negative reaction for CISH (a, ×200). Immunohistochemistry for SV40 T-Antigen control; case of BK-polyomavirus nephropathy showing positive staining of tubular epithelial cell nuclei (b, ×200). BKV-CISH control; case of BK polyomavirus nephropathy showing positive staining of tubular epithelial cell nuclei (c, ×200).

occupying pelvicalyceal system and infiltrating renal parenchyma (Fig. 2; a).

3.1. Immunohistochemistry and CISH

All UCs from immunocompetent hosts, irrespective of grade along with normal mucosa did not show staining for SV40 T-Antigen (Fig. 1; a). CISH performed in 10 cases also showed negative reaction. Renal biopsy with polyomavirus nephropathy used as positive control showed immunopositivity for SV40 T-Antigen and positive CISH reaction (Fig. 1; b & c). Contrary to these results in the case of pelvic UC arising in the redundant graft we observed diffuse and strong nuclear immunopositivity for SV40 T-Antigen in the tumor cells while was negative in the tumor surrounding normal renal parenchyma and ureter (Fig. 2; c & d).

4. Discussion

The question “does BK polyomavirus contribute to oncogenesis of UC in the immunocompetent setting” has been addressed in literature using different techniques. Conventional PCR based studies have demonstrated viral DNA in UC. One such earlier study by Monini et al. demonstrated viral DNA in 50–83% of carcinomas including bladder, prostate and kidney as well as in normal tissue [15]. Similarly, Fioriti et al. in 2003 demonstrated polyomavirus DNA by nested PCR in 55% of the 32 bladder cancers tested while 20 autopsy bladder specimens tested were negative [16]. However only the presence of viral DNA in the carcinoma is insufficient evidence of its role in tumor production, especially since the virus is known to be ubiquitous to the urinary tract [7]. Rollison et al. in a more comprehensive analysis in 2007 used quantitative real time PCR on fresh frozen tissue of 76 UCs along with paired normal urothelium (n = 46) and found only four tumors positive for BKV by both qRT-PCR and conventional PCR, including one with paired normal mucosa [18]. Copy numbers were low and ranged from 0.3 to 15.8 per 1000 cells and all five samples showed negative staining for SV40-T Ag [18]. The authors concluded that BK polyomavirus is unlikely to contribute to oncogenesis in UC. Further Knoll et al. demonstrated lack of viral integration into the host genome in the 16% BKV positive UCs and normal mucosa in their cohort of 55 cases [19].

In contrast, the role of BK polyomavirus in the immunodeficient setting, particularly post-renal transplant is now well established. The pathogenetic model of oncogenesis proposed starts at the point of BKV reactivation in the immunocompromised host and a productive infection [10,11]. Over time mutations in the large T antigen accumulate which enhance viral replication in the urothelium [10,11]. The p53 and pRb inactivation induced by the virus creates an atmosphere conducive to development of further mutations in the infected urothelium and development of malignancy in nonpermissive cells [10,22]. In a recent clinicopathological and molecular study of polyomavirus associated carcinomas of the urinary tract in immunocompromised hosts, 9 cases

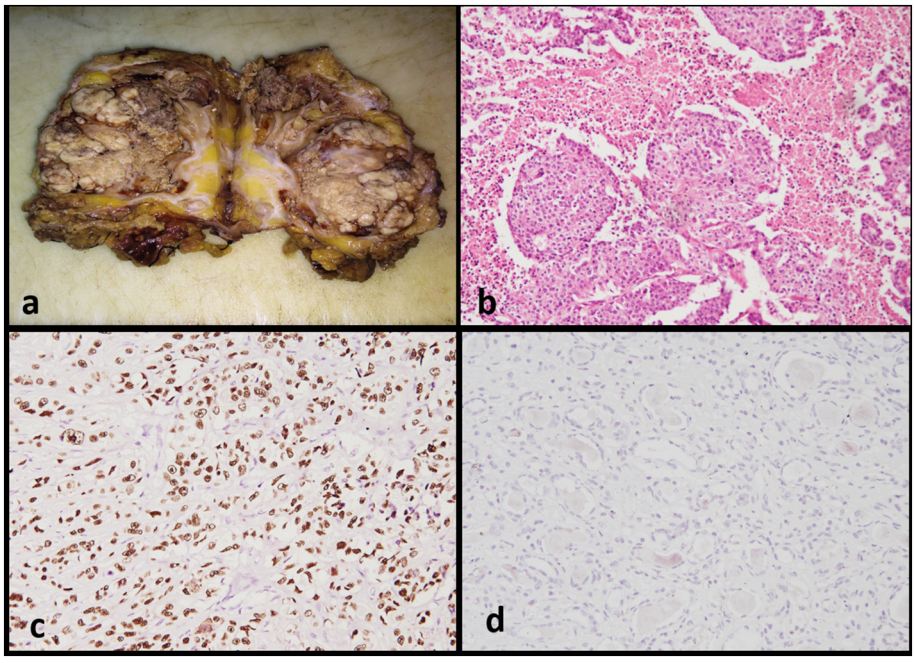

Fig. 2. Gross photomicrograph of a right redundant renal allograft specimen. Cut section showing solid lobulated pelvicalyceal tumor measuring 6.5 × 5.5 × 4 cm (a). Microscopy depicting high grade urothelial carcinoma with areas of necrosis (b, ×200). Diffuse and strong nuclear immunopositivity for SV40 T-Antigen in the tumor cells (c, ×200). Tumor surrounding renal parenchyma immunonegative for SV40 T-Antigen (d, ×200).

were found to be T antigen expressing and 8 were negative for T antigen based on immunohistochemical staining with SV40 T-Antigen [14]. Sequencing studies detected BK virus in both tumor (mean viral load of 12,439, 554 copies/mL) and normal (426,039 copies/mL) from only the T antigen expressing tumors while was negative in the T antigen negative group [14]. There was evidence of viral integration into the host genome at random integration sites [14]. This study validated SV40 T-Antigen immunohistochemistry as a specific test to define BK polyomavirus infection which is the first step to defining its role in oncogenesis. In our own experience with a post renal transplant pelvic UC (previously published), extensive and diffuse SV40 T-Antigen staining was noted in the tumor and was associated with an abnormal p53 immunohistochemistry [8]. The patient underwent surgical resection after course of chemotherapy. SV40 T-Antigen performed on non-neoplastic pelvic and ureteric epithelium in the resected specimen was negative.

This study demonstrates that immunocompetent patients with UC lack significant viral replication in the tumor and in the surrounding non-neoplastic epithelium. This is based on negative SV40 T-Antigen and negative BK polyomavirus specific CISH (in a proportion of cases); both methods which allow for direct visualization of tumor cells unlike most previous studies. Based on pathogenetic models in the immunocompromised host where it has been demonstrated that enhanced viral replication is the first step in viral oncogenesis it therefore appears as a corollary that in the immunocompetent setting BK polyomavirus does not play a role in oncogenesis of UC. With a recent plethora of literature demonstrating presence of BKV DNA in a variety of tumors including renal cell carcinoma [19,21,22] its role in oncogenesis should also be carefully examined as a first step with SV40 T-Antigen immunohistochemistry to demonstrate significant viral replication along with conclusive evidence of viral genome integration before any inferences can be made.

5. Conclusion

BKV is associated with UC, albeit only in the setting of immunosuppression. Our study did not support the association of BKV with UC in immunocompetent hosts.

Compliance with ethical standards

All authors declare no potential conflicts of interest (financial or non-financial). Informed consent was obtained from the patient. No animals were involved in the study.

These results were presented at International Society of Urology conference held in 2016 and published in abstract form in the World Journal of Urology; UP.038 Abstract Book, SUI 2016 Buenos Aires, Argentina.

Funding

This study has been funded through AIIMS, New Delhi intramural research grant awarded to Dr. Geetika Singh.

References

[1] Papadimitriou JC, Randhawa P, Rinaldo CH, et al. BK polyomavirus infection and renourinary tumorigenesis. Am J Transplant 2016;16:398–406.
[2] Reploeg MD, Storch GA, Clifford DB. Bk virus: a clinical review. Clin Infect Dis 2001;33(2):191–202.
[3] Knowles WA, Pipkin P, Andrews N, et al. Population-based study of antibody to the human polyomaviruses BKV and JCV and the simian polyomavirus SV40. J Med Virol 2003;71:115–23.
[4] Ling PD, Lednicky JA, Keitel WA, poston DG, White ZS, Peng R, et al. The dynamics of herpesvirus and polyoma virus reactivation and shedding in healthy adults: 14 months longitudinal study. J Infect Dis 2003;187:1571–80.
[5] Nickeleit V, Mihatsch MJ. Polyomavirus nephropathy in native kidneys and renal allografts: an update on an escalating threat. Transpl Int 2006;19:960–73.
[6] Hirsch HH, Brennan DC, Drachenberg CB, et al. Polyomavirus-associated nephropathy in renal transplantation: interdisciplinary analyses and recommendations. Transplantation 2005;79:1277–86.
[7] Lamarche C, Oris J, Collette S, et al. BK polyomavirus and the transplanted kidney: immunopathology and therapeutic approaches. Transplantation 2016;100(11):2276–87.
[8] Singh G, Swamy A, Kumari K, et al. Mass in nonfunctioning first renal allograft in a recipient of two transplant kidneys. Kidney Int 2019;95:1001–2.
[9] Nickeleit V, Singh HK, Kenan DJ, et al. The two-facet nature of BK polyoma virus: lytic infection or non-lytic large-T-positive carcinomas. J Pathol 2018;246:7–11.
[10] EL Mouallem NJ, Paul AK. BK virus associated urinary bladder cancer in a kidney transplant recipient. A case report and review of the pathogenesis. AJHO 2017;13(3):15–20.
[11] Kenan DJ, Mieczkowski PA, Burger-Calderon R, et al. The oncogenic potential of BK-polyomavirus is linked to viral integration into the human genome. J Pathol 2015;237(3):379–89.
[12] Nickeleit V, Singh HK, Goldsmith CS, et al. BK virus-associated urinary bladder carcinoma in transplant recipients: productive or nonproductive polyomavirus infections in tumor cells? Hum Pathol 2013;44:2870–1.
[13] Roberts IS, Besarani D, Mason P, et al. Polyoma virus infection and urothelial carcinoma of the bladder following renal transplantation. Br J Cancer 2008;99(9):1383–6.
[14] Sirohi D, Vaske C, Sanborn Z, et al. Polyoma virus-associated carcinomas of the urologic tract: a clinicopathologic and molecular study. Mod Pathol 2018;31(9):1429–41.
[15] 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.
[16] Fioriti D, Pietropaolo V, Dal Forno S, 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(3):283–8.
[17] Weinreb DB, Desman GT, Amolat-Apiado MJ, et al. Polyoma virus infection is a prominent risk factor for bladder carcinoma in immunocompetent individuals. Diagn Cytopathol 2006;34:201–3.
[18] Rollison DE, Sexton WJ, Rodriguez AR, et al. Lack of BK virus DNA sequences in most transitional-cell carcinomas of the bladder. Int J Cancer 2007;15(120(6)):1248–51.
[19] Knöll A, Stoehr R, Jilg W, et al. Low frequency of human polyomavirus BKV and JCV DNA in urothelial carcinomas of the renal pelvis and renal cell carcinomas. Oncol Rep 2003;10(2):487–91.
[20] Polesel J, Gheit T, Talamini R, et al. Urinary human polyomavirus and papillomavirus infection and bladder cancer risk. Br J Cancer 2012;106(1):222–6. 3.
[21] Bulut Y, Ozdemir E, Ozercan HI, et al. Potential relationship between BK virus and renal cell carcinoma. J Med Virol 2013;85:1085–9.
[22] Sanjuan NA. The paradox of polyomavirus lytic infection or tumor induction? Medicina (B Aires) 2004;64(1):73–6.