Urothelial carcinoma with prominent squamous differentiation in the setting of neurogenic bladder: role of human papillomavirus infection

Elen B Blochin¹, Kay J Park¹, Satish K Tickoo, Victor E Reuter and Hikmat Al-Ahmadie

Memorial Sloan-Kettering Cancer Center, Department of Pathology, New York, NY, USA

Squamous cell carcinomas of the urinary bladder are rare in the Western world; the majority of cases are reported in countries endemic to *Schistosoma* parasitic infections. Unlike squamous tumors of the uterine cervix or oropharynx, the human papillomavirus (HPV) is not commonly associated with bladder squamous cell carcinomas. We report on two cases of HPV-positive urothelial carcinomas of the urinary bladder with extensive squamous differentiation showing the typical basaloid, poorly differentiated morphology of HPV-associated tumors. These occurred in patients with neurogenic bladders who had long-standing histories of self-catheterization with tumors that tested positive for HPV by *in situ* hybridization. A retrospective review of our institutional database revealed four additional patients with bladder tumors showing squamous differentiation arising in the setting of neurogenic bladder. Review of these cases showed the more common well-differentiated keratinizing appearance of squamous cell carcinomas of the bladder. These tumors showed only patchy positivity for p16 immunohistochemical stain (not the diffuse strong staining seen in HPV-positive tumors), and the one tested case was negative for HPV by *in situ* hybridization. HPV infection and neurogenic bladder have been independently associated with increased risk of developing carcinoma in the urinary bladder; however, this is the first report of squamous tumors arising in the setting of concurrent neurogenic bladder and HPV infection. The morphology of these tumors is similar to that of other high-risk HPV-associated squamous carcinomas with a basaloid, poorly differentiated appearance and little to no keratin formation.

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The human papillomavirus (HPV) has an important role as a human carcinogen, with an estimated 10% of cancers worldwide attributed to it.¹ HPV is a small circular double-stranded DNA virus with more than 100 different types described to date.² The potential of the virus to cause malignant or benign lesions dictates its classification into low-risk (6, 11, 40, 42, and so on) or high-risk (16, 18, 31, 33, 45, 51, and so on) types. The prototypical model for carcinogenesis is squamous cell carcinoma of the uterine cervix, in which nearly all such

carcinomas are associated with HPV infection.^{3,4} The oncogenic role of HPV is also well established for a subset of squamous cell carcinomas in the vulva,⁵ penis,⁶ anus,⁷ and oropharynx.⁸ The possible role of HPV infection in bladder tumors has not been completely elucidated as HPV detection rates vary widely from 0 to 100%, depending on the study.^{9,10}

An intriguing issue has been the possible association of the virus with bladder squamous cell carcinoma. The few published studies^{11–14} on this relationship have shown a low frequency of HPV detection, implying that HPV is not likely to have a major role in the development of bladder squamous cell carcinoma. However, the positive cases identified in the literature suggest that under certain circumstances, such as immunosuppression¹² and persistent condylomatous infection,^{11,15,16} the virus may exert its oncogenic activity in the bladder.

Correspondence: Dr KJ Park, MD, Memorial Sloan-Kettering Cancer Center, Department of Pathology, 1275 York Avenue, New York, NY 10065, USA.

E-mail: gynbreast@mskcc.org

¹These authors contributed equally to this work.

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Interestingly, both low-¹⁷ and high-risk¹⁸ HPV types have been detected in bladder condylomas. While the causative role of HPV infection in bladder cancer remains to be fully elucidated, several other factors have been implicated in its carcinogenesis. Among these are smoking, arsenic and aromatic amines exposure,¹⁹ and chronic irritation associated with *Schistosoma* parasites, chronic urinary tract infection, and calculi.²⁰

Conflicting results have been published on the association of spinal cord injury and bladder carcinomas. Some studies report a 16- to 26-fold increased risk of cancer,^{21,22} while other studies suggest that the risk is comparable to that of the general population.^{23,24} Nevertheless, all groups convincingly show that squamous cell carcinoma of the bladder is overrepresented in this category of patients, comprising 19%²³ to 46.9%^{21,22} of all bladder carcinomas; this is significantly higher than in the general population, where non-schistosomal squamous cell carcinoma constitutes only 1.3–3.2% of bladder cancer.¹¹ It is believed that a significant subset of squamous cell carcinoma arises in a background of squamous metaplasia that develops secondary to irritation related to long-term catheterization and recurrent urinary tract infections associated with neurogenic bladder.

Concurrent HPV infection and prolonged catheterization in bladder cancer has not been previously reported. Here we describe two cases of HPV-positive bladder carcinomas with predominant squamous differentiation in female patients undergoing intermittent catheterization over an extended period of time due to neurogenic bladder.

Materials and methods

Two cases of HPV-positive bladder carcinoma were identified during routine histopathological evaluation at Memorial Sloan-Kettering Cancer Center. In each case, immunohistochemical stains for p16 as well as *in situ* hybridization for high-risk HPV were performed as part of the diagnostic work-up due to the unusual morphologic features of the tumors. Both patients had a long-standing history of neurogenic bladder. This prompted us to perform a retrospective search in the clinical database for bladder cancer with squamous differentiation in patients with a history of neurogenic bladder. A search was conducted for the period 2002–2011 and all cases meeting these criteria were submitted to pathological evaluation. Immunohistochemistry for p16 was performed on the initial two cases using the CINtec p16INK4a Histology kit developed by MTM Laboratories AG, Germany. The *in situ* hybridization probe for high-risk HPV was performed with the HPV III family 16 (high-risk probe) by Ventana (Ventana Medical Systems, Tucson, AZ) with appropriate controls.

Results

Patient 1

A 44-year-old woman presented in February 2010 for evaluation of pelvic pain and hematuria. Her past medical history was significant for lipomeningomyelocele requiring laminectomy at the age of 5 years. She had been using clean intermittent catheterization in the range of 15 to 20 times a day since a very young age. She also reported frequent urinary tract infections. The patient underwent vaginal hysterectomy for prolapse in 2009 with no other pertinent gynecologic history, and there was no specific mention of any previous Pap smear results in this patient. The pelvic pain was felt to be secondary to vaginal wall prolapse; however, an ultrasound showed a bladder abnormality. On cystoscopy, the entire mucosa was white and covered with plaques, and a nodular invasive tumor was identified. The patient underwent a transurethral resection of bladder tumor and two subsequent biopsies. Histological sections from the transurethral resection of bladder tumor showed detached fragments of invasive and *in situ* urothelial carcinoma and squamous cell carcinoma. The invasive component was poorly differentiated and composed of small basaloid cells without prominent keratinization. A spectrum of squamous epithelial atypia was observed in the overlying squamous mucosa equivalent to what would be considered moderate-to-severe dysplasia in the uterine cervix. Koilocytic changes were also noted in the superficial layers of the squamous mucosa. Almost the entire mucosal surface of the bladder showed either squamous metaplasia or squamous dysplasia with very little native urothelium present. The non-neoplastic mucosa (both squamous and urothelial) was negative for p16 and HPV *in situ* hybridization.

The patient received neoadjuvant treatment with gemcitabine and cisplatin followed by cystectomy with partial vaginectomy and bilateral salpingo-oophorectomy. On gross examination the tumor was identified as a 2.0 × 1.7 cm hemorrhagic ulcerated lesion located on the posterior wall and dome of the bladder. Microscopically, the tumor was almost exclusively non-keratinizing squamous cell carcinoma with only focal areas of keratinization invading into the deep muscularis propria and perivesical soft tissue (pathological stage T3; Figure 1). Perineural and vascular invasion were not identified. The urothelium exhibited diffuse squamous features with a basaloid appearance, disordered maturation, loss of polarity, and scattered large atypical cells involving the full thickness of the mucosa consistent with high-grade squamous intraepithelial lesion/carcinoma *in situ* (Figure 1). The same findings were present in bilateral ureters. Vaginal tissue present in the specimen was unremarkable and did not show any signs of viral cytopathic effect. Immunohistochemical studies for

Figure 1 Patient 1: (a) *in situ* squamous cell carcinoma of bladder resembling high-grade squamous intraepithelial lesion of uterine cervix. (b) Invasive and *in situ* squamous cell carcinoma showing diffuse p16 positivity. (c) Positive HPV *in situ* hybridization of non-invasive disease showing punctate integrated pattern of staining. (d) Invasive squamous cell carcinoma of bladder with focal keratinization, deeply muscle invasive. (e) Invasive carcinoma with diffusely positive p16. (f) Positive HPV *in situ* hybridization of invasive component showing punctate integrated pattern of staining.

p16 showed diffuse strong cytoplasmic and nuclear staining in both the *in situ* and invasive components (Figure 1). *In situ* hybridization analysis for high-risk HPV revealed dot-like nuclear labeling in the majority of the invasive carcinoma cells and was seen to a much lesser extent in the *in situ* component (Figure 1). Diffuse nuclear staining, characteristic of the episomal state of the virus, was not seen.

As of the most recent follow-up appointment in February 2012, the patient has no evidence of disease.

Patient 2

A 48-year-old woman presented with hematuria to her local urologist. Her past medical history was significant for paraplegia below T8 after a motor vehicle accident at the age of 23 years. No gynecologic history was reported and specifically, there was no mention of any history of abnormal Pap smears. In 2000, the patient underwent bladder-intestinal augmentation cystoplasty with Mitrofanoff appendicovesicostomy. She had been performing clean intermittent catheterization through the Mitrofanoff canal ever since, complicated by chronic urinary tract infections and development of small bladder capacity. Cystoscopy revealed a tumor that appeared to be limited to the bladder augmentation pouch. Two biopsies from the pouch were performed. The biopsied specimens were histologically similar to those of Patient 1: they were characterized

by invasive urothelial carcinoma with squamous, basaloid, and glandular differentiation, including signet ring cells. The *in situ* component was represented predominantly by squamous carcinoma *in situ* with a small focus of flat (*in situ*) urothelial carcinoma. The non-neoplastic urothelium was diffusely involved by squamous metaplasia. The invasive tumor extended into the lamina propria and foci suspicious for lymphovascular invasion were identified. p16 immunohistochemical stain showed diffuse weak cytoplasmic and nuclear staining in the *in situ* and invasive components. HPV *in situ* hybridization had distinct dot-like nuclear positivity in the invasive part of the tumor and episomal-type nuclear staining in the squamous metaplastic mucosa with low-grade dysplasia. Similar patterns of labeling were seen in both the squamous and glandular components of the tumor. Uninvolved glandular epithelium of the intestinal tissue did not show any staining. Two months after the biopsies, the patient underwent surgical resection including cystectomy, hysterectomy with bilateral salpingo-oophorectomy, and partial vaginectomy. Gross examination revealed a $3.0 \times 1.1 \times 0.3$ cm tan-white firm lesion in the right lateral wall of the neobladder. Microscopically, the bladder was composed of native urothelium and intestinal-type tissue consistent with the augmentation pouch. Squamous cell carcinoma *in situ* as well as metaplastic squamous epithelium extensively involved the urothelial/intestinal mucosa (Figure 2). Glandular dysplasia/adenocarcinoma *in situ* was not

Figure 2 Patient 2: (a) *in situ* squamous cell carcinoma involving augmented intestinal portion of neobladder. Underlying benign intestinal glands are present. (b) *In situ* squamous cell carcinoma of bladder resembling high-grade squamous intraepithelial lesion of uterine cervix. (c) Diffusely positive p16 staining of *in situ* squamous cell carcinoma. (d) Positive HPV *in situ* hybridization of *in situ* carcinoma, showing punctate integrated pattern of staining. (e) Invasive urothelial carcinoma with squamous and glandular differentiation. (f) Invasive squamous cell carcinoma component of tumor. (g) Diffusely positive p16 staining of invasive carcinoma. (h) Positive HPV *in situ* hybridization of invasive squamous and glandular components of the tumor, showing punctate integrated pattern of staining.

apparent. The invasive carcinoma was localized to the urothelial–intestinal junction and was represented predominantly by basaloid non-keratinizing squamous cell carcinoma growing in an infiltrative pattern. A separate focus of invasive adenocarcinoma composed of small- to intermediate-sized cystic spaces lined by a single layer of cuboidal cells was present underneath an area of squamous cell carcinoma *in situ* (Figure 2). The tumor extended into the left ureter, deep muscularis propria, and perivesical soft tissue (pathological stage T3). Perineural and vascular invasion were not identified. Non-neoplastic mucosa showed diffuse squamous metaplasia in the urothelium and ulceration of the intestinal graft. p16 and HPV *in situ* hybridization were negative in the non-neoplastic urothelium and intestinal glands. The entire cervix was coned and submitted for histological evaluation and showed no evidence of dysplasia. Vaginal tissue was also

unremarkable. p16 immunohistochemical stain showed diffuse strong cytoplasmic and nuclear labeling in the *in situ* squamous cell carcinoma and weak positivity in both the squamous and glandular invasive carcinoma (Figure 2). HPV DNA was shown to be present by *in situ* hybridization analysis in the *in situ* and invasive areas (Figure 2). As of the most recent follow-up in April 2012, the patient had no evidence of disease.

A retrospective analysis of 250 cases of urothelial carcinoma with squamous differentiation or pure squamous cell carcinoma of the bladder revealed four patients with a history of neurogenic bladder. Two patients had been undergoing clean intermittent catheterization, one since 1985 following a motor vehicle accident and one since 1986 due to multiple sclerosis. Another patient had been self-catheterizing two to three times a day since 1998 following surgery for endometriosis. The fourth

patient had a clinical picture of neurogenic bladder of uncertain etiology that did not require catheterization. Microscopically, the tumors were composed entirely of squamous cell carcinoma (two cases) or exhibited a prominent squamous cell carcinoma component in an otherwise usual type of urothelial carcinoma. In all four cases, the tumors showed prominent keratinization without the more basaloid appearance seen in our two study cases. p16 immunohistochemical stain showed patchy positivity in one case where it focally labeled urothelial carcinoma *in situ* as well as areas of invasive squamous cell carcinoma. HPV *in situ* hybridization in this case was negative.

Discussion

The association of HPV and tumors in the bladder has been previously reported for benign and malignant lesions, but, true HPV-driven carcinogenesis of bladder is rare, with only a few cases reported in the literature. The association of squamous cell carcinoma and neurogenic bladder has also been described, but this may be a function of the indwelling catheters that are frequently used in these patients. We report on two patients with neurogenic bladder who developed urothelial carcinoma with prominent squamous differentiation displaying morphological features similar to HPV-associated squamous cell carcinoma of the uterine cervix and that were positive for high-risk HPV by *in situ* hybridization. We found no such association in four additional cases associated with neurogenic bladder.

HPV is a non-enveloped, circular, double-stranded DNA virus of approximately 8 kb, which has been implicated in the development of up to 10% of cancers worldwide.^{1,4} It belongs to the *Papillomaviridae* family of viruses and is classified into low-risk and high-risk groups based on the ability to cause malignant transformation. At present, there are over 100 types of papillomaviruses that have been described; new variants are classified when the sequence of the L1 gene is at least 10% dissimilar from any previously described variant. The oncogenic activity of the virus is dependent on the E6 and E7 early viral proteins, which have a major role in malignant transformation by disrupting cell cycle regulation by p53 and the retinoblastoma protein (pRB), respectively. Additionally, HPV targets other important molecules such as c-Myc, hTERT,^{25,26} PDZ-containing proteins,²⁷ and tuberlin,²⁸ leading to overall genomic instability.²⁹ HPV is exclusively epitheliotropic. It is a unique virus that does not bind directly to intact epithelia, but rather requires conformational change at the extracellular basement membrane prior to cellular attachment.³⁰ This mechanism of entry most likely explains the propensity of the virus to infect the cervical transformation zone, where the basement

membrane is frequently exposed, as well as other sites of chronic inflammation with epithelial micro-trauma. Once the virus is in the basal keratinocytes, its replication is coupled to host cell division. To exert its oncogenic potential the virus must be integrated into the host DNA; this usually occurs after long, persistent infections.³¹ The role of HPV has been well established for carcinomas of the cervix, vulva,⁵ penis,⁶ anus,⁷ and oropharynx.²⁰ Most of these tumors are of squamous histology with the exception of cervical adenocarcinoma, and these HPV-associated squamous cell carcinomas of different anatomic locations commonly share basaloid or warty morphology.

The majority of HPV infections resolve spontaneously with the help of the innate immune system.² In patients with immunosuppression, HPV-associated carcinomas are seen at increased frequency, with cervical carcinoma regarded as one of the Acquired Immunodeficiency Syndrome-defining illnesses.^{32,33} Cumulative knowledge about HPV-associated cancers allowed for defining a set of criteria that needs to be met in order for the tumor to be considered HPV-driven. Among these criteria are (1) localization of the virus to the nucleus and (2) the presence of virus in the full spectrum of disease from benign/low-grade lesions to high-grade lesions and invasive carcinomas. Detection of the virus in cervical adenocarcinoma suggests that the transforming activity of the virus is not limited to squamous epithelium.³⁴

Both high-risk¹⁸ and low-risk^{15,17} types of HPV have been detected in condylomata of the bladder. Although these are rare and generally regarded as benign lesions, several studies have shown squamous cell carcinoma arising in the background of condylomata.^{11,16,17,35} It is important to note that patients in these studies were either chronically immunosuppressed^{11,17} or showed persistent condylomatous infection with¹¹ or without¹⁶ associated extensive vaginal condylomata. Inverted papillomas of the bladder have also been examined for HPV with variable results. Gould *et al*³⁶ reported that out of 18 cases, only 1 was positive for HPV 11 (9%), but in a study by Shigehara *et al*,³⁷ DNA from HPV 16 and 18 was identified in 7 out of 8 cases (87.5%) by polymerase chain reaction (PCR) and confirmed by *in situ* hybridization analysis. Reported HPV rates also vary widely for invasive urothelial carcinomas, ranging from 0 to 100%.^{38–40} This variation may be due to the different methods of detection used in the studies. For frozen or fresh tissue, PCR is considered to be the gold standard,⁴¹ although PCR technique is frequently blamed for being oversensitive, subject to contamination, and false-positive results. When interpreting PCR results, it is important to note the strength of the signal, as it is required to have at least one viral copy per cell for transformation. *In situ* hybridization, although not as sensitive as PCR, has been widely applied⁴² with the important advantage of direct visualization of the virus in the cell and

evaluation of the episomal *versus* integrated state of the viral DNA.^{43,44} The optimal detection method, however, still remains unclear and is likely a combination of both molecular detection and direct visualization.

The majority of published studies on bladder urothelial carcinomas use PCR, while some rely on Southern blot analysis for the isolation of the viral capsid or serological testing for antiviral antibody. The use of different techniques and the type of tissue (frozen *vs* paraffin-embedded) does not fully account for the variability in detection rates. High prevalence of HPV has been shown not only in studies relying solely on PCR,^{39,45} but also in studies where PCR was combined with Southern blot,^{9,46} dot blot hybridization,⁴⁷ or *in situ* hybridization.^{48,49} Other studies using the same methods yielded much lower rates of detection.^{10,12,50–53} Unfortunately, it is difficult to interpret the results because not all studies have analyzed non-lesional bladder tissue as control. For example, Anwar *et al*⁹ detected the virus in 33% of healthy bladders, in contrast to Tekin,⁵³ who did not detect it in any of the 10 control cases. Smetana *et al*,⁴⁹ on the other hand, showed the virus in 2 out of 41 samples of non-lesional tissue from the same patients. Regional location does not seem to explain the inconsistencies either. For example, two Japanese groups were among the ones to report both high prevalence and absence of HPV in bladder carcinoma. In a study by Shigehara *et al*,¹³ high-risk HPV DNA was detected in 18 of 117 patients (15%), while Sano *et al*⁵⁴ failed to identify any HPV DNA in their study.

Squamous cell carcinoma of the bladder in particular has also been analyzed in a number of studies.^{12–14} Maloney *et al*¹² showed the presence of HPV 18 by PCR in 1 of 22 patients (4.5%) with squamous cell carcinoma while Shigehara *et al*¹³ and Westenend *et al*¹⁴ did not identify HPV in any of their 4 and 16 cases, respectively.

In clinical practice, p16 is increasingly being used as a surrogate marker for high-risk HPV infection. p16 is a product of the *INK4a* gene on chromosome 9 (9p21) and belongs to a family of tumor suppressor proteins involved in cell cycle regulation. It executes its function by suppressing cyclin-dependent kinase-mediated phosphorylation of pRb to control the cell cycle at the G1–S interphase. In infected cells, the HPV E7 oncoprotein binds and degrades host pRb leading to uncontrolled overexpression of p16.^{55,56} While strong and diffuse cytoplasmic and nuclear p16 expression has been shown to correlate well with HPV status in the cervix^{57–59} and vulva,⁶⁰ it does not appear to have the same predictive value in bladder carcinomas. Cioffi-Lavina *et al*⁶¹ have shown p16 overexpression in 14 out of 38 cases (37%) of bladder squamous cell carcinoma. It is likely that other, non-HPV-related mechanisms are responsible for upregulation of p16 in these cases. In fact, non-HPV-associated p16 overexpression is well established in other tumor types, such as

uterine and ovarian serous carcinomas and non-HPV-associated cervical adenocarcinomas.^{26,62}

The two patients in our study had histories of neurogenic bladder and prolonged catheterization with chronic urinary tract infections, which by themselves are well-established risk factors for the development of bladder squamous cell carcinoma. To our knowledge, this is the first report of HPV-associated bladder carcinoma arising in this setting. Conflicting data exist in the literature regarding the risk of bladder cancer in patients with spinal cord injury and neurogenic bladder. The incidence varies from 0.39%,²⁴ which is comparable to the general population, to 2.4%.²² While the rates of carcinoma vary substantially, all studies showed a strong association of prolonged catheterization with squamous cell carcinoma and adenocarcinoma of the bladder.^{21–24} It is believed that squamous cell carcinoma arises through squamous metaplasia secondary to long-term irritation associated with catheterization and recurrent urinary tract infections.⁶³ Development of adenocarcinoma is less well understood.

Basaloid and warty morphologies are characteristic of HPV-associated squamous cell carcinomas and have been shown to correlate with viral status in oropharyngeal,⁸ vulvar,⁵ penile,³⁸ and anal carcinomas.⁵³ Bladder squamous cell carcinoma very rarely shows basaloid/warty features, which is consistent with the infrequent finding of HPV in bladder squamous cell carcinoma. In fact, it was these morphological features that lead us to perform HPV studies initially. Results of our retrospective analysis confirm the observation. We failed to demonstrate HPV infection in tumors that displayed overt keratinization and did not show basaloid morphology. Aside from our two case studies with a prominent basaloid component, only four such case reports of basaloid squamous cell carcinoma of the urinary tract were found in the English literature.^{64,65} Vakar-Lopez and Abrams⁶⁵ reported a 60-year-old woman who had developed urinary tract incontinence in her 40s due to multiple vaginal deliveries complicated by chronic urinary tract infections and multiple corrective surgeries. The cystectomy specimen contained extensive invasive squamous cell carcinoma with a microscopic component of urothelial cell carcinoma, while the uninvolved urothelium showed squamous metaplasia. Interestingly, *in situ* hybridization for low-risk and high-risk HPV was positive in the areas of squamous metaplasia, but not in the basaloid nests. The second case, reported by Neves *et al*⁶⁴ featured a 54-year-old man with basaloid carcinoma of the bladder associated with a minor small-cell neuroendocrine carcinoma component arising in the background of squamous metaplasia, dysplasia, and carcinoma *in situ*. Of note, the patient had a history of long-standing neurogenic bladder with frequent urinary tract infections secondary to multiple sclerosis. In another case it occurred in the renal pelvis and was associated with a

small-cell carcinoma, while the other arose in the ureter and was associated with a sarcomatous component, consistent with carcinosarcoma.^{66,67} However, no HPV studies were performed in the latter three cases.

Neither one of our patients had documented genital involvement by HPV. Portions of the vaginal tissue submitted with the cystectomy specimens did not show any morphological evidence of viral cytopathic effect in either patient. It is conceivable and likely that the patients had an HPV infection in the genital tract at some point in their lives as HPV is the most ubiquitous sexually transmitted infection with approximately 80% of sexually active individuals harboring the virus during their lifetime.⁶⁸ The virus was then either directly inoculated into the bladder urothelium in the process of catheterization or was transferred by route of ascending infection. Frequent catheterizations over an extended period of time likely contributed to the recurrent infections and irritation of the bladder leading to loss of urothelial integrity and development of squamous metaplasia. Such an environment could be seen as analogous to the transformation zone of the cervix, where there is a continuous substitution of glandular epithelium by squamous cells in response to trauma and infection. In this process, the basement membrane becomes intermittently exposed and serves as an effective binding site for HPV virions, where they undergo conformational change allowing them to enter the basal keratinocytes. The development of an HPV-positive glandular component (adenocarcinoma) in patient 2 is intriguing and could be similar to the process of urothelial tumorigenesis, where glandular-type divergent differentiation is a known phenomenon. The possibility that this adenocarcinoma arose from the intestinal epithelium (appendix) is unlikely due to the absence of HPV DNA in the uninvolved intestinal mucosa. It is more likely that both squamous and glandular components represent divergent differentiation of one histogenesis driven by integrated HPV.

In conclusion, we report two cases of urothelial carcinoma with prominent squamous differentiation associated with high-risk HPV arising in the setting of recurrent urinary tract infections and squamous metaplasia secondary to neurogenic bladder and prolonged catheterization. We hypothesize that repeated catheterization may have an important role in the viral transformation of the epithelium by creating an environment analogous to the cervical transformation zone. Our inability to identify HPV infection in four patients presenting in a similar clinical setting of neurogenic bladder, but with squamous cell carcinoma with prominent keratinization, indicates that urothelial and squamous tumorigenesis in this setting is not exclusive to HPV effect. It is possible that the basaloid morphology may be an important indicator of the viral status in the bladder as it is in other organs. Validation of this hypothesis requires future studies

directed at the patient population undergoing bladder catheterization.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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