We report a 35-year-old man with bladder carcinoma developing 2 months after urethral condyloma acuminatum, with an 8-year history of persistent human papilloma virus-58 infection in the urinary tract. DNA was extracted from paraffin-embedded tissue specimens. Human papilloma virus-6 and -58 were detected in the condyloma, whereas human papilloma virus-58 was detected in the carcinoma. In situ hybridization analysis also demonstrated high-risk human papilloma virus-DNA signals in the condyloma and carcinoma tissues. Immunohistochemistry showed that p16-INK4a and mcm-7, surrogate markers of oncogenic human papilloma virus E7 protein, were weakly expressed in the condyloma tissue but were strongly expressed in the carcinoma tissues, suggesting that human papilloma virus-58 was present in the episomal state in the condyloma, whereas human papilloma virus-58 DNA was integrated into the host cells and its infection may have a role in the development of bladder carcinoma. Human papilloma virus-58 was continuously detected in the urethral brushing samples 8 years after treatment for urethral condyloma, and human papilloma virus-58 infection was still persistent in the urethra.

Key words: condyloma acuminatum – urethra – human papilloma virus – bladder carcinoma

INTRODUCTION

Human papilloma virus (HPV) is known to be the causative agent of condyloma acuminatum and cervical cancer. Several recent studies have also demonstrated etiological roles of high-risk HPV infection in the development of other malignancies, such as carcinoma of the oral cavity, laryngeal carcinoma, penile cancer and anal cancer, and almost 10% of the worldwide cancer burden has been estimated to be linked to HPV infection (1). On the other hand, the reported prevalence of HPV infection in bladder carcinoma ranges from 0 to 81%, and these conflicting results have prevented definitive conclusions being reached regarding the causative role of HPV infection in bladder carcinoma (2–5).

Here, we present a case study suggesting that HPV infection could be persistent in the urinary tract, and that it was likely to have an important etiological role for the development of bladder carcinoma.

CASE REPORT

A 35-year-old man presented with dysuria and genital warts. His past history revealed gonococcal or chlamydial urethritis several years previously. Visual examination identified genital warts measuring 5 mm on the external urethral orifice. Cystourethroscopy also revealed a small non-papillary tumor (Figs 1A and 2) on the urethra extending 5 cm from the
urethral orifice (Figs 1A and 2), and no tumors in the urinary bladder. Transurethral resection was performed in July 2001, and a diagnosis of urethral condyloma acuminatum was made based on histopathological examination. Two months after the operation, the patient presented with gross hematuria and cystoscopy revealed a grain of rice-sized papillary tumor (Fig. 1B) on the trigone of the urinary bladder. Transurethral resection of the bladder tumor was performed in September 2001, and histopathological examination indicated it to be a Grade 1 non-invasive urothelial carcinoma. Although cystoscopic and radiological evaluation revealed no evidence of tumor recurrence, a urethral leukoplakia lesion (Fig. 1C) was found at the same site where the initial urethral condyloma was present in September 2009 (Figs 1C). A brushing cytological examination of the lesion revealed no atypical cells.

After written informed consent, approved by the ethics committee of Kanazawa University Graduate School of Medicine for use of samples, was obtained from the patient, DNA was extracted from paraffin-embedded tumor tissue by microdissection using the Pinpoint Slide DNA Isolation System™ (Zymo Research, Orange, CA). HPV-DNA detection and genotyping were performed using an HPV GenoArray Test Kit (HybriBio Ltd., Chaozhou, China) (6). In situ hybridization (ISH) was performed to detect HPV DNA in tumor tissue using an HPV detection kit according to the manufacturer's instructions (Dako GenoPoint System K0620; Dako, Carpinteria, CA). A wide-spectrum probe (Y1404; Dako) for 13 high-risk HPV DNA (HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) and low-risk HPV DNA (HPV types 6, 11) probes (Y1411; Dako) was hybridized with denatured DNA on tissue samples. In addition, immunohistochemical (IHC) analysis was performed using a Dako ChemMate ENVISION Kit/HRP (DAB)-universal kit (K5007; Dako) to investigate the expression level of oncogenic HPV E7 protein (5). The monoclonal antibodies used were as follows: p16-INK4a (Immuno-Biological Laboratories Co., Ltd., Gunma, Japan) and mcm-7 (Abnova, Taipei, Taiwan).

Based on the results of molecular analysis, HPV6 and HPV58 were detected in the condyloma, and HPV58 was detected in the bladder carcinoma. ISH analysis demonstrated that both high-risk-type HPV-DNA signals and low-risk HPV-DNA signals were observed in the condyloma tissues, and the signals predominantly showed a diffuse pattern (Fig. 3A–C). IHC demonstrated that p16-INK4a and mcm-7 were expressed in the limited area of the condyloma tissue (Fig. 4A and B). On the other hand, ISH showed that high-risk HPV-DNA signals were also detected in the nuclei of the bladder carcinoma cells, and punctate signals for high-risk HPV were observed in the tumor tissue (Fig. 3D and F). No low-risk HPV-DNA signals were detected in the carcinoma cells (Fig. 3E). P16-INK4a and mcm-7 protein were expressed strongly in the carcinoma tissues (Fig. 4C and D), and p16-INK4A was widely expressed in the carcinoma tissues, whereas expression of mcm-7 was often observed on the basal layer of the tumor tissues. Expression levels of these proteins were much higher in bladder carcinoma than
in condyloma. Furthermore, HPV58 was detected again in the brushing samples collected from the leukoplakia, which occurred in the urethra 8 years after the operation.

**DISCUSSION**

In the present case, high-risk-type HPV58 could be detected in bladder carcinoma that occurred in a young patient. It is well known that bladder carcinoma is generally a disease seen in the elderly. Since HPV infection generally occurs after sexual debut in younger age, age is considered to be critical factor for cancer related to HPV infection. Actually, a previous study reported that HPV DNA was detected in 18 (15%) of 117 bladder carcinomas, and demonstrated that younger age (<60 years; odds ratio: 10.9, 95% confidence interval (CI): 2.6–45.3) was one of
the independent factors for detection of HPV in bladder carcinoma (5).

We demonstrated that the same high-risk HPV type was detected in urethral condyloma and bladder carcinoma, suggesting that HPV infection first acquired via the distal urethra by sexual contact could ascend to the urothelium of the bladder. The patient described here had a past history of urethritis, which is associated with high risk for HPV infection (7). Indeed, one previous study indicated that urethritis was associated with an increased risk of bladder carcinoma in males (8). Furthermore, HPV58 was detected in the same urethral site 8 years after treatment of the urethral condyloma lesion. Although we cannot exclude the possibility that this represented reinfection with HPV58, as HPV58 was detected at the same site where the initial urethral condyloma was present, this infection was likely to have been persistent for 8 years. Most incidences of HPV infection are also usually temporary in males, and HPV is considered to be eradicated by the host immune response. Lajous et al. (9) reported a disappearance rate of 89% at ~1 year in men with HPV infection in the external genitalia. The immune response is an important factor for the development of persistent HPV infection, and the patient described here seemed not to readily mount an immune response against viral infection.

In the present case, ISH analysis confirmed the localization of HPV DNA in the tumor tissue. Interestingly, diffuse and punctate patterns of high-risk HPV-DNA signals were observed in the condyloma and bladder carcinoma, respectively. It has been reported that the diffuse patterns represent episomal infection of the HPV genome, while punctate patterns indicate integration of the HPV genome into the host cells (10,11). Although HPV58 was detected in a condyloma sample, ISH analysis showed diffuse signals in the tumor tissue, suggesting that HPV58 infection occurred in the episomal state in the condyloma tissue induced by HPV6 infection. On the other hand, punctate signals for high-risk HPV were observed in the bladder carcinoma, suggesting that HPV58 was likely to be integrated into the carcinoma cells.

Generally, the most prevalent HPV genotypes in patients with condyloma acuminatum are HPV6 and HPV11. However, a previous study demonstrated that high-risk HPV could remain under episomal lesions in external condyloma acuminatum with HPV6 or HPV11 infections (12). We also found that HPV58 might be present under the episomal state in condyloma tissue based on ISH findings. P16-INK4a and mcm-7 expression are linked to HPV-E7 activity, and these molecules are strongly expressed in cervical cancer (13,14). Thus, P16-INK4a and mcm-7 are considered to be surrogate markers of HPV-E7 protein expression in cervical cancer. Our previous study indicated that the levels of p16-INK4a and mcm-7 expression were high in most HPV-positive bladder carcinomas, but these proteins were rarely expressed in HPV-negative cases (5). In the present case, P16-INK4a and mcm-7 protein were also expressed strongly in the carcinoma tissues, whereas these proteins were present in a limited area of the condyloma tissue. These findings suggest that HPV58 was likely to represent episomal infection with weak E7 protein expression in the condyloma, whereas HPV58 infection with high E7 protein expression occurred in the carcinoma. In the present case, HPV58 infection was likely to play an important role for the development of bladder carcinoma, and this case could support a hypothesis of HPV carcinogenesis in the urinary bladder.

Conflict of interest statement
None declared.

References