CASE REPORT DOI 10.1111/j.1365-2133.2007.08094.x

Human papillomavirus type 26 infection causing multiple invasive squamous cell carcinomas of the fingernails in an AIDS patient under highly active antiretroviral therapy

A. Handisurya, A. Rieger, A. Bankier,* A. Koller,† A. Salat,‡ G. Stingl and R. Kirnbauer

*Department of Dermatology, Division of Immunology, Allergy and Infectious Diseases, *Department of Radiology, †Department of Pathology and ‡Department of General Surgery, Medical University of Vienna, Währinger Gürtel 18–20, A-1090 Vienna, Austria*

Correspondence
Reinhard Kirnbauer.
E-mail: reinhard.kirnbauer@meduniwien.ac.at

Accepted for publication
27 April 2007

Key words
human immunodeficiency virus, human papillomavirus type 26, immunosuppression, squamous cell carcinoma

Conflicts of interest
None declared.

Summary

Squamous cell carcinoma (SCC) of the nail unit is a rare disorder. An association with high-risk genital human papillomavirus (HPV) infection has been reported. We report a 28-year-old human immunodeficiency virus (HIV)-infected bisexual man who had multiple invasive SCC of the fingers, infected with the rare type HPV 26. Classification of HPV 26 as high- or intermediate-risk type has been uncertain, due to its rare presence in cervical cancer. Despite successful treatment with highly active antiretroviral therapy (HAART), the patient developed extensive hyperkeratotic nailbed proliferations of all fingers. Tumours were refractory to treatment and invaded into adjacent tissues. X-rays of the hands demonstrated bone invasion, necessitating amputation of distal phalanges of several fingers. Histologically, highly differentiated preinvasive and invasive verrucous SCCs were identified. Molecular DNA typing identified HPV 26 in the SCCs and in some premalignant lesions. By *in situ* hybridization HPV 26 DNA was detected in numerous tumour cells, indicating productive infection with high-level amplification of the viral genome. In the remaining proliferations, high-risk HPV type 58, cutaneous HPVs and a putative new HPV type were identified. HPV 26 infection appears to be causally involved in the development of SCC of the nail unit in this immunosuppressed patient. Timely evaluation of chronic verrucous nailbed tumours is recommended, especially in immunocompromised patients. Identification of HPV 26, besides known high-risk HPV types, may identify patients at risk for developing SCC of the nailbed and possibly at other locations.

Human papillomaviruses (HPVs) are small, nonenveloped, icosahedral tumour viruses, with a double-stranded DNA genome of approximately 8 kb. More than 100 different HPV types have been identified that infect squamous epithelia of skin and mucosa, causing mostly benign papillomas or warts.[1,2] The oncogenic role of high-risk HPV types is firmly established for all cervical dysplasias and cancers, and for a subset of other anogenital and oropharyngeal neoplasias. Certain HPV types cause squamous cell carcinomas (SCCs) of the skin in patients with the rare genodermatosis epidermodysplasia verruciformis (EV),[3] especially on sun-exposed areas, whereas a possible role of HPV in keratinocyte (nonmelanoma) skin cancer of immunocompetent and immunosuppressed individuals is less clear.[4–6]

SCC originating from the nail unit is rare, and misdiagnosis as onychomycosis or verruca vulgaris is quite common. SCC of the finger usually affects older age groups, and men more often than women.[7,8] An association with preceding radiation, chronic trauma or infection, immunosuppression, and exposure to hydrocarbons or arsenic has been reported.[8,9] Infection with high-risk HPV has been causally implicated in the pathogenesis of digital SCC, and the predominant type identified was HPV 16, suggesting that its high oncogenicity largely accounts for it being the main HPV type identified in most cases (similar to cervical cancer). To a lesser extent HPV 31, 33 (our unpublished observation), 34 and 35 have been identified, all closely related phylogenetically to HPV 16, as all are species 9 alpha-papillomavirus[1,7] or species 11 alpha-papillomavirus.[10] Digital SCCs have been reported in patients with concomitant or antecedent genital HPV infection, and a proportion associated with cervical cancer, but most with genital warts or cervical

dysplasia. The presence of the same HPV type in the digital and genital lesions has been identified.[8]

Case report

A 28-year-old human immunodeficiency virus (HIV)-infected bisexual man presented at our department in 2003 with extensive exophytic warts on fingers and toes, genitoanal condylomata acuminata, and perianal bowenoid plaques. Medical history included X-irradiation of anal cancer at 23 years, chronic infection with hepatitis B and C, chronic bronchitis with asthma bronchiale, and hyperuricaemia. Infection with HIV-1 had been diagnosed in 1995, and the nadir CD4+ T-cell count had been 28 cells mm^{-3}. Subsequently he had received several antiretroviral treatment regimens. His current highly active antiretroviral therapy (HAART) consists of lamivudine, tenofovir and ritonavir-boostered atazanavir. HIV replication has been suppressed below the limit of quantification (50 copies mL^{-1}) for 3 years, and his CD4+ T-cell count has risen to approximately 500 mm^{-3}, with a CD4/CD8 ratio of 0·43.

The verrucous acral tumours were initially confined to the nail matrix and the nailbed, and were frequently complicated by painful inflammation of the fingertips. Various conservative and physically destructive methods were repeatedly applied, including keratolytics, podophyllotoxin, imiquimod, cidofovir ointment, and liquid nitrogen. However, the nailbed proliferations remained refractory to conventional therapies. Additionally, acitretin, which has shown modulatory activity on keratinocyte differentiation, was administered orally at a dose of 1 mg kg^{-1} daily for 3 months, but showed no beneficial effect. Warts were surgically removed several times, and eventually the nail matrices were irreversibly destroyed. Nevertheless, tumours relentlessly recurred, several of which progressed across the borders of the nailbeds invading into adjacent periungual skin (Fig. 1a–c). The patient also had recurrent anogenital condylomata with widespread dissemination to adjacent areas. Multiple biopsies of perianal brown to whitish plaques (leucoplakias) (Fig. 1d) revealed high-grade anal intraepithelial neoplasias that were recalcitrant to various conservative and ablative treatments.

Materials and methods

Samples were taken from all fingers, the affected toes, and from paraffin-embedded material from the leucoplakias. DNA was isolated using DNeasy tissue kit (Qiagen, Hilden, Germany) and was tested by two different broad-spectrum polymerase chain reactions (PCRs) for HPV genomes, using degenerate primer pairs PPF1/CP5 or CP4/CP5, as described previously.[11,12] Amplimers were separated on 1·5% agarose gels, stained with ethidium bromide, and analysed by DNA sequencing to determine the HPV type. The HPV 18-positive human cervical cancer cell line HeLa (ATCC CCL-2) was maintained in cell culture and used as a control. To determine analytical sensitivity of the HPV 26 PCR, a known amount of HPV 26 recombinant plasmid (kindly provided by E.M. de Villiers, DKFZ, Heidelberg, Germany) was diluted in DNA isolated from normal skin of a healthy donor, to mimic the situation present in the tumour tissue. Serial dilutions of DNA isolated from the patient, from HPV 26 plasmid plus normal skin, or from HeLa cells were further analysed by PCR

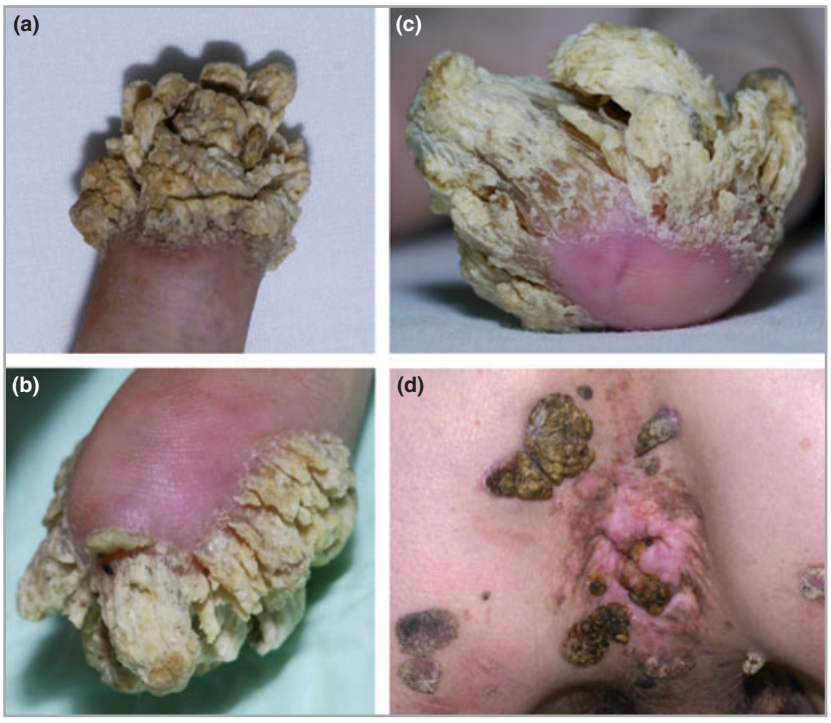

Fig 1. (a–c) Hyperkeratotic verrucous tumours of the fingertips originating from the nailbed. Following surgical removal and destruction of nail matrices, tumours recurred and eventually invaded into adjacent periungual skin. Histology revealed invasive squamous cell carcinomas. (d) Massive involvement of the perianal region with whitish-brown plaques (leucoplakias). Multiple biopsies revealed high-grade squamous intraepithelial neoplasias. Aggregates of hyperkeratotic condylomata acuminata are widely disseminated on the genitalia, perineum and buttocks.

with primers specific for the housekeeping gene β-actin or for HPV 26 E6. HPV 26 E6-specific primer sequences were designed according to published HPV 26 genome sequence (Acc. No. X74472): forward (nt 188–210) 5′-TATATTGCAA-GGAAACCTTACAA-3′, internal reverse (nt 326–347) 5′-TACA-CAGAACATGTATAGCGTC-3′, reverse (nt 549–527) 5′-TTA-CACTTGTGTTTCTGTTTGGC-3′, E6 open reading frame (ORF) spanning forward primer (nt 61–83) 5′-TATAAAAGTAAAAG-GCTAGCTAC-3′ and reverse primer (nt 586–608) 5′-TGCGG-CACCAGATCTAGTATTAC-3′. For *in situ* hybridization, an HPV 26 E6-specific digoxigenin (DIG)-labelled probe was generated by PCR using forward and internal reverse E6 primer and DIG-labelled nucleotides (Roche Diagnostics GmbH, Mannheim, Germany). The labelled probe was further diluted in 2× saline sodium citrate buffer, 50% deionized formamide, 10% dextran sulphate and 250 μg mL^{-1} fish sperm DNA, and *in situ* hybridization was performed on paraffin-embedded tumours using the Rembrandt DISH&HRP Detection Kit (PanPath, Amsterdam, Netherlands) according to the manufacturer's instructions. Swab samples taken from condylomata, perianal plaques and verrucous lesions of fingers and toes were tested for HPV DNA by Hybrid Capture II (hc2) test (Digene Inc., Gaithersburg, MD, U.S.A.).[13]

Results

Conventional X-ray of the right hand in dorsovolar and oblique projections showed extensive papillomatous soft-tissue tumours at the fingertips, and destruction of the bone of distal phalanges of digits I–IV (Fig. 2a, b, arrows). The oblique view additionally revealed osteodestruction of the unguinate process of the index finger (open arrows). Similarly, bony involvement of the distal phalanges of digits I–III of the left hand was observed. X-rays of the toes did not reveal any osteolyses (data not shown). As the bony destruction and clinical aspects strongly suggested invasive malignancies, the patient underwent biopsies followed by disarticulation amputation of terminal phalanges of fingers II–IV of the right hand, and the fifth right toe, under general anaesthesia by a plastic surgeon. Amputation of the right thumb and affected digits of the left hand were postponed to allow the patient to become accustomed to his new handicap, and to observe postoperative medical complications. Systemic treatment with interferon-alpha-2a was initiated (starting with 3×10^6 IU three times weekly with a slow increase to a final dosage of $10{\cdot}5 \times 10^6$ IU three times weekly) as a potential strategy to prevent tumour recurrence and inhibit tumour growth. Perianal condylomata and

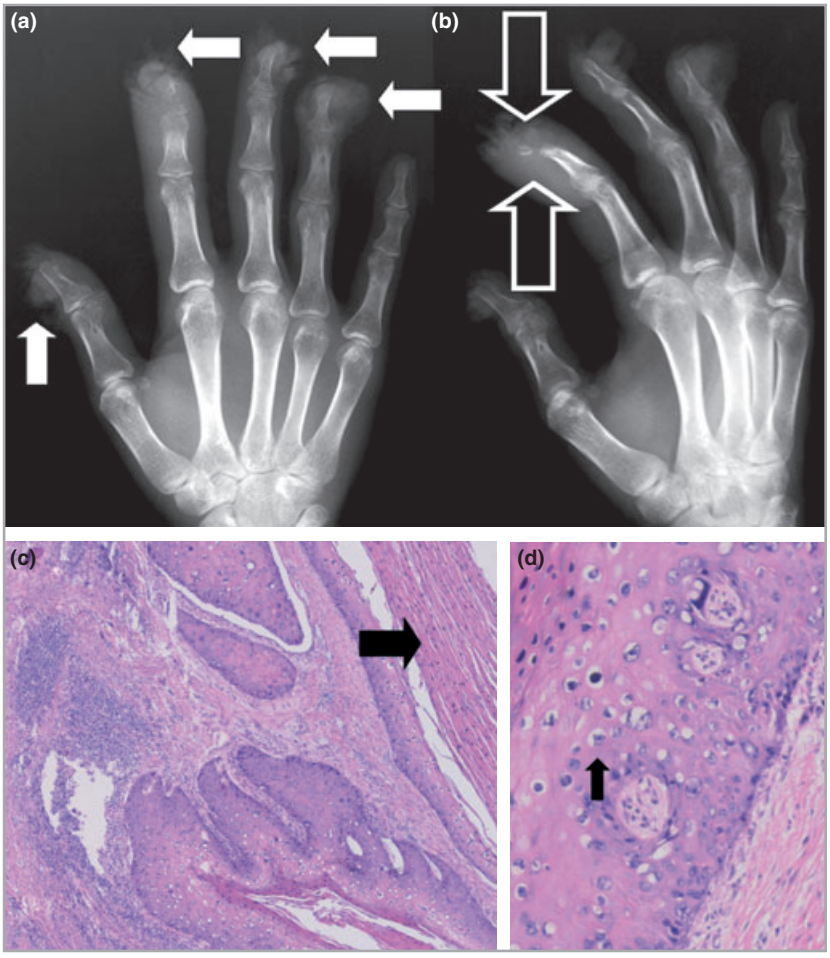

Fig 2. Conventional X-ray of the right hand in dorsovolar (a) and oblique (b) projection. Both radiographic projections show extensive papillomatous soft-tissue tumours at the fingertips (arrows). Oblique view additionally reveals osteodestruction of the unguinate process of the index finger (open arrows). (c) Scanning power view of histological section of the right index finger demonstrates well-differentiated squamous cell carcinoma: infiltration is characterized by expansive growth of squamous tumour nests with pushing borders. The tumour shows massive parakeratosis (arrow). Lymphocytes are present as a demarcation reaction of the peritumoral soft tissue. (d) High-power magnification reveals moderate cellular atypia; mitotic figures are not seen. Nearly all tumour cells show a perinuclear halo (arrow) indicating koilocytes, a typical human papillomavirus-associated phenomenon in epithelial cells (haematoxylin and eosin).

leucoplakias were ablated by a surgeon specialized in proctology (A.S.).

Histological examination of the amputated phalanges revealed invasive SCC. The tumour cells showed a high differentiation level with preserved stratification and massive cornification. Cellular atypia was moderate and mitotic figures were rare. Most cells had a remarkable perinuclear halo indicating koilocytes. The tumour showed an infiltrating growth pattern with complete destruction of the soft tissue and the distal phalanx of the index finger. Multiple biopsies ('mapping') taken from perianal bowenoid plaques revealed multifocal high-grade squamous intraepithelial neoplasias (HSIL) (not shown).

Although metastasis from periungual SCC, independent of the HPV status, is uncommon (2–3%), there are a few reports documenting metastasis to the regional lymph nodes, especially when underlying bones and tendons are infiltrated.[9,14,15] In our patient, regional or distant metastases were not detected by computed tomography.

To detect HPV genomic DNA in material obtained from fingers, toes, condylomata and perianal plaques, PCR was performed using primer pairs PPF1/CP5, which detects at least 64 different HPV types, and CP4/CP5 preferentially amplifying skin-type HPV.[11,12] Amplimers of the expected size were further analysed by DNA sequencing, and the HPV type was identified by BLAST search. Figure 3 (a), lane 1, shows a representative PCR amplimer from DNA isolated from the SCC of the right index finger and primers PPF1/CP5. Surprisingly, HPV 26 was the predominant type detected in about 50% of the samples, isolated from all fingers of the right hand, and from several toes (Table 1). However, we cannot formally exclude the presence of other cutaneous or mucosal HPV types at low levels in the SCC, which were not detected by the employed PCR and hc2 assays. Biological competition with other HPV types, as well as amplification bias for the predominant HPV 26 by PCR, could account for this unusual result. Additionally, skin HPV types 3, 4, 27, 57, 63, 65 and 88 (Kullander *et al.*, manuscript submitted) were detected. The hc2 test for detection of high-risk and low-risk genital HPV was negative for all fingers and most toes, except the left fourth toe (Table 1), indicating high viral load for high-risk HPV for this toe. By further sequence analysis HPV 58, belonging to species 9 of genus alpha-papillomavirus[1] (like HPV 16), was identified in this lesion. Taken together, these results demonstrated the absence of the most common low-risk and high-risk HPVs in the digital SCCs, supporting the hypothesis that the oncogenic effect of the rare type HPV 26 may have been causal to development of invasive digital cancer in this particularly susceptible man. As expected, anogenital condylomata and bowenoid plaques were positive for low-risk and high-risk mucosal HPV types by hc2 test. In addition, HPV 26 was detected by PCR and DNA typing.

Cervical cancers and skin cancers in patients with EV are clonal tumours that typically contain multiple copies of HPV genomes per cell, either episomal and/or integrated into the genome. To obtain further evidence that HPV 26 infection may be causally related to tumour development, the amount of HPV

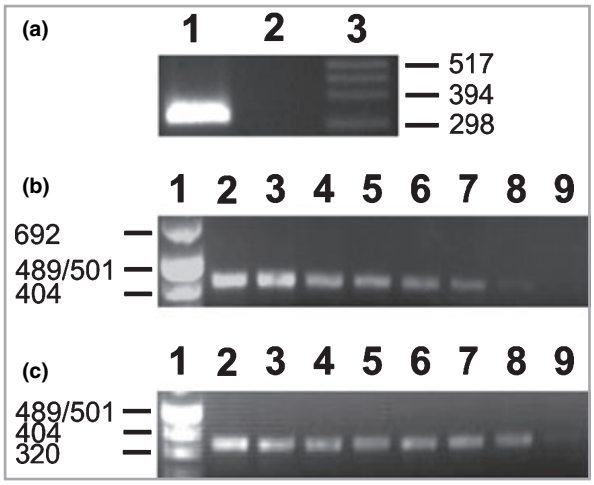

Fig 3. (a) Abundant human papillomavirus (HPV) 26 DNA genome present in the squamous cell carcinoma (SCC) of the fingertip. Polymerase chain reaction (PCR) of DNA isolated from the tumour of the right index finger using primers PPF1/CP5 showed a bright specific band of the expected size at approximately 340 bp, demonstrating the presence of HPV genome in the sample (lane 1). Sequence analysis revealed HPV 26 DNA. Lane 2, negative control; lane 3, DNA molecular weight marker. (b, c) DNA was isolated from paraffin-embedded tissue of the HPV 26-positive SCC of the right index finger. Serial dilutions of DNA (lanes 2–9) were subjected to PCR with primers specific for β-actin and 30 cycles of amplification (b). Detection limit was obtained with 0·1 μL of template DNA (lane 8). Equal amounts of DNA dilutions were analysed for the presence of HPV 26 DNA using HPV 26 E6-specific primers (c). Amplification with only 25 cycles resulted in a strong band at the expected size of 340 bp, with a detection limit at 0·01 μL DNA (lane 9). Comparison with the β-actin detection limit suggests a high abundance of HPV 26 genomes per cell in the DNA isolated from the tumour sample. DNA molecular weight markers (bp) are provided (lanes 1).

Table 1 Human papillomavirus (HPV) skin types present

	HPV type	
	Right	Left
Fingers		
I	26	63
II	26[a]	88
III	26[a]	Not done
IV	26[a]	27
V	27	27
Toes		
I	57	26
II	4	26
III	65	26
IV	65	58[b]
V	26[a]	3

[a]Histologically confirmed squamous cell carcinoma; [b]a high-risk mucosal type related to HPV 16.

26 DNA isolated from the SCC of the right index finger was estimated in relation to the amount of β-actin DNA. DNA was extracted from three paraffin-embedded sections, each at 10 μm, containing approximately 80% tumour tissue. DNA was finally eluted with TE buffer in a final volume of 200 μL. The detection limit for β-actin (PCR at 30 cycles) was determined at 0·1 μL of extracted DNA (corresponding to DNA extracted from 25 HeLa cells; data not shown) (Fig. 3b). The detection limit for HPV 26 was 0·01 μL, from a total volume of 200 μL of extracted DNA (PCR with only 25 cycles, using primers specific for HPV 26 E6) (Fig. 3c). This detection limit corresponds to an estimated 700 000 viral copies, as determined by dilutions of HPV 26 recombinant plasmid spiked into DNA isolated from normal skin. We calculated that $1{\cdot}4 \times 10^{10}$ viral copies were present in the sample extracted from the SCC. These results suggested a high abundance of HPV 26 DNA in the tumour cells. The lower number of cycles for HPV 26 PCR as compared with β-actin PCR, and the presence of HPV 26-negative stromal cells 'diluting' the SCC cells in the biopsy, are both very likely to underestimate the HPV 26 viral load. These results were further confirmed by establishing a real-time PCR method using HPV 26-specific primers. Analysis estimated > 1000 viral copies per cell in the SCC of this patient (Kullander *et al.*, manuscript submitted).

To identify the cells in the SCC that harbour HPV 26 genomes, *in situ* hybridization was performed on paraffin-embedded tumour tissue of the right index finger, using an HPV 26 E6-specific probe. HPV 26 E6 DNA was detected in the nuclei of numerous suprabasal tumour cells (Fig. 4a). In adjacent histological sections, distribution of these HPV 26 DNA-positive cells correlated with the area where koilocytes were most obvious. As a control, an SCC of the skin, taken from an immunocompetent patient, lacked detectable HPV 26 DNA (Fig. 4b). In addition, immunohistochemistry of tissue sections from our patient's SCC showed positive staining of tumour cells with a monoclonal antibody against p16INK4a (data not shown). This cyclin-dependent kinase inhibitor becomes upregulated following inactivation of Rb by E7 oncogene expression in cervical neoplasias, and thus has been used as a biomarker for HPV-associated disease. The control section of the SCC from the same immunocompetent patient as used for the *in situ* hybridization experiments was negative for p16INK4a staining. However, control experiments revealed the functionality of the reagents and antibodies used as well as the correct processing of formalin fixations and paraffin embedding of the sections. In addition, we were unable to examine the tumours for E6 or E7 transcripts, as RNA was found to be degraded following the formalin fixation process.

To determine whether any missense or synonymous nucleotidic changes in the E6 ORF could account for abnormal biological properties of this HPV 26 isolate, DNA was extracted from a histologically confirmed SCC and the DNA sequence of the complete E6 ORF was obtained. By BLAST search, a 100% nucleotide sequence identity was observed between HPV 26 E6 isolated from the SCC and E6 of the HPV 26 prototype sequence (Acc. No. X74472) (not shown).

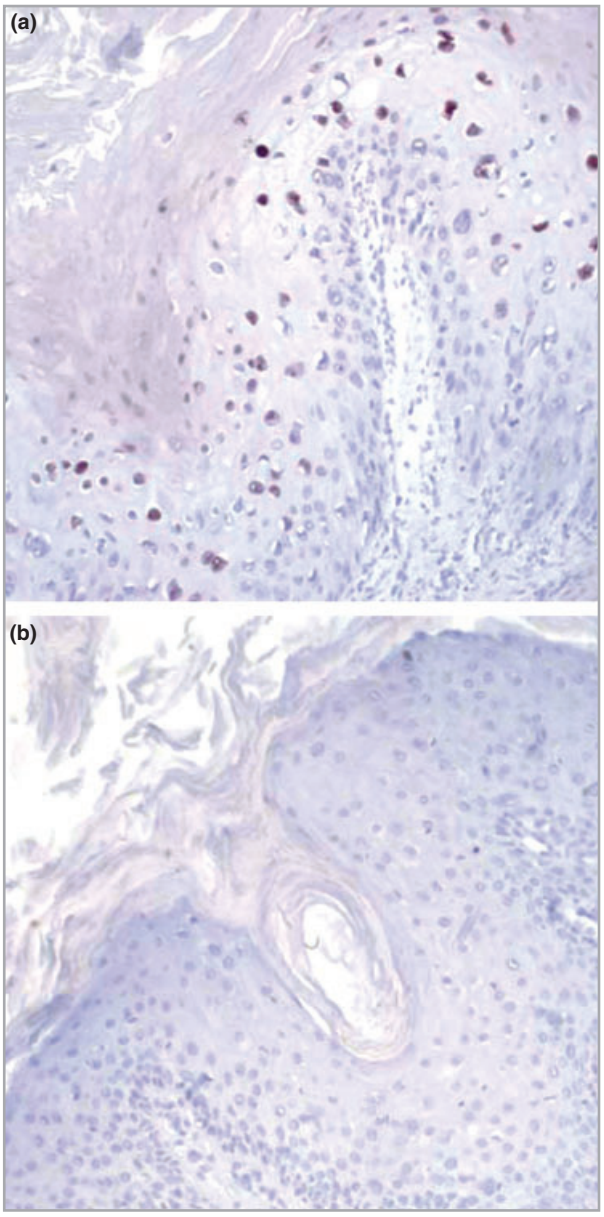

Fig 4. *In situ* DNA hybridization of an invasive digital squamous cell carcinoma (SCC) of the right index finger using human papillomavirus (HPV) 26 E6-specific probes. (a) Amplified HPV 26 DNA was detected in the nuclei of numerous suprabasal tumour cells. (b) The control tumour, an SCC of the skin derived from an immunocompetent patient, was negative for HPV 26 DNA.

Discussion

HPV 26 was first isolated in 1984 from an immunodeficient patient with multiple papillomas.[16] According to the current HPV classification, HPV 26 is categorized phylogenetically as species 5 alpha-papillomavirus, along with 'high-risk' HPV 51, 69 and 82.[1] Muñoz *et al.*[17] have classified HPV 26 as a

probably high-risk type, as HPV 26 was detected in only three of > 1700 cervical carcinomas, but not in > 200 control patients. Due to its very low prevalence, classification of HPV 26 as a high-risk or intermediate-risk HPV has remained uncertain.

Remarkably, almost all reported digital SCCs that have been attributed to HPV were caused by HPV 16 or other species 9 or 11 alpha-papillomavirus.[8,10] To our knowledge there are two other reports of HPV 26 papillomavirus-associated digital SCC, which also occurred in HIV-positive patients.[18,19] In contrast to the case presented herein, in both previous patients only one finger was affected by an SCC. Clinically, one patient displayed a verrucous subungual wart of one finger,[18] and the second patient had warts of multiple fingers.[19] Remarkably, in our patient all fingernails and several toenails were destroyed by enormous verrucous proliferations, several of which had progressed into locally invasive and destructive malignancies.

The presence of HPV 26 genomes in both premalignant and malignant lesions supports the concept that persistent infection with HPV 26 may be responsible for progression into invasive cancer, at least in the setting of immunosuppression. In addition to detecting HPV 26 by a sensitive assay, as in the previous case reports,[18,19] we have also documented that in the SCC each tumour cell contained multiple copies of HPV 26 DNA. The viral load detected is even likely to be an underestimate, due to the dilution factor caused by nonepithelial stromal cells in the biopsy. We also have documented by *in situ* hybridization that vegetative viral DNA replication occurs in the invasive SCC (Fig. 4a).

As all three reported patients with HPV 26-related digital SCC have been infected with HIV, it appears that immunosuppression may either predispose to acquisition of infection by this HPV type, that is not commonly found in malignant tumours attributable to HPV, or to persistence of acral disease and progression into invasive malignancy. This case is, furthermore, a dramatic example where restoration of CD4+ T cells by HAART was not associated with improvement of benign or malignant nongenital or genital lesions attributable to HPV. This finding is in line with previous observations that severely immunosuppressed HIV-infected patients with very low CD4+ T-cell counts at baseline have less complete immune reconstitution after initiation of HAART, despite improvement of CD4+ T-cell counts and suppression of HIV replication below detection levels.[20,21] One explanation might be the persistence of low levels of viral replication with chronic antigen stimulation and T-cell activation, thus preventing a complete immune restoration during HAART.

HPV 26 was also identified in swabs from perianal leucoplakias, which histologically were confirmed as multifocal HSIL. Although we cannot formally exclude an adjunct causal role of HPV 26 coinfection, perianal swab samples were also positive by hc2 test for high-risk (and low-risk) mucosal HPV types, the presence of which suffices to explain the development of perianal intraepithelial neoplasia. We did not further attempt to identify the respective mucosal type(s) at this location. A reasonable proportion of periungual SCC cases in the literature is associated with genital HPV infection, a few of those associated with cervical cancer, but mostly with genital warts or cervical dysplasia.[8] It is likely that our patient's anal cancer was attributable to HPV, developing before initiation of HAART. Compared with the general population, patients with one cancer attributable to HPV are at higher risk of developing a second HPV-related cancer.

Various therapeutic options have been described for HPV-induced digital warts or SCC, including topical treatment, superficial destructive techniques and surgical excision.[7,8,18] Many of these modalities have been applied in our patient (as described above). As warts remained refractory to therapy and signs of invasive malignant transformation were observed, we finally decided to amputate the affected phalanges. As adjunct administration of interferon-alpha has proven beneficial in other HPV-induced tumours,[22] we decided to initiate a systemic treatment with interferon-alpha, to evaluate therapeutic effects on tumour growth of the remaining fingers, and to prevent tumour recurrence on the amputation stumps. Unfortunately, interferon-alpha was ineffective to inhibit growth or induce regression of existing tumours. However, after an observation period of 7–14 months after amputation no signs of tumour recurrence were observed. We refrained from X-ray radiation of the highly differentiated SCC, to avoid possible induction of anaplastic transformation as observed after radiation of verrucous carcinomas.[22,23]

Infection with the rare type HPV 26 may remain underreported unless broad-spectrum HPV PCR systems are used, as screening with the routinely applied hc2 HPV test will not detect HPV 26, whereas high-risk genital-mucosal types including HPV 16, 31, 33 and 58 are readily detected. We suspect that infection with HPV 58, a high-risk type closely related to HPV 16, places our patient at risk for progression to SCC in his fourth left toe (Table 1), although to date no evidence of malignant invasion has been observed.

In conclusion, HPV 26-positive verucous neoplasias of the nailbed may rapidly progress to invasive SCC in the immunocompromised patient. Tumour invasion should be suspected for early recurrent and persistent digital papillomas refractory to treatment. The abundant vegetative HPV 26 DNA replication in an invasive lesion provides convincing evidence for a causal role in tumour development. More experimental and epidemiological data are required to determine whether HPV 26 should be definitively classified as a high-risk type, causing skin cancer at this predilection site.

Acknowledgments

We are thankful to Susanne Drexler, Christopher Nava and Tina Holper for excellent technical assistance, and to Doug Lowy for critical reading of the manuscript.

References

1 de Villiers EM, Fauquet C, Broker TR *et al.* Classification of papillomaviruses. *Virology* 2004; **324**:17–27.

2 Zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer* 2002; **2**:342–50.
3 Kirnbauer R, Lenz P, Okun MM. Human papillomavirus. In: *Dermatology* (Bolognia JL, Jorizzo JL, Rapini RP, eds), Vol. 1. Philadelphia: Elsevier Mosby, 2003; 1217–33.
4 Kiviat NB. Papillomaviruses in non-melanoma skin cancer: epidemiological aspects. *Semin Cancer Biol* 1999; **9**:397–403.
5 Harwood CA, Surentheran T, Sasieni P *et al.* Increased risk of skin cancer associated with the presence of epidermodysplasia verruciformis human papillomavirus types in normal skin. *Br J Dermatol* 2004; **150**:949–57.
6 Forslund O, Lindelöf B, Hradil E *et al.* High prevalence of cutaneous human papillomavirus DNA on the top of skin tumors but not in 'stripped' biopsies from the same tumors. *J Invest Dermatol* 2004; **123**:388–94.
7 Zaiac MN, Weiss E. Mohs micrographic surgery of the nail unit and squamous cell carcinoma. *Dermatol Surg* 2001; **27**:246–51.
8 Alam M, Caldwell JB, Eliezri YD. Human papillomavirus-associated digital squamous cell carcinoma: literature review and report of 21 new cases. *J Am Acad Dermatol* 2003; **48**:385–93.
9 Kopelson PL, Nguyen QH, Moy RL. Verruca vulgaris and radiation exposure are associated with squamous cell carcinoma of the finger. *J Dermatol Surg Oncol* 1994; **20**:38–41.
10 Kawashima M, Jablonska S, Favre M *et al.* Characterization of a new type of human papillomavirus found in a lesion of Bowen's disease of the skin. *J Virol* 1986; **57**:688–92.
11 Petry KU, Menton S, Menton M *et al.* Inclusion of HPV testing in routine cervical cancer screening for women above 29 years in Germany: results for 8466 patients. *Br J Cancer* 2003; **88**:1570–7.
12 Tieben LM, Berkhout RJ, Smits HL *et al.* Detection of epidermodysplasia verruciformis-like human papillomavirus types in malignant and premalignant skin lesions of renal transplant recipients. *Br J Dermatol* 1994; **131**:226–30.
13 Castle PE, Lorincz AT, Mielzynska-Lohnas I *et al.* Results of human papillomavirus DNA testing with the hybrid capture 2 assay are reproducible. *J Clin Microbiol* 2002; **40**:1088–90.
14 Huang KC, Hsu RW, Lee F *et al.* Late inguinal metastasis of a well-differentiated subungual squamous cell carcinoma after radical toe amputation. *Dermatol Surg* 2005; **31**:784–6.
15 McHugh RW, Hazen P, Eliezri YD *et al.* Metastatic periungual squamous cell carcinoma: detection of human papillomavirus type 35 RNA in the digital tumor and axillary lymph node metastases. *J Am Acad Dermatol* 1996; **34**:1080–2.
16 Ostrow RS, Zachow KR, Thompson O *et al.* Molecular cloning and characterization of a unique type of human papillomavirus from an immune deficient patient. *J Invest Dermatol* 1984; **82**: 362–6.
17 Muñoz N, Bosch FX, de Sanjose S *et al.* Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med* 2003; **348**:518–27.
18 High WA, Tyring SK, Taylor RS. Rapidly enlarging growth of the proximal nail fold. *Dermatol Surg* 2003; **29**:984–6.
19 Kreuter A, Brockmeyer NH, Pfister H *et al.* Human papillomavirus type 26-associated periungual squamous cell carcinoma *in situ* in a HIV-infected patient with concomitant penile and anal intraepithelial neoplasia. *J Am Acad Dermatol* 2005; **53**:737–9.
20 Resino S, Rivero L, Ruiz-Mateos E *et al.* Immunity in HIV-1-infected adults with a previous state of moderate-severe immunesuppression and more than 500 CD4+ T cell after highly active antiretroviral therapy. *J Clin Immunol* 2004; **24**:379–88.
21 Giovannetti A, Pierdominici M, Marziali M *et al.* Persistently biased T-cell receptor repertoires in HIV-1-infected combination antiretroviral therapy-treated patients despite sustained suppression of viral replication. *J Acquir Immune Defic Syndr* 2003; **34**:140–54.
22 Geusau A, Heinz-Peer G, Volc-Platzer B *et al.* Regression of deeply infiltrating giant condyloma (Buschke–Lowenstein tumor) following long-term intralesional interferon alfa therapy. *Arch Dermatol* 2000; **136**:707–10.
23 Hagen P, Lyons GD, Haindel C. Verrucous carcinoma of the larynx: role of human papillomavirus, radiation, and surgery. *Laryngoscope* 1993; **103**:253–7.