Comprehensive analysis of human papillomavirus prevalence and the potential role of low-risk types in verrucous carcinoma

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The role of human papillomavirus (HPV) infections in the development of verrucous carcinoma, a well-differentiated variant of squamous cell carcinoma with difficult differential diagnosis, is controversial in the literature. In this study, we analysed verrucous carcinoma from different origins for the presence and activity of a broad spectrum of HPV types, and carefully reviewed the histopathological features. A random series of 27 formalin-fixed, paraffin-embedded specimens of verrucous carcinoma was taken, representing the head and neck region ($n=6$), anogenital area ($n=16$) and extragenital skin region ($n=5$). After review of the histological slides, all samples were subjected to different polymerase chain reaction-based HPV detection techniques, together detecting a total of 83 HPV types, including both mucosal and cutaneous types. Histological revision was carefully performed. Lesions with keratinised papillae, blunt stromal invaginations and minimal cytological atypia were considered verrucous carcinoma. Condylomatous lesions with viral changes were defined as giant condyloma. Verrucous lesions that did not meet those criteria were classified as verrucous hyperplasia. Tumours with stromal infiltration were considered as invasive squamous cell carcinoma. Histological revision revealed that 13 out of 27 cases were verrucous carcinoma (one showing a double infection with HPV 35 and 45), 5 invasive squamous cell carcinomas, 5 verrucous hyperplasia (one with a double infection with HPV 4 and 8), 1 pseudoepitheliomatous hyperplasia and 3 giant condylomas. All three giant condylomas were low-risk HPV positive (HPV 6 and 11) and showed active mRNA transcription. None of the HPV-positive samples tested positive for diffuse p16^{INK4A} staining. In conclusion, our results do not support a causal role of HPV in the development of verrucous carcinoma. Testing for LR-HPV, particularly HPV 6 and 11, may help in the differential diagnosis of lesions suspicious of verrucous carcinoma as those testing positive for LR-HPV most likely represent giant condylomas.

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Verrucous carcinoma is a low-grade, well-differentiated variant of squamous cell carcinoma that was first described by Ackerman in 1948.¹ Verrucous carcinoma typically presents as a slowly enlarging verrucous tumour showing a ‘pushing’ border that compresses the surrounding tissue without true stromal invasion.² Verrucous carcinoma has a benign histopathological appearance with minimal

atypia and few mitotic cells, but behaves locally destructive and seldom has lymph node metastasis.

Because of the absence of epithelial dysplasia and the absence of stromal invasion in the initial phases, most cases are not recognized as being malignant. Therefore, awareness of both the clinician and pathologist is crucial for appropriate diagnosis of verrucous lesions, including the spectrum of verrucous hyperplasia, verrucous carcinoma and stromal invasive squamous cell carcinoma.³ Verrucous carcinoma was originally described as an oral lesion, but subsequent reports identified additional epithelial sites of development, such as skin and anogenital regions. Beside the spectrum of verrucous hyperplasia towards invasive squamous cell carcinoma, other neoplastic lesions like ‘giant

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condyloma' in the anogenital region may show both clinical and histopathological similarities with verrucous carcinoma. Finally, non-neoplastic lesions like lichen simplex chronicus and pseudoepitheliomatous hyperplasia may be considered in the differential diagnosis.²

The pathogenesis of verrucous carcinoma is uncertain.^{4–6} Oral verrucous carcinoma has been associated with the chronic use of tobacco or with the practice of chewing betel nuts,⁷ ultraviolet radiation has been associated with verrucous carcinoma arising from the skin⁸ and chronic lesions like squamous cell hyperplasia and lichen sclerosus have been related to vulvar verrucous carcinoma development.⁹ More recent studies suggest a potential role for human papillomavirus (HPV) in the pathogenesis of verrucous carcinoma, although the literature data are controversial.^{10,11}

HPV are non-enveloped, double-stranded DNA viruses belonging to the family of Papillomaviridae.¹² Papillomaviruses infect epithelial cells and are subdivided into cutaneous and mucosal types. Cutaneous types have been related to both benign skin lesions and non-melanoma skin cancers.¹³ Mucosal HPV types can be found in the epithelial linings of the anogenital, respiratory and upper digestive tracts and are classified as low- or high-risk HPV according to their oncogenic potential.^{14,15} Infections with low-risk types are generally linked to benign wart-like lesions, most of them without risk of carcinogenic transformation. However, occasionally low-risk HPV types have been detected in malignant lesions, such as penile carcinomas.^{16–18} Infection with high-risk HPV has been associated with malignant transformation, and causally related to cervical cancer^{19,20} and a subset of squamous cell carcinomas in the head and neck and anogenital region different from the cervix.^{21–23}

Verrucous carcinoma has been associated with both low-risk (types 6 and 11) and high-risk (types 16 and 18) types of HPV.^{18,24,25} However, a causal relationship between HPV and verrucous carcinoma remains to be proven, specifically in those cases in which a low-risk HPV has been suggested to be involved in the genesis of the tumours.^{5,26}

Some authors have considered verrucous carcinoma and giant condyloma as a spectrum of the same process mainly due to the overlapping histological features,²⁷ suggesting that verrucous carcinoma may evolve from a persistent irritated condyloma. In those cases, low-risk HPV types may initiate the growth process and the chronic epithelial irritation could subsequently contribute to the development of a malignant phenotype.⁹

The aim of this study was to analyse comprehensively verrucous carcinoma from different origins, that is, head and neck region, the anogenital area and extragenital skin for the presence of HPV, including both mucosal and cutaneous types, and to carefully review the histopathological diagnosis. Cases testing positive for low-risk HPV were also

examined for viral E6 mRNA expression and p16^{INK4a} staining to determine their potential contribution to tumour development.

Materials and methods

Patients and Tissue samples

In all, 27 formalin-fixed, paraffin-embedded originally diagnosed verrucous carcinoma from head and neck region ($n=6$), the anogenital area ($n=16$) and the extragenital skin region ($n=5$) were selected from the archives of the Department of Pathology at the VU University Medical Center in Amsterdam, taking into account availability of the blocks. All diagnoses were initially made between 1988 and 2009. All slides were independently reviewed by two pathologists (CM and MB) for consensus diagnosis and to ascertain the presence of a lesion in the sections used for HPV analysis. The revision was performed without knowing the HPV status of the samples. Verrucous carcinoma was defined as a squamous tumour with thick, heavily keratinised papillae and blunt, broad stromal invaginations of well-differentiated squamous cells with minimal cytological atypia and rare mitosis extending into the underlying stroma with broad pushing rather than infiltrating border. Giant condyloma was defined as a verrucous tumour with condylomatous appearance and viral changes, including koilocytosis (perinuclear cytoplasmic clearing with peripheral condensation of the cytoplasm) and significant cytonuclear changes such as irregular nuclear contours, hyperchromasia and binucleation/multinucleation. Verrucous lesions that did not meet the criteria for verrucous carcinoma or condyloma were classified as verrucous hyperplasia. Tumours with destructive stromal infiltration were considered as invasive squamous cell carcinoma.

Two formalin-fixed, paraffin-embedded condylomata acuminata with a single HPV 6 infection were included as controls for transcription analysis. Table 1 shows the clinicopathological data of the selected tissue samples.

Tissue Preparation and Nucleic-Acid Isolation

Formalin-fixed, paraffin-embedded samples were serially sectioned (10 sections $\times$ 10 μ m) on a microtome.

The first and last sections (3 μ m) were H&E stained for histomorphological confirmation of tumour presence. In-between sections were collected in RNase-free reaction tubes (one for DNA isolation and one for RNA isolation). Sectioning and sample preparation were carried out with the highest measures to avoid contamination and cross-contamination. Paraffin blocks lacking tissue were cut in-between the patient samples as controls to assure the lack of contamination. None of these control samples were positive in the HPV polymerase chain reaction

Table 1 Overview of clinicopathological data of verrucous lesions analysed

ID	Sex	Age (years)	Location	Original diagnosis	Consensus diagnosis	HPV status
1	Female	78	Vulva	Verrucous carcinoma	Verrucous carcinoma ^a	Negative
2	Female	82	Anus	Verrucous carcinoma	Invasive squamous cell carcinoma	Negative
3	Male	34	Anus	Verrucous carcinoma	Giant condyloma ^a	HPV 6
4	Female	70	Vulva	Verrucous carcinoma	Verrucous carcinoma	Negative
5	Female	88	Vulva	Verrucous carcinoma	Invasive squamous cell carcinoma	Negative
6	Female	74	Vulva	Verrucous carcinoma	Invasive squamous cell carcinoma	Negative
7	Male	78	Penile	Verrucous carcinoma	Verrucous hyperplasia	HPV 4, 8
8	Female	78	Vulva	Verrucous carcinoma	Invasive squamous cell carcinoma	Negative
9	Female	61	Perineum	Verrucous carcinoma	Giant condyloma ^a	HPV 6
10	Male	66	Penile	Verrucous carcinoma	Verrucous hyperplasia	Negative
11	Male	50	Penile	Verrucous carcinoma	Verrucous hyperplasia	Negative
12	Female	52	Skin	Verrucous carcinoma	Verrucous hyperplasia	Negative
13	Female	81	Vulva	Verrucous carcinoma	Invasive squamous cell carcinoma	Negative
14	Female	80	Vulva	Verrucous carcinoma	Giant condyloma ^a	HPV 11
15	Female	94	Vulva	Verrucous carcinoma	Verrucous carcinoma ^a	Negative
16	Female	59	Head and neck	Verrucous carcinoma	Verrucous carcinoma	HPV 35, 45
17	Female	92	Vulva	Verrucous carcinoma	Verrucous carcinoma ^a	Negative
18	Male	39	Head and neck	Verrucous carcinoma	Verrucous carcinoma	Negative
19	Male	57	Head and neck	Verrucous carcinoma	Verrucous hyperplasia	Negative
20	Male	68	Skin	Verrucous carcinoma	Verrucous carcinoma	Negative
21	Female	50	Vulva	Verrucous carcinoma	Verrucous carcinoma	Negative
22	Female	68	Skin	Verrucous carcinoma	Verrucous carcinoma	Negative
23	Female	79	Head and neck	Verrucous carcinoma	Verrucous carcinoma	Negative
24	Male	76	Skin	Verrucous carcinoma	Verrucous carcinoma	Negative
25	Female	64	Head and neck	Verrucous carcinoma	Verrucous carcinoma	Negative
26	Male	58	Skin	Verrucous carcinoma	Pseudoepitheliomatous hyperplasia	Negative
27	Male	66	Head and neck	verrucous carcinoma	Verrucous carcinoma	Negative
28	Female	33	Vulva	Condyloma acuminata	Condyloma acuminata	HPV 6
29	Female	21	Vulva	Condyloma acuminata	Condyloma acuminata	HPV 6

^aCytomorphological signs of viral activity (ie koilocytosis, double nuclei, coarse granula).

(PCR) assay, indicating adequate avoidance of contamination.

DNA extraction was performed by incubation of deparaffinised sections in 0.1 mg/ml proteinase K solution overnight at 55 °C. Subsequently, proteinase K was heat-inactivated by incubation of the sections at 100 °C for 10 min, and samples were spun down and cooled down at -20 °C for 1–2 min. DNA was isolated using NucliSens easyMAG platform isolation system (bioMérieux, Boxtel, The Netherlands), according to the manufacturer's instructions.

RNA was isolated by digestion of deparaffinised sections in 0.1 mg/ml proteinase K solution overnight at 52 °C, followed by RNA extraction with TRIzol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's protocol. The final RNA pellet was washed twice with 200 µl of 70% ethanol, air-dried and re-suspended in 20–50 µl of RNase/DNase free water.

DNA and RNA yields were quantified spectrophotometrically using the Nanodrop ND-1000 (Isogen Life Science, IJsselstein, The Netherlands).

HPV DNA Detection and Typing

To assess the quality of the DNA, β-globin PCR analysis was performed using the primers BGPCO3 and BGPCO5 as described previously.²⁸ The PCR products were analysed by electrophoresis on a 1.5%

agarose gel. All samples were β-globin PCR-positive, indicating adequate sample quality.

Mucosal HPV DNA was detected by the L1-region-based, GP 5+/6+ PCR as described by Jacobs *et al.*²⁹ Mucosal HPV DNA were typed by Reverse Line Blot (RLB) as described previously.³⁰ This system allows the typing of both high-risk HPV (types 16, 18, 26, 30, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, 70, 73, 82i and 82m) and low-risk HPV (6, 11, 34, 40, 42, 43, 44, 54, 55, 57, 61, 64, 71, 72, 81 and CP6108).

In addition, we used the SPF10-LiPA system (SPF10 HPV LiPA, version 1; Labo Bio-Medical Products, Rijswijk, The Netherlands), as described previously,^{31,32} to test for the presence of the remaining low-risk HPV types HPV 53 and 74.

Cutaneous HPV DNA were detected and typed by L1-region-based BGC-HPV PCR-RLB as described previously,³³ detecting 24 β and γ HPV types (HPV types 4, 5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 48, 49, 50, 60 and 65). Additional cutaneous types 1, 2, 3, 7, 10, 27, 28, 29, 40, 41, 43, 57, 63, 75, 76, 77, 80, 88, 91, 92, 93, 94, 95 and 96 were tested for using the PM-PCR reverse hybridization assay method (Diassay BV, Rijswijk, The Netherlands) as described by de Koning *et al.*⁹ All samples were tested in duplicate and all duplicate assays were highly (25/27; 92.6%) concordant both with respect to HPV positivity and typing results.

Table 2 Primers designed to detect E6 mRNA expression of the low-risk HPV types

HPV type	Sequence	Target gene	Amplicon size (bp)
HPV6	F: 5'-GTGCAAGACGTTTAATCTATCTATGC-3' R: 5'-TATGAATAAATCTCTGCTGTGGTC-3'	E6	91
HPV11	F: 5'-CACACTCTGCAAATTCAGTGCG-3' R: 5'-CACACAACCTTTAGGTTCTTATAGGC-3'	E6	92
snRNP U1A	F: 5'-CAGTATGCCAAGACCGACTCAGA-3' R: 5'-GGCCCCGGCATGTGGTGCATAA-3'	Housekeeping gene	215

F, forward; R, reverse.

The U1 small nuclear ribonucleoprotein-specific A protein was included as a quality control of the procedure.

Reverse transcriptase-PCR

Before reverse transcriptase-PCR (RT-PCR) analysis, RNA samples were RQ1-DNase-treated according to the manufacturer's recommendations (Promega, Leiden, The Netherlands). Subsequently, reverse transcription using AMV RT (Promega) of RNA into cDNA was performed essentially as described before.³⁴ A reaction without RT was run in parallel for each specimen to control for DNA contamination.

To assess the quality of all RNA specimens, RT-PCR for the housekeeping gene encoding the U1 small nuclear ribonucleoprotein-specific A protein (snRNP U1A, 215 bp) was performed. Primers, annealing temperature and amplicon length are listed in Table 2.

Immunohistochemical staining for p16^{INK4a}

Immunohistochemical analysis for p16^{INK4a} was performed on all the HPV-positive samples. First, sections from formalin-fixed, paraffin-embedded tissues and appropriate positive and negative control tissues were deparaffinised. Then, for all samples, endogenous peroxidase activity was blocked by incubating sections in hydrogen peroxide. Antigen retrieval was performed by heating at 100 °C for 15 min in 10 mM sodium citrate buffer (pH 6.0) in a microwave oven (600 W). After blocking of nonspecific binding by pre-incubation with normal rabbit serum, sections were incubated with primary monoclonal antibodies against p16^{INK4a} (CINtec clone ink4a; MTM Laboratories Heidelberg, Germany). Biotinylated rabbit anti-mouse Fab fragment was applied as a secondary antibody for 1 h, followed by incubation with avidin-biotin complex.

Signals were detected using diaminobenzidine and the sections were counter-stained with haematoxylin. Sections treated with PBS instead of the primary antibody were used as negative controls. Cervical carcinoma sections were included in each staining experiment as positive controls for p16^{INK4a}.

Results

HPV detection

To analyse comprehensively the presence of HPV, we applied four different PCR-based techniques

allowing the detection of a total of 83 HPV types, including both mucosal and cutaneous types. HPV DNA, as a single or multiple infection, was detected in 18.5% (5/27) of the lesions analysed, and included low-risk HPV, high-risk HPV and cutaneous HPV types.

Revision revealed that 13 lesions were compatible with verrucous carcinoma, 5 with stromal invasive squamous cell carcinoma and 5 with verrucous hyperplasia. Giant condylomas were diagnosed in 3 cases and 1 case showed pseudoepitheliomatous hyperplasia. Taken both results together, all 3 giant condylomas were low-risk HPV positive, 2 anal lesions containing HPV 6 and the giant condyloma of the vulva containing HPV 11. A double infection with HPV 35 and 45 was found in 1 (1/13) verrucous carcinoma, which was located in the oral cavity and a double infection with HPV 4 and 8 was detected in 1 (1/5) verrucous hyperplastic lesion located in the penis. All invasive squamous cell carcinomas and the pseudoepitheliomatous hyperplastic lesion were HPV negative. Table 1 shows the HPV detection and typing results in relation to the histopathological diagnosis of all samples. In Figure 1 representative photographs of the different lesions are shown.

E6 mRNA expression

As all samples positive for low-risk HPV were upon review diagnosed as giant condylomas, which has previously been suggested as a starting point of verrucous carcinoma development,^{9,27} we next measured HPV E6 mRNA expression in these samples to determine viral activity. We included two HPV 6-positive condylomata acuminata as controls for transcriptional assays.

RT-PCR analysis revealed E6 mRNA expression in all 3 giant condylomas and both control condylomata acuminata (Figure 2).

p16^{INK4a} immunostaining

To determine the presence of a so-called transforming infection, as is particularly seen in high-grade premalignant cervical lesions and cancers associated with a high-risk HPV infection, we subjected all samples testing positive for HPV to p16^{INK4a} staining. The low-risk HPV-positive samples either

Figure 1 Verrucous carcinoma is a verrucous tumour with blunt broad stromal invaginations of well-differentiated squamous cells with minimal cytological atypia and rare mitosis extending into the underlying stroma with broad pushing rather than infiltrating border (a, b). Invasive squamous cell carcinoma present with destructive stromal infiltration (c, d). Giant condyloma is a verrucous tumour with condylomatous appearance and viral changes, including koilocytosis (perinuclear cytoplasmic clearing with peripheral condensation of the cytoplasm) and significant cytonuclear changes, such as irregular nuclear contours, hyperchromasia and binucleation/multinucleation (e, f). Differential diagnoses of verrucous carcinoma includes verrucous hyperplasia (g, h) or pseudo-epitheliomatous hyperplasia (i, j). Squares indicate locations of pictures shown in the right panels.

did not show any staining or showed sporadically cells with weak, mainly cytoplasmic staining.

The high-risk HPV-positive verrucous carcinoma (HPV 35/45) and the verrucous hyperplasia containing skin HPV (HPV 4/8) were also negative for p16^{INK4A} staining.

p16^{INK4A} was also performed on both control condylomata acuminata. One showed a focal patchy staining pattern and the other was negative for p16^{INK4A}.

Discussion

As a potential role of HPV in the development of verrucous carcinoma is still a matter of debate, we extensively explored the presence of HPV in 27 samples taken from the archives of the Department of Pathology of the VUMC and originally diagnosed as verrucous carcinoma. Testing for a total of 83 HPV types, including both mucosal and cutaneous types,

Figure 1 Continued.

revealed HPV positivity in 18.5% (5/27) of the verrucous tumours.

Careful histopathological revision without prior knowledge of the HPV status revealed 13 verrucous carcinomas, 5 verrucous tumours with focal stromal invasion being defined as invasive squamous cell carcinoma, 5 verrucous hyperplasias, 3 giant condylomas and 1 pseudoepitheliomatous hyperplasia. A double infection with high-risk HPV types (HPV 35 and 45) was detected in a verrucous carcinoma of the head and neck region, and a double infection with cutaneous HPV types (HPV 4 and 8) was found in a verrucous hyperplastic lesion of the penis. Hence, only 1 out of 13 (7.7%)

verrucous tumours revised as verrucous carcinoma was HPV positive. All three verrucous tumours that were revised as giant condylomas were positive for low-risk HPV: two giant condylomas of the penis were positive for HPV 6 and one giant condyloma of the vulva was positive for HPV 11. These results suggest that HPV testing can help in the differentiation between verrucous carcinoma and giant condyloma in difficult cases, favouring verrucous carcinoma in case of HPV negativity and giant condyloma in case of HPV positivity.

The prevalence of HPV in verrucous carcinoma in our study was rather low, although the literature data are inconsistent. While some studies on vulvar

Figure 1 Continued.

and penile verrucous carcinoma have failed to detect HPV DNA,^{35–37} others have described HPV DNA positivity rates of up to 50%.^{2,17,38}

Revision of the verrucous tumours, initially diagnosed as verrucous carcinoma, suggests that these lesions can be misclassified. Verrucous carcinoma and giant condylomas may share architectural and morphological similarities complicating the differential diagnosis between these entities. Moreover, stromal invasion can be very focal in a lesion with an appearance of a verrucous carcinoma in the bulk of the tumour. However, it has been accepted that tumours with stromal invasion should be classified as invasive squamous cell carcinoma.^{2,39} In addition, as known from the literature, the exact distinction between verrucous hyperplasia and verrucous carcinoma is not clearcut and the exact criteria are lacking.^{3,40}

Previous studies concluded that verrucous carcinoma from mucosal areas are related to mucosal HPV types and verrucous carcinoma located in cutaneous areas are not specifically linked to any specific group of HPV.⁴¹ In this study, we found that mucosal types were indeed restricted to mucosal lesions, that is, high-risk HPV in a verrucous carcinoma of the head and neck region and low-risk HPV types in giant condylomas of the anus and vulva, whereas cutaneous HPV types were detected in a verrucous hyperplastic lesion of the penis.

Several hypotheses have been proposed to explain a potential role of HPV infection in the development of verrucous carcinoma. For high-risk HPV types, it has been well established that the viral genes *E6* and *E7* drive malignant transformation in case of cervical squamous cell carcinomas and adenocarcinomas.⁴² For verrucous carcinoma arising from the head and

neck region, it has been reported that high-risk HPV are opportunistic viruses enhancing other carcinogenic factors such as tobacco or alcohol.^{43,44} In case of low-risk HPV types, sequence variations increasing their transforming potential has been described.²⁶ Moreover, it has also been suggested that malignant transformation may be more related to the host response to the HPV infections than to the virus itself.¹¹

Giant condylomas have been considered as a different spectrum of the same entity as verrucous carcinoma,^{27,45} suggesting that a chronic irritated condylomata caused by a low-risk HPV could evolve to a more aggressive lesion as a verrucous carcinoma.^{38,46}

RT-PCR analysis of the verrucous carcinoma testing positive for low-risk HPV revealed *E6* mRNA expression in all of them, indicating that the virus is actively present. However, the absence of diffuse p16^{INK4A} staining is in agreement with earlier observations that low-risk HPV do not interfere with the cell cycle, as is seen upon pRb inactivation by high-risk HPV E7. The same pattern (*E6* mRNA expression and the absence of diffuse p16^{INK4A} positivity) was observed in the two control condylomata acuminata analysed, strengthening the hypothesis that condylomata acuminata, giant condyloma and verrucous carcinoma belong to a different spectrum of the same entity. Also, the fact that the oral verrucous carcinoma testing positive for HPV35 and 45 was p16^{INK4A} negative indicates that the virus is most likely not involved in the development of the oral verrucous carcinoma. Recent studies have proposed diffuse p16^{INK4A} staining as the first step in an algorithm for detecting clinically relevant HPV infections in squamous cell

Figure 2 Agarose gel read-outs of reverse transcriptase polymerase chain reactions (PCRs) determining mRNA expression of human papillomavirus (HPV) 6 and HPV 11 *E6* and the housekeeping gene *U1A*, respectively, in low-risk HPV-positive samples and condylomata acuminata. (a) HPV 6 *E6* and *U1A* mRNA expression in two giant condylomas that were HPV 6 DNA positive (b). HPV 6 *E6* and *U1A* mRNA expression in two condylomata acuminata (c). HPV 11 *E6* and *U1A* mRNA expression in a vulvar giant condyloma. RT + and RT – represent the results with and without reverse transcriptase added to the reverse transcriptase reaction before PCR.

carcinoma of head and neck, as the overexpression of p16^{INK4A} is strongly related with the viral oncogene activity.⁴⁷ The absence of a causal relation in the oral verrucous carcinoma is also supported by the fact that the PCR signals obtained for HPV35 and 45 DNA were rather weak.

From these data, we conclude that HPV is unlikely to be causally related to verrucous carcinoma development. In addition, testing for low-risk HPV

may aid in histopathological diagnosis of lesions suspected of verrucous carcinoma as the low-risk HPV-positive cases are most likely to represent giant condylomas.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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