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Genomic characterization of a novel human papillomavirus (HPV-117) with a high viral load in a persisting wart☆

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ABSTRACT

Warts from immunosuppressed organ transplant recipients (OTR) persist over years and may progress into non-melanoma skin cancer. Human papillomaviruses (HPV) are considered the causal agents for the development of such warts. We isolated the novel type HPV-117 from a persisting wart by rolling circle amplification. One hundred eighteen warts from immunocompetent patients (IC) and 49 warts from OTR were analyzed by HPV-117 E6 type-specific PCR. As inferred from a phylogenetic analysis, the new type HPV-117 belonged to alpha-PV species 2, including the most similar types HPV-10 and HPV-94. The general prevalence of HPV-117 in warts was 2% in IC (2/118), and 12% in OTR (6/49). The high viral load in dysplastic cells of a *Verruca vulgaris* was shown by *in situ* hybridization. Our results suggest an active role of the novel type in the development of cutaneous warts of OTR.

Introduction

The complete genome of more than 100 human papillomavirus (HPV) types has been characterized. HPV are classified into mucosotropic (alpha-PV), cutaneotropic (beta-PV and gamma-PV) and cutaneous, wart-associated types (species 2 and 4 of alpha-PV, mu-PV and nu-PV) based on sequence similarities and clinical manifestations (de Villiers et al., 2004). Most HPV types have been initially isolated and identified from genital/mucosal lesions, and PCR based methods have identified a huge number of L1 gene fragments of putatively novel cutaneous HPV types (Forslund et al., 1999, 2003). By convention, a new PV type requires the isolation and characterization of the entire genome (de Villiers et al., 2004), and viral genomic fragments are not sufficient to identify novel PV types.

In the general population, cutaneous warts are induced by alpha-PV species 2 (HPV-3, -10, -28, -29, -77 and -94) and species 4 (HPV-2, -27 and -57) as well as by mu-PV (HPV-1 and -63) and nu-PV (HPV-41). HPV-2, -27 and -57 are the most prevalent types with this respect (Rübben et al., 1993, 1997). From a phylogenetic perspective, cutaneotropic alpha-PV species 2 and species 4 may derive from the otherwise mucosotropic alpha-PV (Muñoz et al., 2003).

A large number of organ transplant recipients (OTR) develop warts and wart-like lesions during immunosuppression. Presence of cutaneous warts increase with time after transplantation, and 39% of 560 OTR have developed *Verrucae vulgares* after 7 years (Bouwes Bavinck et al., 2007). Cutaneous warts of OTR persist over years, are often atypical and can show dysplasia. Moreover, they are associated and co-localize with non-melanoma skin cancer, indicating that benign warts may progress into malignant skin cancer during immunosuppression (Blessing et al., 1989; Euvrard et al., 2003).

A more diverse range of cutaneotropic HPV types has been reported in warts of OTR compared to immunocompetent patients (IC) (Shamanin et al., 1994; Obalek et al., 1992; Harwood et al., 1999). However, a subpopulation of warts from OTR does not appear infected with known HPV types, although they show all characteristics of HPV-induced skin alterations (Köhler et al., 2009). Here, we report the complete genome of a novel, wart-associated HPV type isolated from a persisting wart of an OTR, who has been previously considered HPV negative. We examine the histological localization of the novel type by *in situ* hybridization and the prevalence in warts of IC ($n=118$) and of OTR ($n=49$) using type-specific PCR.

Results and discussion

The novel type had the principle genome organization found in the majority of mucosotropic alpha-PV types, consisting of the four large genes E1, E2, L2 and L1 and the three smaller genes E6, E7 and E5 (García-Vallvé et al., 2005). The complete genome of HPV-117 comprised 7895 bp, and the L1 nt sequence showed the highest similarity of 87.5% with both HPV-10 and HPV-94, as inferred from a

☆ The GenBank/EMBL/DDBJ accession number for the sequence of HPV-117 reported in this paper is GQ246950.

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sequence similarity matrix (available at http://htcc.pt-dlr.de/dateien/KoehlerAlpha.xls). Primary sequence analysis, including position and length of the ORF, are summarized in Table 1. The non-coding region was located upstream of the E6 gene, and downstream of the L1 gene, and comprised 675 nt. Potential polyadenylation signals in this region were present for the early genes (nt 4421) and the late genes (nt 7447).

The alignment for the multi-gene phylogenetic analysis was 2421 aa positions in length, and 1553 of these sites (64%) were parsimony-informative (55.5 per terminal taxon). Fig. 1 shows the best-scoring ML-tree (−ln = 67264.44), with the statistical support values for the two phylogenetic approaches used. alpha-PV were monophyletic, irrespectively whether the data were analyzed under the likelihood criterion (bootstrap support value from ML analysis: 100 LBS) or the Bayesian approach (Bayesian posterior probability: 1.00 BPP). Low-risk human alpha-PV types were a paraphyletic assemblage, from which PV clades with other character traits derived. Such clades included non-human PV that were polyphyletic, as well as high-risk human alpha-PV types. Cutaneous wart-associated alpha-PV types constituted two monophyletic groups (alpha-PV species 2 and 4) that were only distantly related to each other. The new type HPV-117 belonged to alpha-PV species 2 (100 LBS, 1.00 BPP), but the closest relative could not be determined unequivocally (either HPV-10 or HPV-94). The results support the principle correspondence between phylogenetic relationships and viral properties within mucosotropic alpha-PV (Muñoz et al., 2003).

The ability of PV to induce proliferation of the infected cells has been attributed mainly to the two viral oncogenes E6 and E7. The corresponding oncoproteins bind host cellular proteins, with the tumor suppressors, p53 and retinoblastoma protein (pRb), as major targets (Münger et al., 2004). The E7 ORF of the vast majority of PV contains the conserved pRb-binding core sequence LxCxE (Boulet et al., 2007). However, this motif is absent from a subset of alpha-PV species 2 including HPV-117 (Figs 1 and 2). The lack of a pRb-binding motif is rare among PV and is only known from ruminant delta-PV and epsilon-PV as well as from a few solitary other types. Moreover, this character trait may correlate with specific pathologies (Narechania et al., 2004).

A number of zinc-binding domains is characteristic for the two oncogenes E6 and E7 (Nomine et al., 2006; Liu et al., 2006). The E7 ORF of HPV-117 exhibited one regular zinc-binding domain at aa position 50 [CxxC(x)$_{29}$CxxC] (Fig. 2). However, the E6 ORF contained only one of two regular zinc-binding domains [CxxC(x)$_{29}$CxxC] (data not shown). The first started at aa position 29 and was separated by 36 aa from the mutated second motif. The loss of the second zinc-finger domain at nt position 417 was due to a missense mutation (A instead of G), resulting in a replacement of tyrosin for cystein at aa position 105 (C105Y). This mutation was also found in sequences amplified from 8 warts that were infected with HPV-117 using type-specific primers. Thus, the loss of the second zinc-finger domain is not due to experimental artefacts, but is biologically present in the novel type HPV-117.

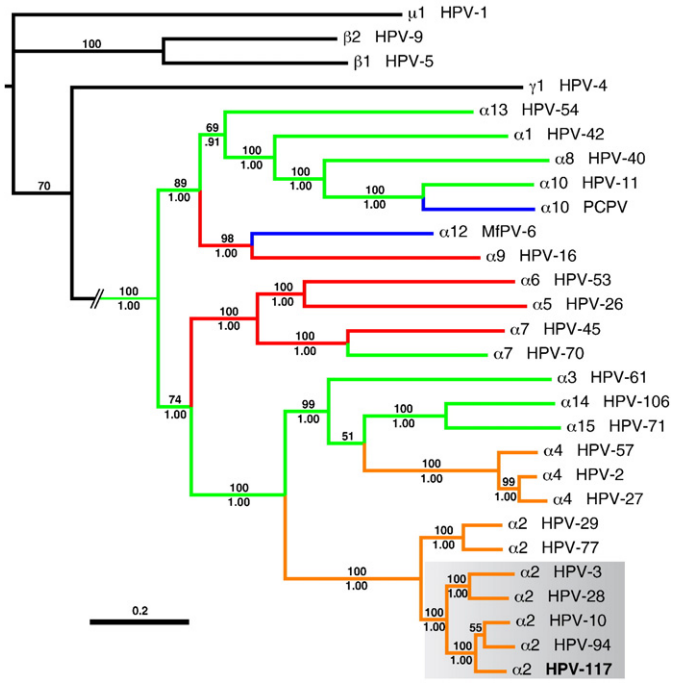

Fig. 1. HPV-117 belongs to alpha-PV species 2. ML tree of PV comprising a representative set of 24 alpha-PV, as inferred from a combined E1–E2–L2–L1 amino acid (aa) analysis (1553 parsimony-informative aa positions). HPV-117 is highlighted by bold lettering. PV character traits are colored green (human alpha-PV low-risk types), red (human alpha-PV high-risk types), blue (non-human alpha-PV types) and ochre (wart-associated alpha-PV types), respectively. Branch lengths are drawn to scale (outgroup types are in black). Scale bar, number of aa substitutions per site. Numbers on branches are bootstrap support values to clusters of the right of them (above: ML criterion; below: Bayesian posterior probabilities; values under 50 and 0.90 are not shown). HPV types lacking the pRb-binding motif (see Fig. 2) are highlighted by the shaded box.

As a component of alpha-PV species 2, HPV-117 had a putative E5 ORF (nt 4152-4295) between the genes E2 and L2, as it has been also described for the closely related type HPV-94 (de Villiers and Gunst, 2009). In alpha-PV, four types of E5 proteins have been identified, with different biochemical properties and corresponding to phylogenetic groups (Bravo and Alonso, 2004). The E5beta-protein is present in alpha-PV species 2, 3, 4, 14 and 15 including HPV-117 and has a distinct hydrophobic profile over its aa sequence.

Overall, little is known about the causal HPV types in warts of immunosuppressed OTR due to the small number of studies analyzing the presence of HPV in warts of this cohort. Cutaneous (beta-, and gamma-PV) and wart-associated HPV types (species A2 and A4 of alpha-PV, mu-, and nu-PV) were found in 76% (37/49) of OTR by PCR based methods. HPV-117 was detected in warts from 2% (2/118) of the general population and from 12% (6/49) of OTR. Of these HPV-117 positive warts, multiple infections with additional cutaneous HPV

Table 1
Primary sequence analysis of the HPV-117 genes and sequence similarity to the closely related types HPV-10 and HPV-94.

Gene(s)	Genome length	E6	E7	E1	E2	E4[a]	L2	L1
Number of nucleotides [*nt*]	7895	444 (103–546)	264 (525–788)	2040 (795–2834)	1149 (2776–3924)	333 (3359–3688)	1392 (4433–5824)	1602 (5721–7322)
Number of amino acids [aa]	–	147	87	679	382	110	463	533
Molecular weight [kDa][b]	–	19.8	11.7	91.7	51.6	14.9	62.5	72.0
Sequence similarity [percentage nt (percentage aa)]	HPV-10	86 (82)	91 (86)	90 (92)	87 (83)	87 (80)	86 (89)	88 (92)
	HPV-94	86 (79)	93 (88)	89 (91)	88 (84)	87 (79)	84 (87)	88 (91)

Putative E5 ORF (nt 4152–4295).
[a] Putative E4 without ATG.
[b] Average molecular weight of about 135 Da.

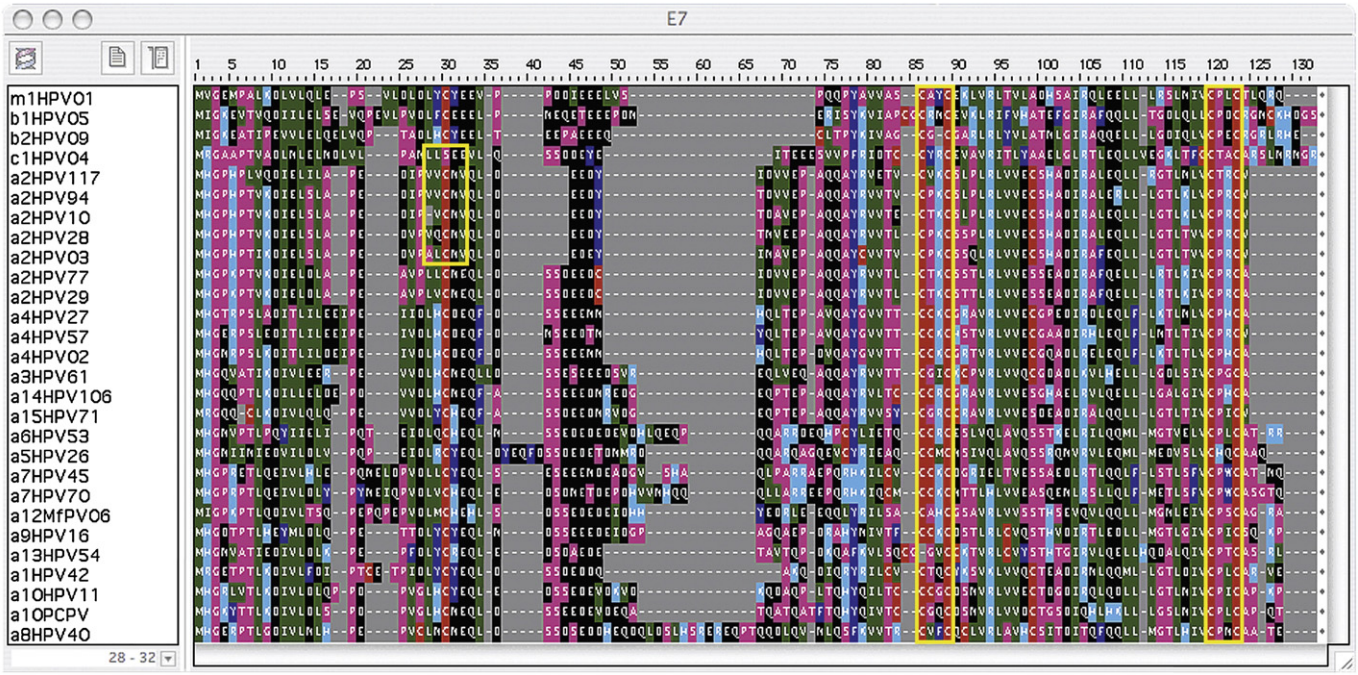

Fig. 2. A subset of alpha-PV species 2 do not exhibit a pRb-binding motif in the E7 open reading frame (ORF). Alignment of the E7 ORF of different PV types. Note that some alpha-PV species 2, constituting a monophyletic group (Fig. 1) and including the novel type HPV-117, lack the pRb-binding motif LxCxE at alignment positions 28–32 (indicated by a yellow box). Additionally, the dimeric C-terminal zinc-binding domain [CxxC(x)$_{29}$CxxC] constraining the protein's fold is indicated by yellow boxes.

types were found in both lesions of IC and in 4/6 of OTR, while single infections were present in 2/6 warts of OTR. Moreover, the novel type was present in the nucleus of dysplastic keratinocytes of the wart shown by *in situ* hybridization (Fig. 3). The high viral load, and the presence in the lesion, suggests that HPV-117 is the etiological agent of this cutaneous wart.

In conclusion, HPV-117 belongs to alpha-PV species 2, whose types induce cutaneous warts and share viral properties. The new type is localized in dysplastic keratinocytes and is a widely distributed type among OTR. To further determine the pathogenicity of the novel type, functional analyses of viral genes in human cells and human primary keratinocytes, and their interactions with UV-radiation, are required. Our study may contribute to a better understanding how benign warts may progress into malignant skin cancer during immunosuppression.

Materials and methods

Patients

The *Verruca vulgaris* from an immunosuppressed renal transplant recipient (42-year-old male) persisted over 24 months. The wart was removed from the back of the right hand by deep curettage to the dermis at the time of surgery. The biopsy was divided into two halves, one was snap frozen in liquid nitrogen and stored at −80 °C, and the other half of the biopsy was fixed in formalin and embedded in paraffin. Paraffin embedded sections of the *Verruca vulgaris* were used for histological evaluation and *in situ* hybridization. Moreover, we examined 167 paraffin embedded warts (confirmed by histology), including 118 IC and 49 immunosuppressed OTR. This study was approved by the local ethics committee at the Charité, University Hospital, Berlin, Germany (number Si. 248) and was conducted according to the Declaration of Helsinki.

HPV detection and isolation of new types

DNA was isolated from tissue using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). HPV detection of cutaneous types (beta-, and gamma-PV) and wart-associated types (species A2 and A4 of alpha-PV, mu-, and nu-PV) was performed by PCR based methods as previously described (Köhler et al., 2009). To obtain novel HPV type(s), rolling circle amplification (RCA) of the episomal viral DNA was performed with a TempliPhi 500 Amplification kit (Amersham Biosciences, Munich, Germany). We followed an optimized protocol using additional nucleotides to obtain higher folds of amplification as previously described (Schulz et al., 2009). Purified DNA of the complete HPV genome was cloned into pUC19 (Invitrogen, Karlsruhe, Germany) at the *Bam*HI restriction site, and sequencing was performed by primer walking (GENterprise, Mainz, Germany). The clone, and the corresponding complete genome sequence, was submitted to the International Reference Centre for Papillomaviruses at the German Cancer Research Centre, Heidelberg, Germany. The novel type was designated HPV-117 (GenBank accession no. GQ246950).

Protein prediction and phylogenetic analyses

Open reading frames (ORF) were predicted using 'ORFinder' (http://www.ncbi.nlm.nih.gov/gorf/gorf.html). They were confirmed by the manual alignment of nucleotide (nt) and amino acid (aa) sequences to homologous regions of most similar PV types in 'BioEdit' (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Primary sequence analysis of the predicted proteins was performed with 'ProtParam' (www.expasy.ch/tools/protparam.html), 'Prosite' (Hulo et al., 2008) and 'PSORT II' (http://psort.ims.u-tokyo.ac.jp/form.html).

The taxon sample for phylogenetic analyses covered the currently known diversity of wart-associated PV types, embedded in a representative set of alpha-PV, and comprised 28 complete PV sequences (including HPV-117 and 4 outgroup taxa; see Supplementary Table S1). After exclusion of the highly divergent E4 gene region, an aa alignment (comprising the genes E1–E2–L2–L1) was constituted using 'MAFFT' (v6.523; (Katoh et al., 2005); http://align.bmr.kyushu-u.ac.jp/mafft/software/). The final matrix is available at http://htcc.pt-dlr.de/dateien/KoehlerAlpha.nex. A sequence identity matrix of a

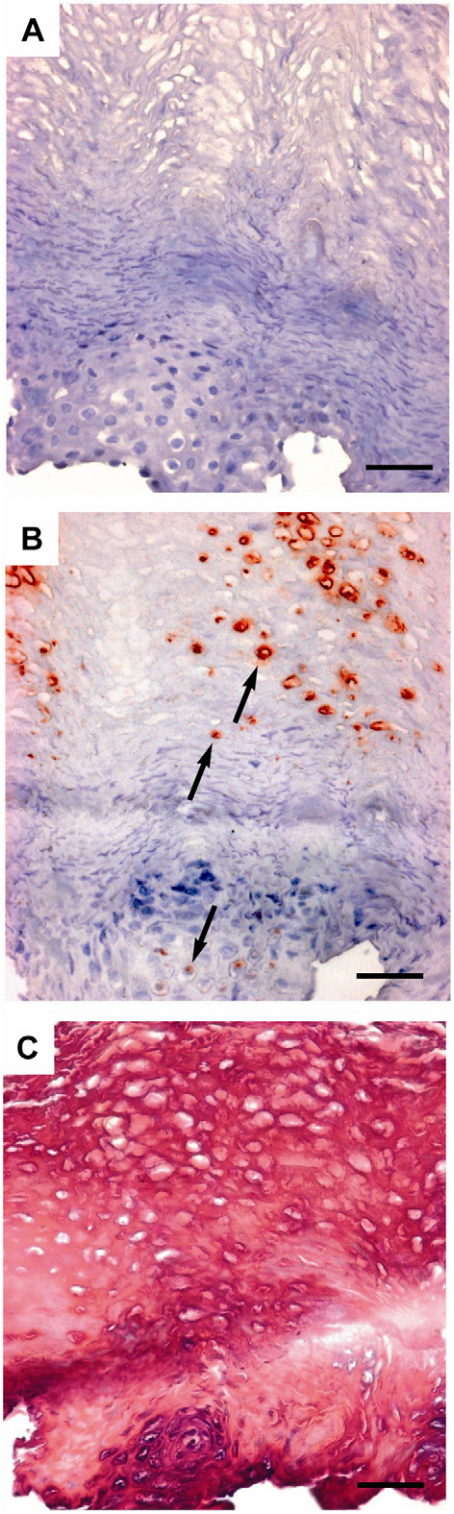

Fig. 3. HPV-117 DNA detection in *Verruca vulgaris* by *in situ* hybridization. Three consecutive sections of a 24-month persisting wart from an immunosuppressed renal transplant recipient (42-year-old male). (A) Negative control, the section was stained with cutaneotropic HPV-8. (B) The section was stained with the type-specific genome of HPV-117 that was isolated from this wart. HPV-117 DNA (red spots marked by arrows) was only present in the nucleus of dysplastic keratinocytes. (C) Staining with hematoxylin and eosin. Histology showed characteristic features of *Verruca vulgaris*: Vacuolar transformation and parakeratosis was observed in the thickened horny layer as well as acanthosis in the spinous and granular layer of the epidermis (scale bar = 50 μm).

L1 gene nt alignment also constituted in 'MAFFT' was generated by 'BioEdit' (v7.0.0; (Hall, 1999). Phylogenetic analyses were conducted using 'RAxML' (Stamatakis, 2006; Stamatakis et al., 2008); http://www.phylo.org/portal/Home.do) and 'MrBayes' v3.1.2 (Huelsenbeck and Ronquist, 2001), applying the rtREV substitution model (Dimmic et al., 2002) as previously described (Gottschling et al., 2007). Bayesian calculations were carried out by using the resources of the Computational Biology Service Unit from Cornell University, which is partially funded by Microsoft Corporation. We ran two independent analyses of 4 (1 cold and 3 heated) mcmc chains with 6,000,000 cycles, sampled every 1000th cycle, with an appropriate burn-in (10%).

In situ hybridization

In situ hybridization of paraffin-embedded tissue sections (5 μm) was performed as previously described (Nafz et al., 2007). Briefly, the tissue was digested with proteinase K and pre-hybridized. Hybridization was conducted with pre-hybridization buffer, supplemented with 500 ng biotinylated HPV genome DNA (HPV-117 or HPV-8, both cloned in pUC19) using a biotin-nick translation kit (Roche, Mannheim, Germany). After the washing steps, slides were incubated with a streptavidin-horseradish peroxidase (HRP) conjugate, and the signal was amplified with the biotin derivative and amplification reagent biotinyltyramid (Perkin Elmer, Norwalk, CT). Red precipitate of 3-amino-9-ethylcarbazol chromogen substrate (AEC; Dako, Hamburg, Germany) activated by HRP represent HPV DNA, and nuclei were counterstained with hematoxylin.

Type-specific PCR

To determine the prevalence of the novel type, we analyzed cutaneous warts of IC and OTR. HPV-117 E6 type-specific PCR was performed with forward and reverse primers (5′-GCCTCAAACATTGG-GAACAT-3′ and 5′-CAGTCGTCGTCGTTCGTTAC-3′). Each amplification mix contained in a final volume of 50 μl 0.5 μmol L^{-1} of each gene-specific primer, 0.2 mmol L^{-1} dNTPs, 1.5 mmol L^{-1} $MgCl_2$ in 1× PCR buffer (Qiagen), 0.3 units of Taq polymerase (Qiagen) and 1 μL of template DNA. The corresponding PCR was performed under the following conditions: initial denaturation at 94 °C for 4 min; 40 cycles comprising the steps 94 °C for 45 s, 60 °C for 45 s, 72 °C for 45 s, and final elongation at 72 °C for 4 min. The PCR products with a size of 152 bp were separated by electrophoresis in 2% agarose gel, and the isolated products were sequenced. The sequences were aligned using the software program CodonCode Aligner (version 3.0, CodonCode Corporation, Dedham, USA) and were compared to the prototype sequence of HPV-117.

Conflict of interest statement
The authors state no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.virol.2009.12.023.

References

Blessing, K., McLaren, K.M., Benton, E.C., Barr, B.B., Bunney, M.H., Smith, I.W., Beveridge, G.W., 1989. Histopathology of skin lesions in renal allograft recipients—an assessment of viral features and dysplasia. Histopathology 14, 129–139.

Boulet, G., Horvath, C., Vanden Broeck, D., Sahebali, S., Bogers, J., 2007. Human papillomavirus: E6 and E7 oncogenes. Int. J. Biochem. Cell Biol. 39, 2006–2011.
Bouwes Bavinck, J.N., Euvrard, S., Naldi, L., Nindl, I., Proby, C.M., Neale, R., Abeni, D., Tessari, G.P., Feltkamp, M.C., Claudy, A., Stockfleth, E., Harwood, C.A., 2007. Keratotic skin lesions and other risk factors are associated with skin cancer in organ-transplant recipients: A case-control study in The Netherlands, United Kingdom, Germany, France, and Italy. J. Invest. Dermatol. 127, 1647–1656.
Bravo, I.G., Alonso, Á., 2004. Mucosal human papillomaviruses encode four different E5 proteins whose chemistry and phylogeny correlate with malignant or benign growth. J. Virol. 78, 13613–13626.
de Villiers, E.M., Gunst, K., 2009. Characterization of seven novel human papillomavirus types isolated from cutaneous tissue, but also present in mucosal lesions. J. Gen. Virol. 90, 1999–2004.
de Villiers, E.M., Fauquet, C., Broker, T.R., Bernard, H.U., zur Hausen, H., 2004. Classification of papillomaviruses. Virology 324, 17–27.
Dimmic, M.W., Rest, J.S., Mindell, D.P., Goldstein, R.A., 2002. rtREV: An amino acid substitution matrix for inference of retrovirus and reverse transcriptase phylogeny. J. Mol. Evol. 55, 65–73.
Euvrard, S., Kanitakis, J., Claudy, A., 2003. Skin cancers after organ transplantation. N. Engl. J. Med. 348, 1681–1691.
Forslund, O., Antonsson, A., Nordin, P., Stenquist, B., Hansson, B.G., 1999. A broad range of human papillomavirus types detected with a general PCR method suitable for analysis of cutaneous tumours and normal skin. J. Gen. Virol. 80, 2437–2443.
Forslund, O., Ly, H., Reid, C., Higgins, G., 2003. A broad spectrum of human papillomavirus types is present in the skin of Australian patients with non-melanoma skin cancers and solar keratosis. Br. J. Dermatol. 149, 64–73.
García-Vallvé, S., Alonso, Á., Bravo, I.G., 2005. Papillomaviruses: different genes have different histories. Trends Microbiol. 13, 514–521.
Gottschling, M., Stamatakis, A., Nindl, I., Stockfleth, E., Alonso, Á., Bravo, I.G., 2007. Multiple evolutionary mechanisms drive papillomavirus diversification. Mol. Biol. Evol. 24, 1242–1258.
Hall, T.A., 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.
Harwood, C.A., Spink, P.J., Surentheran, T., Leigh, I.M., de Villiers, E.M., McGregor, J.M., Proby, C.M., Breuer, J., 1999. Degenerate and nested PCR: A highly sensitive and specific method for detection of human papillomavirus infection in cutaneous warts. J. Clin. Microbiol. 37, 3545–3555.
Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.
Hulo, N., Bairoch, A., Bulliard, V., Cerutti, L., Cuche, B.A., de, C.E., Lachaize, C., Langendijk-Genevaux, P.S., Sigrist, C.J., 2008. The 20 years of PROSITE. Nucleic Acids Res. 36, D245–D249.
Katoh, K., Kuma, K., Toh, H., Miyata, T., 2005. MAFFT version 5: improvement in accuracy of multiple sequence alignment. Nucleic Acids Res. 33, 511–518.
Köhler, A., Meyer, T., Stockfleth, E., Nindl, I., 2009. High viral load of human wart-associated papillomaviruses (PV) but not beta-PV in cutaneous warts independent of immunosuppression. Br. J. Dermatol. 161, 528–535.
Liu, X., Clements, A., Zhao, K., Marmorstein, R., 2006. Structure of the human Papillomavirus E7 oncoprotein and its mechanism for inactivation of the retinoblastoma tumor suppressor. J. Biol. Chem. 281, 578–586.
Münger, K., Baldwin, A., Edwards, K.M., Hayakawa, H., Nguyen, C.L., Owens, M., Grace, M., Huh, K., 2004. Mechanisms of human papillomavirus-induced oncogenesis. J. Virol. 78, 11451–11460.
Muñoz, N., Bosch, F.X., de Sanjose, S., Herrero, R., Castellsague, X., Shah, K.V., Snijders, P. J., Meijer, C.J., 2003. Epidemiologic classification of human papillomavirus types associated with cervical cancer. N. Engl. J. Med. 348, 518–527.
Nafz, J., Köhler, A., Ohnesorge, M., Nindl, I., Stockfleth, E., Rösl, F., 2007. Persistence of *Mastomys natalensis* papillomavirus in multiple organs identifies novel targets for infection. J. Gen. Virol. 88, 2670–2678.
Narechania, A., Terai, M., Chen, Z., DeSalle, R., Burk, R.D., 2004. Lack of the canonical pRB-binding domain in the E7 ORF of artiodactyl papillomaviruses is associated with the development of fibropapillomas. J. Gen. Virol. 85, 1243–1250.
Nominé, Y., Masson, M., Charbonnier, S., Zanier, K., Ristriani, T., Deryckère, F., Sibler, A.P., Desplancq, D., Atkinson, R.A., Weiss, E., Orfanoudakis, G., Kieffer, B., Travé, G., 2006. Structural and functional analysis of E6 oncoprotein: Insights in the molecular pathways of human papillomavirus-mediated pathogenesis. Mol. Cell 21, 665–678.
Obalek, S., Favre, M., Szymanczyk, J., Misiewicz, J., Jabłońska, S., Orth, G., 1992. Human papillomavirus (HPV) types specific of Epidermodysplasia verruciformis detected in warts induced by HPV3 or HPV3-related types in immunosuppressed patients. J. Invest. Dermatol. 98, 936–941.
Rübben, A., Krones, R., Schwetschenau, B., Grussendorf Conen, E.I., 1993. Common warts from immunocompetent patients show the same distribution of human papillomavirus types as common warts from immunocompromised patients. Br. J. Dermatol. 128, 264–270.
Rübben, A., Kalka, K., Spelten, B., Grussendorf-Conen, E.I., 1997. Clinical features and age distribution of patients with HPV 2/27/57-induced common warts. Arch. Dermatol. Res. 289, 337–340.
Schulz, E., Gottschling, M., Bravo, I.G., Wittstatt, U., Stockfleth, E., Nindl, I., 2009. Genomic characterization of the first insectivoran papillomavirus reveals an unusually long, second non-coding region and indicates a close relationship to *Betapapillomavirus*. J. Gen. Virol. 90, 626–633.
Shamanin, V., Glover, M., Rausch, C., Proby, C., Leigh, I.M., zur Hausen, H., de Villiers, E. M., 1994. Specific types of human papillomavirus found in benign proliferations and carcinomas of the skin in immunosuppressed patients. Cancer Res. 54, 4610–4613.
Stamatakis, A., 2006. RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690.
Stamatakis, A., Hoover, P., Rougemont, J., 2008. A rapid bootstrap algorithm for the RAxML Web servers. Syst. Biol. 57, 758–771.