

Novel Human Papillomavirus Type 174 from a Cutaneous Squamous Cell Carcinoma

Boštjan J. Kocjan, Andrej Steyer, Martin Sagadin, Lea Hošnjak, Mario Poljak

Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

B.J.K. and A.S. contributed equally to this work.

We report the cloning and characterization of a novel human papillomavirus (HPV), now officially recognized as HPV-174, isolated from a cutaneous squamous cell carcinoma. HPV-174 has a genome organization characteristic of cutaneous HPV types, clusters to the genus *Betapapillomavirus*, species $\beta 2$, and is most closely related to HPV-145.

Received 22 May 2013 Accepted 28 May 2013 Published 5 July 2013

Citation Kocjan BJ, Steyer A, Sagadin M, Hošnjak L, Poljak M. 2013. Novel human papillomavirus type 174 from a cutaneous squamous cell carcinoma. *Genome Announc.* 1(4): e00445-13. doi:10.1128/genomeA.00445-13.

Copyright © 2013 Kocjan et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/3.0/).

Address correspondence to Mario Poljak, mario.poljak@mf.uni-lj.si.

Human papillomaviruses (HPVs) from the genus *Betapapillomavirus* (β -PV) have been found in association with various benign and malignant cutaneous lesions, including nonmelanoma skin cancer in immunocompromised individuals; however, the role of these viruses in tumor formation is still unclear (1). Currently, over 40 β -PV types have been completely characterized and officially recognized, and are placed into 6 different species (2). We report the complete genome sequence of a novel β -PV type isolated from a 64-year-old male patient suffering from a facial squamous cell carcinoma.

A partial HPV L1 gene sequence of 373 bp (ENA accession number HF970576) was obtained using β -PV broad-range nested PCR (1) from a specimen additionally containing HPV-9 and a putative subtype of HPV-150 (FR822732). A complete viral genome was preamplified using rolling-circle amplification (3) and then amplified using inverse long-range PCR with primer pair V001FwLNG3 (5'-CCAACGAACCTTAGGCAAC-3', nucleotides [nt] 6310 to 6328) and V001RwLNG3 (5'-TCCTGACCTGCTTTGGC-3', nt 6309 to 6293). The resulting amplicon was cloned using a TOPO XL PCR cloning kit (Invitrogen, Carlsbad, CA) and sequenced at Microsynth AG (Balgach, Switzerland) using a primer-walking strategy. The sequence of a complete viral genome was assembled and characterized using Vector NTI Advance 11 software (Invitrogen) and phylogenetically evaluated using a maximum-likelihood algorithm, as described previously (4). A reference clone, covering the full genome of HPV-174, was deposited in March 2013 in the Reference Centre for Papillomaviruses (recently moved to Stockholm, Sweden), where its sequence was verified and the genotype officially named in May 2013.

The complete genome of HPV-174 has 7,359 bp and a G+C content of 40.2%. Its genome organization is typical of cutaneotropic HPVs, containing a classical long control region (LCR) and five early (E1, E2, E4, E6, and E7) and two late (L1 and L2) genes but no E5 open reading frame (ORF). Multiple binding sites for transcriptional regulatory factors (such as AP-1, NF-1, and SP-1) were identified within the LCR genomic region using SIGSCAN software V4.05 (5), in addition to two consensus palin-

dromic E2-binding sites (ACC-N₆-GGT), two putative TATA boxes (TATAAA) of the E6 promoter, and the putative polyadenylation site (ATATAA) necessary for processing viral late gene mRNA transcripts (6, 7). Two characteristic zinc-binding domains [CxxC(x)₂₉₋₃₀CxxC], separated by 36 amino acids, were identified in the putative E6 protein and one was identified in E7. The E7 protein additionally contains an LxCxE motif responsible for binding to the cell retinoblastoma protein (7, 8). The HPV-174 largest putative protein, E1, contains 601 amino acids, and the conserved ATP-binding site (GPPDTGKS) of the ATP-dependent helicase is located in its carboxyterminal part (9). The putative E4 gene has an initiation codon and is completely positioned within the E2 gene. No leucine-zipper domain was observed in the putative E2 protein (7). According to the results of phylogenetic analysis, HPV-174 clusters to the β -PV species $\beta 2$ and is most closely related to HPV-145. To conclude, the genetic characterization of HPV-174 expands the heterogeneity of members of species $\beta 2$ of the genus β -PV. The role of HPV-174 in the development of skin cancer remains elusive.

Nucleotide sequence accession number. The complete genome sequence of HPV-174 is available in the ENA, GenBank, and DDBJ databases under the accession number [HF930491](https://www.ncbi.nlm.nih.gov/nuccore/HF930491).

ACKNOWLEDGMENT

No financial support was received.

REFERENCES

1. Potočnik M, Kocjan BJ, Seme K, Luzar B, Babič DZ, Poljak M. 2006. Beta-papillomaviruses in anogenital hairs plucked from healthy individuals. *J. Med. Virol.* 78:1673–1678.
2. Bernard HU, Burk RD, Chen Z, van Doorslaer K, Zur Hausen H, de Villiers EM. 2010. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology* 401: 70–79.
3. Rector A, Tachezy R, Van Ranst M. 2004. A sequence-independent strategy for detection and cloning of circular DNA virus genomes by using multiply primed rolling-circle amplification. *J. Virol.* 78: 4993–4998.
4. Kocjan BJ, Hošnjak L, Seme K, Poljak M. 2013. Complete genome

- sequence of a novel human betapapillomavirus, HPV-159. *Genome Announc.* 1(3):e00298-13. doi:[10.1128/genomeA.00298-13](https://doi.org/10.1128/genomeA.00298-13).
5. Prestridge DS. 1991. Signal scan: a computer program that scans DNA sequences for eukaryotic transcriptional elements. *Comput. Appl. Biosci.* 7:203–206.
 6. Tachezy R, Duson G, Rector A, Jenson AB, Sundberg JP, Van Ranst M. 2002. Cloning and genomic characterization of *Felis domesticus* papillomavirus type 1. *Virology* 301:313–321.
 7. de Villiers EM, 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.
 8. Lehoux M, D'Abramo CM, Archambault J. 2009. Molecular mechanisms of human papillomavirus-induced carcinogenesis. *Public Health Genomics* 12:268–280.
 9. Titolo S, Pelletier A, Sauvé F, Brault K, Wardrop E, White PW, Amin A, Cordingley MG, Archambault J. 1999. Role of the ATP-binding domain of the human papillomavirus type 11 E1 helicase in E2-dependent binding to the origin. *J. Virol.* 73:5282–5293.