Virology 499 (2016) 82–90

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Comparison of human papillomavirus type 16 replication in tonsil and foreskin epithelia

Mohd Israr [b,1], Jennifer Biryukov [a], Eric J. Ryndock [c], Samina Alam [a], Craig Meyers [a,*]

[a] Department of Microbiology and Immunology, The Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033, USA
[b] Department of Pediatrics, Division of Allergy and Immunology, The Feinstein Institute for Medical Research, Northwell Health, Manhasset, NY 11030, USA
[c] Molecular and Cellular Oncogenesis Program, The Wistar Institute, Philadelphia, PA 19104, USA

ARTICLE INFO

Article history:
Received 27 June 2016
Returned to author for revisions
29 August 2016
Accepted 6 September 2016

Keywords:
Human papillomavirus
Head and neck cancer
Oropharyngeal cancer
HPV capsid proteins

ABSTRACT

Human papillomavirus (HPV) is well recognized as a causative agent for anogenital and oropharyngeal cancers, however, the biology of HPV infection at different mucosal locations, specifically the oral cavity, is not well understood. Importantly, it has yet to be determined if oral tissues are permissive for HPV infection and replication. We investigated for the first time the titers, infectivity, and maturation of HPV16 in oral epithelial versus genital epithelial tissue. We show that infectious HPV16 virions can be produced in oral tissue. This demonstrates, for the first time, that infectious virus could be spread via the oral cavity. HPV16 derived from oral tissue utilize a tissue-spanning redox gradient that facilitates the maturation of virions over time. Maturation is manifested by virion stability and increased susceptibility to neutralization with anti-HPV16 L1 antibodies. However, susceptibility to neutralization by anti-HPV16 L2 specific antibodies decreases during the maturation of HPV16 virions in oral tissue.

1. Introduction

In a recent report to the nation published in the Journal of the National Cancer Institute, it was reported that death rates continued to decline for all cancers combined for men and women of all major racial and ethnic groups and for most major cancer sites (Jemal et al., 2013). However, the incidence rates increased for oropharynx human papillomavirus- (HPV-) associated cancers (Jemal et al., 2013). There have also been substantial changes in the burden of cancer affecting HIV-infected individuals in the U.S. during a 12-year period spanning the introduction of highly active anti-retroviral therapy (HAART). As a result of these temporal changes, non-AIDS-defining malignancies have come to comprise the majority of cancers in HIV-infected persons during the HAART era (Engels et al., 2008). Among these emerging malignancies, HPV-associated cancers of the oral cavity/pharynx increased significantly following an AIDS diagnosis (Engels et al., 2008; Grulich et al., 2010). HPV is rapidly changing the landscape of head and neck squamous cell carcinoma (HNSCC). However, the mechanisms and natural history of oral HPV infection and oncogenic progression is poorly understood. Extrapolation of knowledge of HPV-associated infection and oncogenicity in the genital tract is not satisfactory to our understanding of oral HPV disease. HPV is necessary, but not sufficient, to cause invasive cervical cancer. Although HPV is an important cause of oropharyngeal cancer, HPV infection is neither necessary nor sufficient to cause HNSCC that can occur in the absence of HPV. Therefore, HPV-HNSCC needs to be studied as its own entity.

HPV16 is the most dominant HPV type, accounting for (> 90%) HPV-associated HNSCCs. HPV16 is also the same type that commonly leads to HPV-associated anogenital cancers (Marur et al., 2010). While the lifecycle of HPV16 has been extensively studied in anogenital tissues, little has been reported on the HPV16 life cycle in oral tissues. In fact, direct evidence that oral tissue can support HPV replication of infectious virus particles is lacking.

2. Methods

2.1. Keratinocyte cultures and generation of cell lines

Primary human tonsil (HTLK) and primary human foreskin keratinocytes (HFK) were isolated and grown from tonsil and foreskin tissues, respectively, according to the method described previously (Conway et al., 2009a, 2009b). HPV16+ cell lines were generated as previously described (McLaughlin-Drubin et al., 2004). In short, pBSHPV16 plasmid DNA was digested with BamH1 to linearize the viral DNA and separate it from the vector sequence. A total of 10 μg viral DNA was electroporated into HTLKs or HFKs

* Corresponding author.
E-mail address: cmm10@psu.edu (C. Meyers).
[1] Authors contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.virol.2016.09.004
0042-6822/

using a Gene Pulser (Bio-Rad Laboratories, Hercules, CA) (Conway et al., 2009a, 2009b). Immortalized keratinocytes stably maintaining HPV16 genomes following electroporation were cultured with J2 3T3 feeder cells and maintained in E-medium.

2.2. Southern blot hybridization

Immortalized HTLK cell lines were characterized by Southern blot hybridization, as previously described, to determine the state and quantity of the HPV16 genome within the immortalized keratinocytes (Meyers et al., 1992a). Briefly, total cellular DNA was isolated from the immortalized cell line. To linearize the viral genome, 5 μg of the total cellular DNA was digested with BamHI. Samples were then separated by gel electrophoresis on a 0.8% agarose gel, transferred onto a GeneScreen Plus membrane (New England Nuclear Research Products) and probed with a HPV16 specific, whole genome probe, as previously described (McLaughlin-Drubin et al., 2004, 2003; Meyers et al., 1992a, 1997).

2.3. Production of native HPV in organotypic raft cultures

Organotypic (raft) cultures were grown as previously described (Meyers et al., 1992a, 1997). Immortalized HTLK and HFK lines persistently infected with HPV16 were seeded onto collagen matrices consisting of rat-tail type 1 collagen and containing J2 3T3 feeder cells. Following cell attachment and growth to confluence, the matrices were lifted onto stainless steel grids and fed with E-medium supplemented with 10 μM 1,2-dioctanoyl-*sn*-glycerol (C8:O; Sigma Chemical Company) via diffusion from below. Raft cultures were allowed to stratify and differentiate for 10 days, 15 days, or 20 days.

2.4. Histochemical analyses

Raft culture tissues were harvested at different time points, fixed in 10% neutral buffered formalin, and embedded in paraffin. Four-micrometer sections were cut and stained with hematoxylin and eosin (H&E) (Meyers et al., 1992b, 1997).

2.5. HPV Isolation and optiprep purification of virions

HPV infected raft tissues were harvested as has been described (Conway et al., 2009a, 2009b, 2011a, b). Viral preparations (VPs) were prepared as previously described (Conway et al., 2009a, 2009b, 2011a, b; Cruz and Meyers, 2013). Briefly, two rafts were dounce homogenized in 500 μl of phosphate buffer (0.05 M sodium phosphate [pH 8.0]/2 mM $MgCl_2$). Homogenizers were rinsed with 250 μl of phosphate buffer. Then, un-protected genomes were digested by the addition of 1.5 μl (375 U) of benzonase to 750 μl of VP, followed by incubation at 37 °C for 1 h. Samples were brought to 1 M NaCl by adding 188 μl of ice-cold 5 M NaCl. Then, samples were vortexed and centrifuged at 4 °C and 10,500 rpm for 10 min. The supernatants were stored at −80 °C for further experiments. Optiprep purification was performed as described previously (Conway et al., 2009a, 2009b, 2011a, b). Briefly, OptiPrep gradients were prepared by underlaying 27%, 33%, and 39% Optiprep. Gradients were allowed to diffuse for 1 h at room temperature. Then, 600 μl of clarified, benzonase-treated virus preps was layered on top of the gradient. Tubes were then centrifuged in a SW55 rotor (Beckman) at 234,000 × *g* for 3.5 h at 16 °C. After centrifugation, 11–500 μl fractions were carefully collected, top to bottom, from each tube.

2.6. Titration of HPV16

HPV16 titers were measured as previously described (Conway et al., 2009a, 2009b, 2011a, b; Cruz and Meyers, 2013). Briefly, viral genomes were released from a benzonase treated virus preparation by re-suspension in 200 μl HPRT DNA extraction buffer (400 mM NaCl /10 mM Tris-HCl [pH 7.4]/10 mM EDTA, pH 8.0), 2 μl 20 mg/ml proteinase K, and 10 μl 10% SDS for 2–4 h at 37 °C. Following extraction, the DNA was purified by phenol-chloroform extraction and ethanol precipitated overnight at −20 °C. To quantify the viral genomes, a Thermo Scientific Maxima SYBR Green qPCR kit was utilized. Amplification of the HPV16 E2 open reading frame (ORF) was performed using 0.3 μM of forward primer 5′-CCATATAGACTATTGGAAACACATGCGCC-3′ (nucleotides (Zhao et al., 2000) 2839 to 2868) and 0.3 μM of reverse primer 5′-CGTTAGTTGCAGTTCAATTGCTTGTAATGC-3′ (nt 2960 to 2989). Amplification of the E2 ORF of serially diluted pBSHPV16 DNA, ranging from 10^8-10^4 copies/μl served to generate a standard curve. A Bio-Rad iQ5 Multicolor Real-Time qPCR machine and software were utilized for PCR amplifications and subsequent data analysis.

2.7. Infectivity assays

All infectivity studies were performed using HaCaT keratinocytes. HaCaT cells were seeded 50,000 cells/well in 24-well plates and infectivity assays were performed as previously described (Conway et al., 2009a, 2009b, 2011a, b; Cruz and Meyers, 2013). Briefly, cells were incubated with virus for 48 h at 37 °C/5% CO_2 followed by mRNA harvest using a RNeasy kit (Qiagen). Infections were analyzed using a RT-qPCR based assay detecting levels of the E1ȆE4 splice transcript. HPV16 E1ȆE4 was detected using the forward primer 5′-GCTGATCCTAGCAACGAAGTATC-3′, the reverse primer 5′-TTCTTCGGGTGCCCAAAGGC-3′, and the probe 5′- (6-FAM)CCCGCCGCGACCCATACCAAAGCC(BHQ-1)-3′. For neutralization assays, virus was incubated for 1 h at 37 °C prior to infection with the conformation-dependent anti-L1 antibody H16. V5 (1:1000 dilution; a kind gifts from Neil Christensen, Penn State College of Medicine) or the anti-L2 antibodies RG-1 (1:100 dilution; a kind gift from Richard Roden, Johns Hopkins) and L2#4 (1:100 dilution; a kind gift from Tadahito Kanda, the RIKEN institute) followed by the infectivity assay as described previously (Conway et al., 2009a, 2009b, 2011a, b; Cruz and Meyers, 2013).

2.8. Western blot analysis

Aliquots (70 μl) from OptiPrep fractions were boiled for 10 min in 6% 2-mercaptoethanol (2-ME) loading buffer and then loaded onto 8–12% polyacrylamide gels. To detect HPV16 L1, the Camvir-1 anti-HPV16 L1 monoclonal antibody (BD Pharmingen) was used at a dilution of 1: 2500 according to the manufacturer's recommendations. To determine the relative quantity of L1 present in the bands, a Bio-Rad G8-800 calibrated densitometer along with Quantity One analysis software was used.

2.9. Identification of free sulfhydryls and disulfide bonds

Frozen sections of HPV16-infected tonsil and foreskin epithelial tissue were stained with DACM (*N*-[7-Dimethylamino-4-Methylcoumarinyl]-Maleimide) to detect free sulfhydryls and disulfide bond-specific sulfhydryls as previously described (Ogawa and Taneda, 1979; Ogawa et al., 1979). Tissue sections were imaged on a Nikon Eclipse 80i microscope and images were taken with a CoolSnap Cf2 camera and NIS-elements 3.10 software.