Virology 433 (2012) 385–394

Contents lists available at SciVerse ScienceDirect

Virology

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

# Murine skin and vaginal mucosa are similarly susceptible to infection by pseudovirions of different papillomavirus classifications and species

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ARTICLE INFO

*Article history:*
Received 17 May 2012
Returned to author for revisions
19 June 2012
Accepted 27 August 2012
Available online 15 September 2012

*Keywords:*
Papillomavirus
Pseudovirions
Murine model
Skin infection
Tissue tropism
Murine papillomavirus
*In vivo* imaging

ABSTRACT

Depending upon viral genotype, productive papillomavirus infection and disease display preferential tropism for cutaneous or mucosal stratified squamous epithelia, although the mechanisms are unclear. To investigate papillomavirus entry tropism, we used reporter pseudovirions based on various cutaneous and mucosal papillomavirus species, including the recently identified murine papillomavirus. Pseudovirus transduction of BALB/c mice was examined using an improved murine skin infection protocol and a previously developed cervicovaginal challenge model. In the skin, HPV5, HPV6, HPV16, BPV1 and MusPV1 pseudovirions preferentially transduced keratinocytes at sites of trauma, similar to the genital tract. Skin infection, visualized by *in vivo* imaging using a luciferase reporter gene, peaked between days 2–3 and rapidly diminished for all pseudovirion types. Murine cutaneous and genital tissues were similarily permissive for pseudovirions of HPV types 5, 6, 8, 16, 18, 26, 44, 45, 51, 58 and animal papillomaviruses BPV1 and MusPV1, implying that papillomavirus' tissue and host tropism is governed primarily by post-entry regulatory events in the mouse.

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## Introduction

Papillomaviruses (PV) comprise a group of small, non-enveloped DNA viruses that infect humans and many other vertebrate species. To date, more than 180 genotypes have been characterized and classified into 29 genera (Bernard et al., 2010), based on the nucleotide sequence relatedness of the PV major capsid protein L1. Productive infection and induction of neoplasia is species-specific and PVs that affect humans (HPV) are clustered in five of these genera, namely alpha, beta, gamma, mu and nu. Additionally, HPVs can be designated as either a cutaneous or mucosal type based on the preferential productive lesion/papilloma formation in stratified squamous epithelia of skin or mucosa, respectively. However, these designations represent infection preferences, rather than absolute requirements for infection of a specific anatomical area. For example, HPV 2, a common cause of verrucae vulgares on the skin, can also be detected in benign vulvar warts of children (Aguilera-Barrantes et al., 2007) and in oral lesions (de Villiers, 1989). HPV 8, which is responsible for the development of non-melanoma skin cancer in Epidermodysplasia verruciformis patients and possibly in non-genetically predisposed individuals (Gewirtzman et al., 2008), has been detected in some oropharyngeal tumors (Lindel et al., 2009). Conversely, mucosal HPVs can also infect skin sites. High-risk mucosal HPV types were detected in digital Bowen's diseases and squamous cell cancers (SCC), some of these in association with concomitant or antecedent genital malignancies (Forslund et al., 2000; Kreuter et al., 2009). High-risk mucosal HPV 26 has been detected in a few cervical carcinomas, but recently has more often been found in SCC of the fingers and toes of individuals infected with HIV-1 (High et al., 2003; Kreuter et al., 2005; Handisurya et al., 2007).

Tissue tropism cannot be explained solely by the phylogeny of PV types. For instance, the mucosal HPV types 6 and 11, which share 85% sequence identity within their L1 genes, show differences in their predilection for specific sites. HPV 6 is more commonly found in anogenital warts than HPV 11, while HPV 11 is found more frequently in laryngeal papillomas. The closely related type HPV 13, which has 78% sequence identity to both HPV 6 and 11, causes focal epithelial hyperplasia of the oral cavity and has not been detected in either anogenital or laryngeal papillomas (Syrjanen, 2003). HPV 7 and 40, sharing 87% L1 nucleotide sequence identity, also affect different anatomical sites. HPV 7 is responsible for Butcher's warts, particularly on

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0042-6822/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.virol.2012.08.035

the hands of meat handlers, while HPV 40 causes mucosal papillomas of the anogenital and laryngeal tract. It is important to note that infection is often identified as a clinically apparent lesion, so apparent tropism may often correspond to sites at which papillomatous changes can be induced, with the possibility that sites of asymptomatic infection remain underappreciated.

The reasons for the observed tissue tropism are unclear, despite several attempts to clarify the factors responsible for preferential PV infection and/or induced neoplasia at specific sites. Characterization of the genetic elements that control tissue tropism has suggested that the long control (LCR) or upstream regulatory region serve to influence the tropism of HPV types (Steinberg et al., 1989). This region contains enhancer elements that show some tissue or cell type specificity and likely play a role in the initial expression of the viral genes after virus infection (Howley and Lowy, 2007). Differences in the capacity of transcriptional activation have been shown for the LCR of cutaneous HPV 5 and mucosal HPV 16, using cell lines of different derivations (Mistry et al., 2007). Furthermore, prediction of transcription factor binding sites revealed that, while some binding sites are present in all LCRs, *e.g.,* AP-1, other binding sites are restricted to certain genera or even HPV types (García-Vallvé et al., 2006). These differences in the LCR may singly, or in combination, have an impact on the preferential infection of distinct types of epithelia by the individual PV genotype.

Another factor in the determination of tissue tropism could be the interaction of the PV capsid proteins with host attachment factors. *In vivo*, attachment to negatively charged heparan sulfate proteoglycans (HSPG) on the basement membrane (BM) is regarded as the initial step leading to infection, at least for types examined until now: HPV 5, HPV 16, HPV 31 and HPV 45 (Johnson et al., 2009; Schiller et al., 2010). After undergoing a conformational change followed by furin cleavage of the L2 minor capsid protein, the L1 major capsid protein is thought to bind to a still undetermined secondary receptor on the cell surface, leading to internalization of the virus (Kines et al., 2009). It has been suggested that differences in the surface charge of PV L1 proteins may influence viral attachment, and modeling of the net surface charges has suggested that cutaneous HPV exclusively display negatively charged L1 surfaces, whereas positively charged L1 surfaces were predominantly modeled for mucosal alpha-PV (Mistry et al., 2008). Whether these hypothetical differences affect infectious entry tropism *in vivo* is unclear. HSPGs do show great variability across individual cell types, due to differences in sulfation patterns and other chemical modifications of the heparan sulfate side chains, which are the target of initial PV binding (Turnbull et al., 2001). This heterogeneity might theoretically skew the binding of virions towards cells or the BM of cutaneous and/or mucosal origins. However, it is important to note that HSPGs are invariably negatively charged and would therefore be expected to interact with positively charged peptides on the virion surface.

To investigate whether the tissue specificity of cutaneous and mucosal PV types is determined at the level of infectious entry, from BM binding through establishment of the genome in the nucleus to initiate transcription, we have determined the *in vivo* infectivity of PV pseudovirions (PsV) concomitantly at a cutaneous and a mucosal site. Cutaneous exposure was achieved using an improved murine model for skin infection and mucosal exposure employed a previously published cervicovaginal challenge model of PV transmission (Roberts et al., 2007). The use of PsV allowed the delivery of an identical reporter plasmid (pseudogenome), thereby eliminating the potential confounding variability in gene expression for native PV genomes. Additionally, PsV infection can be readily and repeatedly quantified *in vivo* in an animal over an extended time course, using luciferase gene expression as a marker of infection. We included PsV of the recently identified mouse papillomavirus, Mus PV 1 (Ingle et al., 2011), to assess the possibility that infection tropism or kinetics might be influenced by a natural host *versus* a heterologous host species.

## Results

### *PsV preparations of different PV types have variable particle to infectivity ratios in vitro*

PsV representative of cutaneous (HPV 5, 8), low- (HPV 6, 44) and high-risk mucosal HPV types (HPV 16, 18, 26, 45, 51, 58), and two animal PV, bovine PV type 1 (BPV 1) and the recently identified murine PV, Mus PV 1, were generated by encapsidation of the reporter plasmid pCLucf, which contains a luciferase expression cassette (Johnson et al., 2009). In addition to luciferase, this reporter plasmid also expresses enhanced green fluorescent protein (GFP), which means the *in vitro* infectivity of PsV carrying pCLucf can be monitored by flow cytometric measurement of GFP expressing cells or luciferase. To evaluate the relative infectivity of cutaneous and mucosal PV types, the amount of encapsidated pCLucf reporter plasmid for various PsV stocks was calculated by quantitative PCR (qPCR) and compared to the GFP-transducing potential of the stock on a per cell basis of 293TT cells *in vitro*. Surprisingly, the observed particle to infectivity ratios were highly variable for different PV types (Table 1). The lowest mean ratios of 23, 37, 53 and 28 (*i.e.,* most potent per particle infectivity) were observed for the high-risk mucosal HPV types 45, 58 and 26 and the new murine PV, Mus PV 1, respectively. The highest mean ratio of 4811 was obtained with preparations of cutaneous HPV 5. The ratios were similar among different preparations of the same PsV type (Table 1). The slight variabilities observed are likely due to minor differences in the passage number or physiological state of the producing cell line 293TT at time of PsV production. No correlation between the calculated ratios and the genera or tissue tropism of the PVs was evident. Furthermore, because empty capsids might compete with the transduction of the pseudogenome, we also determined the number of L1 capsids per encapsidated pCLucf reporter plasmid. The range of the calculated ratios was between 1.14 and 5.51 present in the stock. No correlation was observed between reporter plasmid encapsidation efficiency and infectivity (Table 1).

### *An improved murine skin challenge model allows efficient and consistent infection with PV PsV*

We next established an efficient and reliable method for infection of murine skin that could be used to compare the

**Table 1**
Ratio of reporter plasmid copies *vs.* infectious units or L1 capsids.

| Pseudovirion types | Reporter plasmid copies/ infectious units (mean ± SD) | Capsid equivalents/ reporter plasmid |
|---|---|---|
| HPV 5 | 4811 ± 2249 | 4.70 |
| HPV 6 | 73 ± 21 | 5.51 |
| HPV 8 | 635 ± 777 | 1.35 |
| HPV 16 | 67 ± 26 | 3.78 |
| HPV 18 | 171 ± 16 | 1.47 |
| HPV 26 | 53 ± 34 | 4.41 |
| HPV 44 | 360 ± 13 | 3.36 |
| HPV 45 | 23 ± 33 | 1.33 |
| HPV 51 | 116 ± 28 | 1.14 |
| HPV 58 | 37 ± 62 | 2.50 |
| Mus PV | 28 ± 15 | 2.54 |
| BPV 1 | 174 ± 56 | 1.34 |