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The HPV16 and MusPV1 papillomaviruses initially interact with distinct host components on the basement membrane

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ABSTRACT

To understand and compare the mechanisms of murine and human PV infection, we examined pseudovirion binding and infection of the newly described MusPV1 using the murine cervicovaginal challenge model. These analyses revealed primary tissue interactions distinct from those previously described for HPV16. Unlike HPV16, MusPV1 bound basement membrane (BM) in an HSPG-independent manner. Nevertheless, subsequent HSPG interactions were critical. L2 antibodies or low doses of VLP antibodies, sufficient to prevent infection, did not lead to disassociation of the MusPV1 pseudovirions from the BM, in contrast to previous findings with HPV16. Similarly, furin inhibition did not lead to loss of MusPV1 from the BM. Therefore, phylogenetically distant PV types differ in their initial interactions with host attachment factors, but initiate their lifecycle on the acellular BM. Despite these differences, these distantly related PV types displayed similar intracellular trafficking patterns and susceptibilities to biochemical inhibition of infection.

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Introduction

Papillomaviruses (PVs) are a family of small, non-enveloped viruses that infect humans and many other vertebrate species. PVs infect mucosal and cutaneous squamous epithelia, where they cause disease by stimulating epithelial proliferation (Howley et al., 2013). Despite similarity in genomic organization and capsid structure, productive infection with PVs is generally species-specific (Chan et al., 1992; Parsons and Kidd, 1942). The 8 kb PV genome is encapsidated within a 55–60 nm non-enveloped capsid comprised of two virion proteins. There are 360 copies of L1, the major capsid protein, arranged into 72 capsomers. Each of these capsomers may contain one copy of L2, the minor capsid protein (Buck et al., 2005b). L1 can assemble in the absence of L2 to produce non-infectious virus-like particles (VLPs). VLPs are able to attach and enter cultured cells similarly to infectious virus (Day et al., 2003). Therefore, L2 is not considered to play a role in the initial attachment of the virus, although it may interact with cell surface proteins following capsid attachment (Kawana et al., 2001; Woodham et al., 2012; Yang et al., 2003). Furin/proprotein convertase activation of L2 precedes endocytosis and is essential for efficient infection (Richards et al., 2006). L2 is also essential for the successful delivery of the genome to nucleus (Day et al., 2004).

Studies of the early events in PV infectious entry have produced different results, depending on the in vitro or in vivo model system utilized. Monolayer cell culture has been instrumental to the basic understanding of PV binding and entry. Although a consensus model has not yet been achieved, multiple independent studies utilizing pseudovirus (PsV) indicate an initial interaction with HSPGs, furin cleavage of L2 and internalization via a non-clathrin, non-caveolar pathway that most closely resembles macropinocytosis (Cerqueira et al., 2013; Day et al., 2008; Giroglou et al., 2001; Joyce et al., 1999; Knappe et al., 2007; Schelhaas et al., 2012; Selinka et al., 2007, 2003; Spoden et al., 2008). Despite the advances afforded from in vitro studies, cells adapted to long-term culture have undergone numerous modifications and may not adequately reflect the in vivo situation. We have recently utilized a murine cervicovaginal challenge (CVC) model to dissect early in vivo infection events (Kines et al., 2009). The initial description of this model identified a requirement for epithelial wounding and access to the acellular basement membrane (BM) for initial capsid binding (Roberts et al., 2007). The interpretation was that epithelial cells were infected as they migrated over the BM during the wound healing process. Further analysis of HPV16 PsV infection in this system allowed us to compare parameters of in vitro and in vivo infection. These studies identified several differences between the systems, with the most significant being that the initial HSPG-dependent capsid binding and

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furin cleavage occurs on the BM in vivo, prior to interaction with the epithelial cells, whereas in vitro these events can occur on the cell membrane (Day et al., 2008, 2012). The recent identification of a mouse papillomavirus, MusPV1 (also termed MmuPV1) (Ingle et al., 2011), now allows us to utilize the murine model with a homologous virus type. Although this eliminates the caveat of a heterologous virus-host system, the fact that MusPV1 is presumably a cutaneous type must be considered if differences in initial host interactions were detected in comparison to the mucosotropic HPV16. Based on its phylogeny, MusPV1 is a member of the Beta+Xi-PV super taxon within the genus Pi-PV, which is phylogenetically distant from the alpha genus human types (Schulz et al., 2012). Indeed the interaction of HPV5, a cutaneous beta human type, with specific HSPGs has been shown to differ relative to that of the mucosotropic alpha types, HPV16, HPV31 and HPV45 (Johnson et al., 2009). Additionally carrageenan, a highly sulfated polysaccharide, does not efficiently prevent infection with HPV5 PsV in vitro, whereas picomolar concentrations block infection with all examined mucosotropic types (Buck et al., 2006).

In this study, we have detailed the binding and infection events of MusPV1 PsV in the murine CVC model and compared these interactions to those of HPV16. As with HPV16, we find that MusPV1 PsV infection is initiated on the BM, confirming the importance of this extracellular site in the PV lifecycle. Infection can be prevented by the addition of exogenous heparin and by furin inhibition. However, the mechanism of inhibition is apparently different, revealing a non-HSPG binding factor for MusPV1 on the murine BM. We have also compared the binding and entry of these viruses in vitro, in cultured keratinocytes of both human and murine origin. The two virus types exhibited no differences in their profiles of biochemical inhibition among the cell types examined. However, the cells of murine and human origin exhibited marked variations in these profiles.

Results

Examination of MusPV1 interactions in vivo

Utilizing the murine CVC model, we examined the interaction of MusPV1 pseudovirions with host tissue. We have previously established that MusPV1 pseudovirions are infectious in the CVC model and require tissue disruption similarly to infection with HPV16 and other HPV pseudovirions (Handisurya et al., 2012; Roberts et al., 2007). Here we found that MusPV1 particles bound to the acellular basement membrane (BM) at an early time point (4 h) and were found associated with epithelial cells following re-epithelialization (18 h) (Fig. 1, panels A and B). These tissues were co-stained with an antibody recognizing nidogen to delineate the BM. This association of the capsids with the BM at early time points and their relocalization to the epithelium at later time points replicates our findings with HPV16 (Kines et al., 2009). In that study, we demonstrated that HPV16 interacted with HSPG on the BM and that cleavage of HS moieties with heparinase III prevented binding of HPV16 capsids to the BM and subsequent infection. In contrast, we found here that heparinase III treatment did not affect the in vivo infectivity of MusPV1, although the control experiments clearly showed the previously described diminution of HPV16 infection (Fig. 2A). We examined the distribution of MusPV1 capsid binding at 4 h post-instillation either in the presence of heparinase digestion buffer as a negative control (Fig. 1C) or following heparinase III digestion (Fig. 1D). Heparinase treatment did not visibly affect the interaction of capsids with the BM. To confirm that heparinase treatment resulted in efficient removal of HS moieties from the BM, we stained these tissues with an anti-HS antibody coincidently with anti-L1 staining. This analysis showed clearly that MusPV1 bound well to BM that exhibited no detectable anti-HS reactivity (Supplemental Fig. 2).

Although it is unlikely that the initial interaction of the MusPV1 capsid is to BM HSPG, it was unclear from this experiment whether HSPG interactions subsequent to the initial BM interaction might be critical, as HS moieties would be regenerated by the tissue during the time course of the infection. Their regeneration could allow transfer of BM-bound virions to newly synthesized HSPG; indeed the increased infection observed with MusPV1 following heparinase digestion (Fig. 2A) may reflect this propensity. Therefore, we decided to examine the effect of exogenous heparin, an established competitor for HSPG interaction, on in vivo MusPV1 infection, reasoning that the pre-association of heparin with the capsids could prevent a putative heparin-dependent step that occurs subsequent to the initial BM interaction. As shown in Fig. 2B, the instillation of exogenous heparin did repress in vivo infection by MusPV1. This observation probably implies the existence of an HSPG-dependent step in infection subsequent to the HSPG-independent BM binding. Consistent with this possibility, heparin, like heparinase III treatment, did not noticeably affect the initial binding of the particles with the BM (Fig. 1E). By contrast, at 18 h post-instillation, the heparin-treated capsids were not found in association with the epithelium (Fig. 1F), unlike the untreated capsids (Fig. 1B).

We reasoned that heparin inhibition might not result in a durable decrease in infection, as the initial tissue binding is not prevented and heparin may possibly dissociate from the capsid over time, allowing infection to progress. Therefore we examined infection at 48 and 72 h post-infection. A marginal increase in infection by 72 h was observed. HPV16 infection remained unchanged during this time. As expected in the event of infection, MusPV1 capsid association with the vaginal epithelium was detectable at these later time points (data not shown).

MusPV1 infection is furin-dependent

All PV PsV examined so far have been found to require furin for in vitro infection, and the furin-dependence of in vivo HPV16 infection has been established (Kines et al., 2009; Richards et al., 2006). Furin cleaves the amino terminus of the L2 protein during infectious entry in cultured cells and on the BM in vivo, and in vivo inhibition of furin cleavage of HPV16 capsids results in their premature release from the BM. Our working model of in vivo HPV16 infection suggests that furin cleavage of L2 induces a change in capsid morphology that results in exposure of a buried neutralization epitope on L2 and a secondary receptor binding site(s) on L1. Exposure of this putative L1 site(s) allows for stable association with the epithelial target cells, and inhibition of furin cleavage prevents this association. However, reduced affinity for the BM HSPG causes loss of the HPV16 capsids from the tissue (reviewed in Day and Schiller (2009)).

Given that the initial in vivo interactions of MusPV1 appeared to differ from those of HPV16, we examined the effects of furin inhibition on MusPV1 infection. After confirming that in vitro infection with MusPV1 PsV was inhibited with decanoyl-RVKR-cmk, a potent inhibitor of furin and other proproteins convertases (data shown as part of Fig. 9), we then determined that it also inhibited in vivo MusPV1 infection, similarly to HPV16 (Fig. 2C). However, when we examined the distribution of MusPV1 capsids within the murine genital tract in the presence of the furin inhibitor, we found extensive association with the BM at both the 4 h (Fig. 3A) and 18 h time points (Fig. 3B). Although the 4 h time point result is similar to what we have observed with HPV16, the HPV16 capsids are lost from the BM by the 18 h time point under conditions of furin inhibition. Consistent with the ability of the furin inhibitor to prevent MusPV1 infection, decanoyl-RVKR-cmk treatment did not result in the detectable transfer of capsids to the epithelium seen at the 18 h time point, unlike the untreated capsids at this time (Fig. 1B). Therefore, the