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GEM

Mus musculus Papillomavirus 1: a New Frontier in Animal Models of Papillomavirus Pathogenesis

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ABSTRACT Animal models of viral pathogenesis are essential tools in human disease research. Human papillomaviruses (HPVs) are a significant public health issue due to their widespread sexual transmission and oncogenic potential. Infection-based models of papillomavirus pathogenesis have been complicated by their strict species and tissue specificity. In this Gem, we discuss the discovery of a murine papillomavirus, Mus musculus papillomavirus 1 (MmuPV1), and how its experimental use represents a major advancement in models of papillomavirus-induced pathogenesis/carcinogenesis, and their transmission.

KEYWORDS animal models, human papillomavirus, murine papillomavirus, papillomavirus, viral oncology, viral pathogenesis, MmuPV1

Viruses significantly affect human health, and the prevention, control, and treatment of viral infections require a fundamental understanding of their pathogenesis. Such knowledge demands investigation not only at the molecular and cellular levels but also in the organisms they infect. Animal viruses are vital to our understanding of human viruses, including infection, transmission, host responses, and pathogenesis. Moreover, they provide critical preclinical models in which to identify preventative and therapeutic approaches to human viral infections and disease. Such models have provided pivotal insight into viruses such as influenza virus, herpesviruses, Zika virus, and emerging viruses (1–5). Studying human viruses using genetically tractable and cost-effective animal models is often complicated by their strict species tropism. This is true for human papillomaviruses (HPVs), thus limiting the use of infection models to study these common and important human pathogens.

Papillomaviridae is a large and diverse family of nonenveloped, double-stranded circular DNA viruses that by and large exhibit rigid species and tissue tropism. There are more than 220 formally accepted types that infect humans (6–8). Human papillomaviruses infect stratified squamous epithelia in the oral cavity and upper respiratory tract, the anogenital tract, and the skin and cause a range of pathologies, from warts (papillomas) to dysplasia and cancer. HPV genotypes are classified by their oncogenic potential. Low-risk HPV types cause benign skin, oral/respiratory, and genital papillomas, whereas high-risk HPVs cause cancer (9). High-risk mucosal HPVs are the etiological factors of nearly all cervical cancers, a large number of vaginal, penile, and anal cancers, and a subset of head and neck cancers, particularly of the oropharynx (10). Certain high-risk HPVs, such as HPV16 and HPV18, cause the majority of HPV-associated cancers (11–13). Cutaneous HPVs are also linked to certain types of skin malignancies (14). Given their broad diversity, prevalence, and oncogenic potential, HPVs are one of the top infectious causes of human cancer, causing approximately 5% of cancers worldwide (10, 15).

The public health threat of mucosotropic HPVs is exacerbated by their being the most common sexually transmitted infection in the United States (16). While most infections are cleared (17), persistent HPV infections can be established and are a major

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risk factor for progression to cancer (18, 19). Particular high-risk HPVs such as HPV16 are more likely to establish persistent infections, contributing to their oncogenic potential (20). HPV persistence is often accompanied by viral genome integration into host DNA, which occurs at random sites but with a preference for chromosomal fragile sites, genes, and enhancers (21–24). Integration is thought to potentiate HPV-mediated oncogenic progression by increasing the amount and stability of transcripts of the viral oncogenes E6 and E7 (25, 26), providing a selective growth advantage to cells (27). High-risk HPV E6 and E7 are well-validated, potent oncogenes. The highly multifunctional proteins they encode contribute to carcinogenesis at least in part by inactivating the major tumor suppressors p53 and pRb, both common targets of DNA tumor viruses (28, 29).

Prophylactic HPV vaccines are a significant milestone in the effort to control HPV-mediated cancers (30). In Australia, where HPV vaccination is mandatory for young girls and boys, cervical cancer is predicted to be eliminated as a public health problem within the next 20 years (31). However, inadequate vaccine availability and vaccination coverage have allowed HPV to remain a significant public health issue elsewhere (32, 33). Vaccination is also ineffective against preexisting HPV infections. For these reasons, there remains a pressing need to study HPV infection and persistence and the contribution of host and environmental factors to HPV transmission and subsequent disease. Many of the underlying mechanisms that govern these aspects of HPV pathogenesis have not been fully elucidated. Tractable animal models of papillomaviral pathogenesis are essential to advance our understanding of these viruses. In this Gem, we discuss existing comparative models of HPV pathogenesis and disease and focus on new and emerging models utilizing a murine papillomavirus, Mus musculus papillomavirus 1 (MmuPV1). These MmuPV1-based models have the potential to transform our ability to study the molecular basis of PV infection and pathogenesis and provide an opportunity to identify therapeutic interventions to control HPV transmission and disease.

EXISTING ANIMAL MODELS OF PAPILLOMAVIRUS PATHOGENESIS

As a result of the stringent host species specificity of PVs, animals do not support productive HPV infections. Researchers therefore rely on the use of animal PVs to establish infection models in their respective hosts or use genetically engineered mouse models, such as transgenic mice, to study the role of specific HPV genes in neoplastic disease.

Animal models of papillomavirus pathogenesis. Modern sequencing techniques and increased sampling have started to reveal the broad diversity of animal papillomaviruses (8, 34–36). Animal models have contributed significantly to our understanding of papillomaviral pathogenesis, tissue tropism, and disease (for reviews, see references 37 to 40). The first animal papillomavirus studied was cottontail rabbit papillomavirus (CRPV), described in the 1930s as causing papillomas in rabbits (41). CRPV was subsequently found to promote cutaneous malignancies (42) and provided insights into a variety of virus-host interactions for PVs with cutaneous tropism (43). A mucosotropic PV, rabbit oral papillomavirus (ROPV), was later isolated from domestic rabbits and paved the way for studies in the oral mucosa as well as male and female genital tissues (44, 45). Given their divergent tropisms, CRPV and ROPV were useful models to study the underlying molecular mechanisms of PV tissue tropism. These models, along with canine oral PV (COPV) (46), were heavily utilized in testing vaccines, leading to the current HPV prophylactic vaccines (for reviews, see references 39, 47, and 48). Papillomaviruses that infect the multimammate rat species *Mastomys natalensis* (Mastomys natalensis papillomavirus 1 [MnPV1]) and *Mastomys coucha* (Mastomys coucha papillomavirus 2 [McPV2]) can establish persistent infections and promote tumorigenesis in the skin and anogenital tissues, respectively (49, 50). The Rosl group has extensively studied various aspects of PV biology using these *Mastomys* models (for a review, see reference 51), contributing novel observations related to viral mRNA splicing patterns *in vivo* (52) and the role of environmental factors such as UV radiation in cutaneous PV-associated carcinogenesis (53).

Comparative models of PV pathogenesis have also been established in cattle and nonhuman primates. Bovine papillomaviruses (BPVs) cause various pathologies in cattle (54) and horses (55). BPV-1 was the first fully sequenced PV genome (56) and was the subject of many *in vitro* transformation studies and the first papillomavirus transgenic mouse model (57). BPV-1 studies facilitated the discovery of the viral E5 oncoprotein (58, 59), which is also expressed by high-risk mucosal HPVs (for a review, see reference 60). The evolutionary proximity of nonhuman primates to humans made these species attractive models for studying HPV pathogenesis. Papillomaviruses have been associated with cutaneous and mucosal disease in colobus monkeys, rhesus macaques, and chimpanzees (35, 61–66). Some of these PVs show close sequence similarity to high-risk HPVs and are associated with neoplastic disease, including precancerous lesions and carcinomas (65, 67, 68). The first preclinical models of PV sexual transmission also were in nonhuman primates (67, 69). While the contribution of these animal models to our understanding of PV infection and pathogenesis cannot be overstated, they present challenges related to cost, availability of technical reagents, and genetic tractability.

HPV transgenic mouse models. The most widely used, well-characterized, and technically well-supported animal used as a model system remains the laboratory mouse, *Mus musculus* (70). Until the discovery of a murine papillomavirus, the PV field lacked an infection-based system to model HPV-mediated carcinogenesis in laboratory mice. Instead, researchers employed genetically engineered, transgenic mice (for a review, see reference 71). The first such model involved insertion of 1.69 tandem copies of the BPV-1 genome into the mouse genome (57). These transgenic mice developed cutaneous fibropapillomas and fibrosarcomas, both of which contained replicating extrachromosomal BPV-1; however, the virus remained transcriptionally inactive in asymptomatic skin (72). This model established the feasibility of using transgenic mice to study PV-associated diseases. The high-risk mucosotropic HPV16 E6 and E7 oncogenes were then studied using transgenic mice (73–76), providing key insights into their contributions to tumorigenesis, including the abilities of E6 to inhibit apoptosis through p53-dependent and -independent means (74, 77, 78) and E7 to induce hyperplasia through its inactivation of the tumor suppressor pRb and dysregulation of E2F-dependent gene expression (77, 79). Epidermis-specific expression of E6 and E7 also induced skin tumors (76). A subsequent generation of transgenic mice directed HPV16 protein expression to the natural site of infection, the basal cells of the stratified squamous epithelia, using the keratin 14 (K14) promoter. These models expressed either the entire early region of the HPV16 genome (80) or the individual HPV16 viral oncogene E5 (81), E6 (82), or E7 (83). These transgenic mice have been instrumental in modeling HPV-induced progressive neoplastic disease and cancer development in the cervix (84–88), head and neck (89, 90), and anus (91). HPV-transgenic mice have allowed researchers to establish the relative potencies of individual HPV oncogenes in neoplastic disease (88, 89, 92–95), the role of host factors in HPV-associated disease (84–86, 90, 96–100), and therapeutic treatment efficacy (99, 101–103). Transgenic mice have also been developed to study cutaneotropic HPVs, such as HPV8 (104) and HPV38 (105), and their role together with UV radiation in promoting cutaneous disease and carcinogenesis (106–109). Clearly, HPV-transgenic models have provided a vital platform to study various aspects of papillomavirus-induced disease *in vivo*. However, their ability to model other key events during PV infection and pathogenesis is limited (Fig. 1A).

MmuPV1 DISCOVERY AND MOLECULAR VIROLOGY

Researchers have long sought an infection-based model of papillomaviral pathogenesis in laboratory mice to study aspects of viral pathogenesis not possible in transgenic mice, such as virus replication, persistence, transmission, and infection-mediated carcinogenesis (Fig. 1A). Until recently, no murine papillomavirus had been discovered. In 2011, Ingle and colleagues reported the isolation of a murine papillomavirus (MmuPV1) from cutaneous papillomas present on the skin of immunodeficient NMR1-*FoxN1*$^{nu/nu}$ mice (110). A highly similar MmuPV1 variant and a novel PV were subsequently isolated from normal skin of a house mouse (*Mus musculus*) and a wood

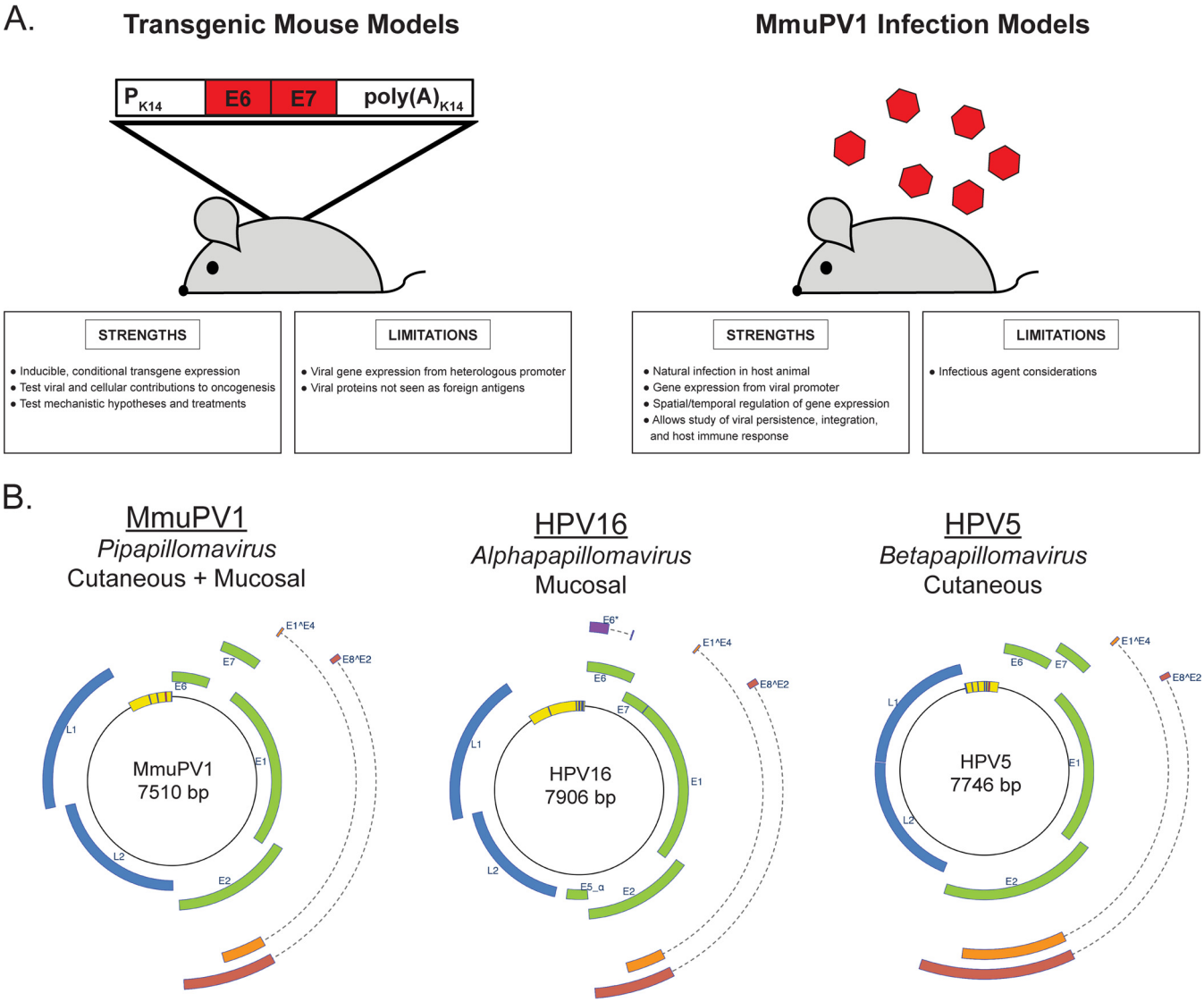

FIG 1 Comparison of HPV transgenic models and MmuPV1 infection-based models. (A) Strengths and limitations of HPV transgenic mouse models and MmuPV1 infection-based models. (B) MmuPV1 genomic organization, classification, and tissue tropism compared to an *Alphapapillomavirus*, HPV16, and a *Betapapillomavirus*, HPV5. All viral genomes were generated and images adapted from the PAVE database (36; http://pave.niaid.nih.gov).

mouse (*Apodemus sylvaticus*), respectively (111). Phylogenetically, MmuPV1 is classified in the genus *Pipapillomavirus* and is most closely related to other rodent papillomaviruses (40, 112). The genomic organization of MmuPV1 is comparable to that of other PV genomes, including common HPV genotypes (Fig. 1B). The MmuPV1 double-stranded DNA circular genome is composed of a noncoding upstream regulatory region (also known as the long control region) and early and late regions containing 7 translational open reading frames (ORFs). The early region ORFs encode the early (E) proteins E1, E2, E4, E6, and E7. Two late (L)-region ORFs encode the major and minor capsid proteins L1 and L2, respectively. MmuPV1 also expresses two spliced gene products, E1^E4 and E8^E2. There is no E5 ORF in the MmuPV1 genome, a trait that is shared with cutaneotropic beta HPVs and that differs from mucotropic alpha HPVs (Fig. 1B).

Despite their similarities, there are molecular and biochemical differences between MmuPV1 and HPVs. MmuPV1 shares little nucleotide sequence similarity (<70%) with other PVs (112), with MmuPV1 and HPV16, a prototypic oncogenic alpha PV, being 49.8% identical in sequence. In high-risk alpha HPVs, the major viral oncogenes E6 and E7 are transcribed from a single early promoter. In MmuPV1, E6 and E7 are transcribed

from two separate early viral promoters (113). HPV16 E6 and E7 proteins share approximately 45% and 40% sequence identity with their MmuPV1 counterparts, respectively (112). At first glance, these differences call into question the use of MmuPV1 as a model for HPV-associated carcinogenesis, but experimental studies demonstrate that activities associated with transformation by high-risk alpha HPV oncoproteins are retained in MmuPV1 E6 and E7. High-risk alpha HPV E7 proteins contain an LXCXE motif, which binds pocket proteins, including the RB1 tumor suppressor. In MmuPV1, the LXCXE motif is present in E6 but not E7 (111, 112). However, MmuPV1 E7 is similar to the gamma PV HPV197 E7 protein in that it binds RB1 through LXCXE-independent mechanisms (114). Another possibility is that MmuPV1 E7 has RB1-independent oncogenic potential. White and colleagues recently reported that MmuPV1 E7 binds to the cellular nonreceptor protein tyrosine phosphatase PTPN14 (115), a protein targeted for HPV16 E7-mediated degradation to impair keratinocyte differentiation in a process independent of RB1 binding (116). The MmuPV1 E6 protein inhibits Notch and transforming growth factor β (TGF-β) signaling, both tumor suppressor pathways, to delay differentiation and promote proliferation, functions shared with high-risk beta HPV E6 proteins (117, 118). Like high-risk beta HPV E6 proteins, MmuPV1 E6 does not bind directly to p53. Schulz et al. noted that the C terminus of the MmuPV1 E7 protein contains a putative PDZ-binding motif, a feature present in alpha HPV E6 that interacts with cell polarity and motility proteins (111). Therefore, MmuPV1 E6 and E7 appear to retain multiple, potentially tumorigenic properties. Most importantly, MmuPV1 clearly exhibits oncogenic potential, as discussed below. Additional studies are required to further characterize binding partners of the MmuPV1 E6 and E7 proteins and to determine whether and to what extent these interactions contribute to pathogenesis.

MmuPV1 EXHIBITS EXPANDED TROPISM IN MICE

Given its isolation from cutaneous papillomas and its genomic similarities with cutaneous beta HPVs, MmuPV1 was initially considered a cutaneotropic virus (110). MmuPV1 infects and causes disease at cutaneous sites, including the tail, muzzle, back, and ears (110, 119–125). There are conflicting reports on whether dorsal skin supports MmuPV1 infection, suggesting strain- and skin-specific variability in susceptibility to MmuPV1 infection (120, 123, 126, 127). Importantly, mucosal epithelia also support experimental MmuPV1 infection in immunodeficient mice (119, 123, 127–130), and studies in our laboratory have verified this expanded tissue tropism in immunocompetent *FVB/N* mice (Fig. 2A) (131–133). Lateral transmission in *FoxN1*$^{nu/nu}$ immunodeficient mice between experimentally infected cutaneous sites and oral and vaginal mucosae (134) provides further evidence for dual tropism. Like MmuPV1, certain beta HPVs also exhibit dual tropism (135, 136). The expanded tissue tropism of MmuPV1 has facilitated the development of novel models of PV pathogenesis in both cutaneous and mucosal epithelia, described below.

CURRENT MmuPV1 MODELS OF PAPILLOMAVIRUS INFECTION AND PATHOGENESIS

Virus entry and species and tissue tropism. MmuPV1 provides an opportunity to explore mechanisms that govern PV species and tissue tropism. Much of the research comparing MmuPV1 and HPV entry and tropism has been performed using *in vivo* cervicovaginal and/or cutaneous infections with pseudoviruses (PsV), which do not contain the viral genome but rather a reporter gene (e.g., that encoding luciferase or green fluorescent protein) encapsidated into virus-like particles composed of papillomaviral capsid proteins L1 and L2. Initial studies using MmuPV1 and HPV PsVs revealed similar mechanisms for virus entry (137); however, some differences have emerged. Day and colleagues found that, while MmuPV1 PsV initiates infection at the basement membrane, it does so, at least initially, in a heparan sulfate proteoglycan (HSPG)-independent manner (138), unlike HPV16 and other mucosotropic alpha PVs, which associate with the basement membrane using HSPG-dependent mechanisms. Interestingly, cutaneotropic beta PVs, like HPV5, interact with heparin moieties in a manner that

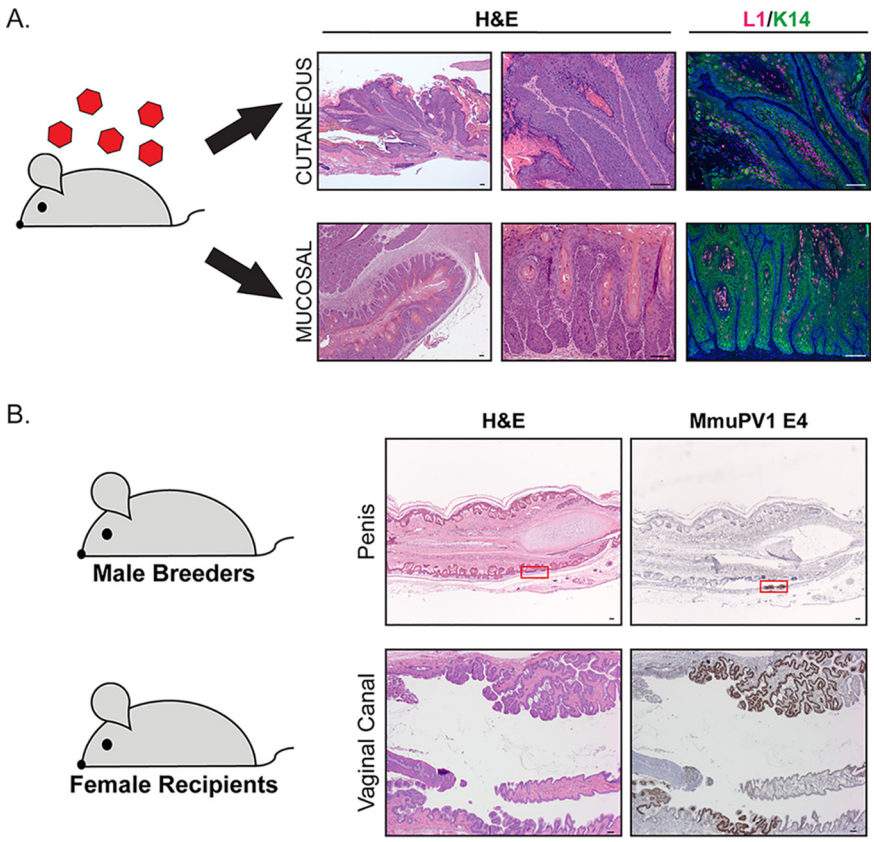

FIG 2 MmuPV1 exhibits expanded tissue tropism in mice and is a sexually transmitted virus. (A) MmuPV1 infects cutaneous and mucosal epithelia in immunocompetent mice, causing papillomas in skin (top row) and neoplastic disease in the female cervicovaginal mucosal epithelium (bottom row). Representative images of H&E (hematoxylin and eosin)-stained tissue show pathology. Productive MmuPV1 infection is indicated by immunofluorescence for MmuPV1 L1 protein (green), and keratin staining (red) highlights the epithelium. Bars = 100 μM. (B) MmuPV1 is a sexually transmitted virus. Shown are male (penile epithelium; top row) and female (vaginal canal; bottom row) reproductive organs of *FVB/N* mice that acquired MmuPV1 through sexual transmission. Representative images of H&E-stained tissue are shown, and RNA *in situ* hybridization for the E4 transcript (brown) indicates infected regions of epithelia (the red box highlights the region in male penile epithelium). Bars = 100 μM.

is also distinct from that of alpha PVs (139). Nevertheless, postentry intracellular trafficking of MmuPV1 PsV is similar to that of HPV PsV (138). These findings illuminate key differences and similarities in papillomavirus entry processes that are consistent with a previous report finding variations in how accurately animal PV replication models the HPV life cycle (140). Such limitations are inherent to animal models and warrant caution in extrapolating findings made in the use of MmuPV1 as a model for HPVs.

Studies of host immune response. A major strength of infection-based models is the opportunity they provide to study the host immune response during a natural infection. Initially, several laboratories reported that MmuPV1 fails to infect and/or efficiently promote disease in common strains of immunocompetent mice (110, 120, 121, 123–125, 141). However, subsequent studies have revealed a more nuanced relationship. Immunocompetent *SENCAR* and S/RV/Cri-*ba/ba* bare mice are susceptible to MmuPV1 infection and disease when high virus titers are used (110, 121, 123), an observation confirmed in *FVB/N* mice in our laboratory (133, 142). Outbred immunocompetent SKH1 mice are also susceptible (122, 125). Mice of the common *C57BL/6* strain seem particularly resistant to MmuPV1-induced disease (121, 123–125, 132), while they develop antibody responses and biomarkers for MmuPV1 infection in asymptomatically infected skin (121, 123, 125, 141), demonstrating that the genetic background of mice affects susceptibility to MmuPV1 infection.

The different sensitivities of various murine strains to MmuPV1 have helped define a role for the immune system, and more specifically, adaptive immunity, in regulating MmuPV1 infection. MmuPV1 was originally isolated from *FoxN1*$^{nu/nu}$ mice, which are homozygously null for the *FoxN1* gene, resulting in athymic mice that are T-cell deficient (143). Using a combination of approaches, researchers discovered that T-cell deficiency renders mice susceptible to MmuPV1 infection and disease (110, 120, 121, 123–125, 127, 129, 134, 141). For instance, several other strains of mice carrying the *FoxN1* genetic mutation are also susceptible to MmuPV1 infection (for a review, see reference 40). Various regimens of T-cell depletion revealed that complete T-cell deficiency correlates with susceptibility to MmuPV1 infection (121, 125). Systemic immunosuppression, induced with continuous cyclosporine treatment (121) or UV radiation (124), also can make resistant strains of mice more susceptible to MmuPV1 infection.

Several labs are using MmuPV1-based models to explore PV immunity. Pretreatment with an MmuPV1 L1 rabbit antiserum prevented MmuPV1-induced papillomas (120). Likewise, hyperimmune serum from mice immunized with MmuPV1 virus-like particles (VLPs) prevented MmuPV1 infection in highly susceptible T-cell-deficient strains of mice (127). Many studies continue to define the role of T-cell antitumor immunity to MmuPV1-based disease. The Roden lab revealed that MmuPV1 E6- and E7-specific CD8$^+$ T-cell responses promote papilloma clearance and regression and that adoptively transferred E6-specific CD8$^+$ T cells alone prevent MmuPV1-dependent tumor growth in nude mice (122, 125). In another study, MmuPV1-induced cutaneous tumors in T-cell-deficient mice regressed following adoptive transfer of hyperimmune splenocytes from congenic mice (144). Our laboratory discovered that a host stress keratin, keratin 17, is upregulated following MmuPV1 infection and prevents T cell recruitment to confer protection against cutaneous disease (142), highlighting a critical virus-host interaction involved in PV pathogenesis. Recently, a provocative study used an MmuPV1 cutaneous infection model to argue that immunity to cutaneous PVs protects against UV- and chemical-induced skin cancer (145). However, data from our laboratory contradict these findings, in that UV B radiation (UVB) treatment and MmuPV1 infection led to the development of cutaneous squamous cell carcinomas (SCCs) (124). Ongoing studies continue to evaluate other aspects of immunity, including the role of interferon signaling and neutrophil infiltration in MmuPV1 infection and disease (125, 126). More comprehensive reviews on MmuPV1 models and host immune response have been written (40, 146).

Studies of MmuPV1 viral gene products and host factors. The alpha HPV E6 and E7 proteins, in addition to their oncogenic properties (28), function during the maintenance and productive phases of the HPV life cycle (147, 148). We found that MmuPV1-induced papilloma formation requires the E6 protein, as an E6-null (E6^{STOP}) mutant MmuPV1 mutant failed to induce papillomas in nude mice (118). Likewise, an MmuPV1 E6 mutant that cannot bind MAML1 (E6^{R130A}) also failed to induce papillomas. These findings suggest that E6 protein functions, including its inhibition of Notch signaling, are critical to MmuPV1-induced pathogenesis. Using antisera against MmuPV1 L1 and L2, Handisurya and colleagues found that during MmuPV1 cutaneous infections, L1 is expressed throughout all epithelial layers in papillomas instead of just suprabasal layers and is localized to the cytoplasm in the absence of L2, suggesting that a unique pattern of late gene expression and virion assembly may occur during the MmuPV1 life cycle (120). Additional studies are necessary to characterize the role of other MmuPV1 proteins in the viral life cycle, pathogenesis, and carcinogenesis.

The lack of an E5 protein is a key difference between MmuPV1 and high-risk alpha HPV genomes (Fig. 1B). We infected the skin and female reproductive tracts of *K14E5* HPV16 transgenic mice with MmuPV1 to determine the effect of epithelial E5 expression on MmuPV1-associated pathogenesis (133). In MmuPV1-infected *K14E5* mice, skin lesions exhibited earlier onset, higher incidence, and reduced frequency of spontane-

ous regression compared to nontransgenic mice. Moreover, estrogen-treated *K14E5* mice were more likely to develop cervicovaginal cancers than their nontransgenic counterparts. Therefore, HPV16 E5 potentiates MmuPV1 pathogenesis. Further studies are necessary to determine mechanisms of E5 function in this context, which could involve the role of HPV16 E5 in suppressing immune responses (149). Complementation studies combining HPV16 transgenic mice and MmuPV1 infection provide a unique platform to study the role of high-risk alpha HPV proteins in pathogenesis.

New MmuPV1-based infection models provide important opportunities to study host factors that promote PV persistence, a key risk factor for subsequent malignant progression (18, 19). In the MmuPV1 cervicovaginal model, persistent infections are established in the mucosal epithelia of immunocompetent *FVB/N* mice that persist for at least 10 months (131, 132). Estrogen increases the severity of disease, and this correlates with the establishment of persistent infections (132). Notably, MmuPV1 viral copy numbers are highest during the estrus phase in immunodeficient mice (130). That estrogen is being revealed as a potential persistence factor is just one example of how MmuPV1 has the potential to illuminate roles of host factors in PV pathogenesis and disease.

MmuPV1 cancer models. MmuPV1 E6 delays differentiation and promotes proliferation in keratinocytes *in vitro* and is necessary for papilloma development *in vivo* (118). However, there is relatively little published biochemical evidence for the transforming activity of the MmuPV1 viral proteins. That being said, there are now multiple studies demonstrating that MmuPV1 displays oncogenic potential *in vivo*. In cutaneous sites, immunodeficient nude mice experimentally infected with MmuPV1 developed poorly differentiated, locally invasive tumors that histologically resembled human trichoblastomas (123). Squamous cell carcinomas were also observed at cutaneous sites of secondary MmuPV1 infections in nude mice at 9.5 months postinfection (134). *FVB/NJ* mice infected with MmuPV1 and treated with UVB were found to develop SCC of the skin, demonstrating that MmuPV1 can cause cancer in immunocompetent mice (124).

MmuPV1 also displays oncogenic potential in mucosal tissues. Cladel et al. first reported that heterozygous nude mice (*FoxN1*$^{nu/+}$) infected for 7.5 months with MmuPV1 develop carcinoma *in situ* in the female reproductive tract (126). Later, it was found that high-grade precancerous lesions and SCC developed in the female reproductive tract of immunocompetent *FVB/N* mice at 6 months postinfection (132). Similar to its role as a cocarcinogen in HPV16 transgenic mice (84–86, 88), estrogen significantly increased the incidence and severity of high-grade disease and cancer in MmuPV1-infected *FVB/N* mice (132). Further exploration of the role of estrogen and of whether anti-estrogen drugs are efficacious in preventing and treating disease, as is the case in HPV16-transgenic mice (101, 102), is warranted in MmuPV1-based infection models of cervicovaginal disease. These established and emerging MmuPV1 models show great potential for studying all stages of papillomavirus-mediated carcinogenesis, the role of host cofactors, and therapeutic treatments.

Models of PV transmission. MmuPV1 models have the potential to identify unexplored facets of PV transmission relevant to public health. Mucosal HPV transmission occurs most frequently through sexual contact, and HPV infections are the most common sexually transmitted infections in the United States (16). Epidemiological data are used to understand HPV sexual transmission in humans (150); however, laboratory models to study the underlying molecular mechanisms involved in papillomavirus sexual transmission are lacking. While rhesus macaque PV 1 (RhPV-1) is sexually transmitted (67, 69), the cost, scalability and ethical considerations in the use of nonhuman primates limit its application to the study of sexual transmission of papillomaviruses. MmuPV1 was recently described as being sexually transmitted (131). Female *FVB/N* donor mice, experimentally infected in their cervicovaginal tracts with MmuPV1, transmitted MmuPV1 to untreated *FVB/N* male breeders through sexual transmission, indicating that the penile epithelium supports MmuPV1 infection

(Fig. 2B). The infected male breeders subsequently transmitted MmuPV1 to untreated naive *FVB/N* recipient female mice (Fig. 2B). Approximately one-third of these recipient female mice acquired MmuPV1 infections, some transient and some prolonged, through natural sexual transmission. Therefore, MmuPV1 can be sexually transmitted in wild-type laboratory mice in the absence of any environmental or genetic manipulation. This powerful new animal model of natural PV sexual transmission promises to provide new insights into the molecular dynamics of PV sexual transmission in both male and female reproductive organs.

An MmuPV1 model has also provided potential evidence for blood-borne PV transmission (151). MmuPV1 was introduced into immunodeficient *FoxN1*$^{nu/nu}$ mice via tail vein injection; infections developed at prewounded cutaneous and mucosal sites, and virus was detected in the stomach. Furthermore, naive mice receiving blood from MmuPV1-infected animals developed disease in both cutaneous and mucosal epithelia. While these results are intriguing, they should be carefully interpreted. Experimentally wounded immunodeficient mice used in this study are highly susceptible to lateral transmission and infection by MmuPV1, which could be inadvertently introduced through a variety of environmental routes (animal bedding, handling, etc.). Therefore, the incorporation of appropriate mock-infected and unwounded controls into such experiments is critical for conclusive interpretation. Nevertheless, MmuPV1 promises to uncover new molecular insights into PV transmission.

CONCLUSIONS AND FUTURE PERSPECTIVES

The discovery of MmuPV1 has ultimately provided a practical and genetically tractable infection-based system to model HPV infection, transmission, and pathogenesis. Preclinical animal models of viral pathogenesis are inherently limited in their ability to unequivocally recapitulate every aspect of human infection and disease. However, as discussed in this Gem, MmuPV1-based infection models hold incredible promise for providing insight into facets of HPV pathogenesis that were difficult to study before their development. For these reasons, established and emerging MmuPV1 infection models represent a new frontier in animal models of PV pathogenesis and promise to transform our understanding of HPV-associated human disease.

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