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Molecular Mechanisms of Merkel Cell Polyomavirus Transformation and Replication

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Abstract

Viral infection underlies a significant share of the global cancer burden. Merkel cell polyomavirus (MCPyV) is the newest member of the human oncogenic virus family. Its discovery over a decade ago marked the beginning of an exciting era in human tumor virology. Since then, significant evidence has emerged to support the etiologic role of MCPyV in Merkel cell carcinoma (MCC), an extremely lethal form of skin cancer. MCPyV infection is widespread in the general population. MCC diagnoses have tripled over the past 20 years, but effective treatments are currently lacking. In this review, we highlight recent discoveries that have shaped our understanding of MCPyV oncogenic mechanism and host cellular tropism, as well as the molecular events occurring in the viral infectious life cycle. These insights will guide future efforts in developing novel virus-targeted therapeutic strategies for treating the devastating human cancers associated with this new tumorigenic virus.

INTRODUCTION

Merkel cell polyomavirus (MCPyV) belongs to the *Polyomaviridae* family and is the most recently discovered human oncogenic virus (1, 2). Accumulating evidence supports a causative role for MCPyV in the development of a highly lethal form of malignancy, Merkel cell carcinoma (MCC) (1, 3). While the primate simian virus 40 (SV40) has been shown to induce tumor growth only in laboratory animal models (4) and the etiological role of BK virus (BKV) in human cancer remains controversial (5), MCPyV has become the first polyomavirus to be associated with human cancer (1, 3). MCC usually develops in the skin but can quickly disseminate to lymph nodes and other organs. With a 5-year disease-associated mortality rate of ~46% (1, 2, 6–10), MCC is considered one of the most aggressive forms of skin cancer. Although rare, the incidence of MCC has increased by more than 95% in the United States since 2000 (11). Excessive exposure to sunlight and ultraviolet (UV) radiation, advanced age, and immunosuppression are the principal risk factors for MCC development (9, 12–16).

MCPyV was discovered in 2008 by the Chang & Moore team (1), which is adept at hunting for cancer viruses as etiologic agents in human malignancies. Because MCC disproportionally affects immunosuppressed and elderly individuals, the team reasoned that the disease is likely caused by an infectious agent (1). Building on their previous success in discovering Kaposi sarcoma–associated herpesvirus as the causative oncogenic virus of Kaposi sarcoma (17), they developed a transcriptomic sequencing approach called digital transcriptome subtraction to identify foreign transcripts expressed in human MCC tumors. Using this approach, they first identified a polyomavirus large tumor (LT) antigen transcript homologous to known polyomavirus tumor antigens in the MCC tumor genome. Viral genome walking was then combined with 3′ rapid amplification of complementary DNA ends to retrieve the complete sequence of this virus—MCPyV (1) (**Figure 1**).

MCPyV is a small, nonenveloped, icosahedral virus with a circular, double-stranded DNA genome of ~5,400 base pairs (2, 18) (**Figure 1**). Phylogenetically, it is quite distant from other known human polyomaviruses (HPyVs) and SV40 but more closely related to viruses within the chimpanzee and gorilla polyomavirus subgroup (19, 20). The viral genome is divided by a noncoding regulatory region (NCRR) into early and late regions (2, 21, 22) (**Figure 1**). The NCRR contains the viral origin (Ori) of replication and bidirectional promoters for viral transcription (21, 22). The early region of MCPyV houses the MCPyV tumor antigen locus, which encodes four differentially spliced messenger RNA (mRNA) transcripts corresponding to the LT antigen, small tumor (sT) antigen, and 57-kDa tumor (57kT) antigen isoform, as well as the overprinted gene named alternative LT open reading frame (ALTO) (1, 2, 23) (**Figure 1**). As discussed below, LT and sT antigens are the best-studied viral proteins that regulate both MCPyV replication and host cell proliferation. Very little is known about the function of 57kT. ALTO is expressed during MCPyV genome replication in HEK293 cells (23). However, it does not appear to directly contribute to viral genome replication in this cell culture setting but may play an accessory role in the viral life cycle (23). Like other polyomaviruses, MCPyV also encodes a microRNA (miRNA), termed miR-M1 (2, 23–25) (**Figure 1**). The late region of MCPyV encodes the two capsid proteins, virus protein 1 (VP1) and virus protein 2 (VP2), which function as the major and minor subunits of the viral capsid, respectively (26–28) (**Figure 1**). Unlike other polyomaviruses, MCPyV does not encode an agnoprotein.

Since its discovery, MCPyV has been recognized as a ubiquitous virus that asymptomatically infects most individuals commencing in early childhood (29–32). Studies in the past decade have established a causal role of MCPyV in MCC (33, 34). Among them, one suggested that nearly all MCCs carry MCPyV (35), but the others concluded that about 80% of all MCC cases are MCPyV

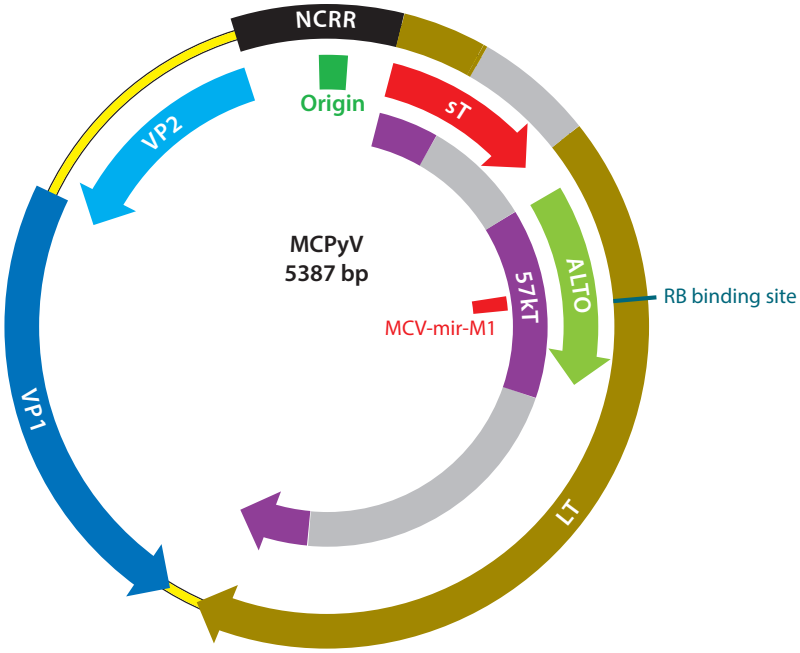

Figure 1

The genome structure of MCPyV. This schematic diagram depicts the NCRR, early genes, late genes, and microRNA miR-M1 encoded by the MCPyV genome. Abbreviations: 57kT, 57-kDa tumor antigen; ALTO, alternative large tumor open reading frame; LT, large tumor antigen; MCPyV, Merkel cell polyomavirus; NCRR, noncoding regulatory region; RB, retinoblastoma; sT, small tumor antigen; VP, virus protein.

positive while the rest do not harbor MCPyV and may instead be caused by a distinct pathoetiology (36–39). Although high mutational burdens were found in the MCPyV-negative MCCs, MCPyV-positive MCC tumors tend to harbor very few somatic mutations (40–42), thereby holding MCPyV gene products responsible for driving tumorigenesis in this majority group of MCCs. In light of these revelations, this review aims to highlight important recent discoveries that explain how this small oncogenic virus infects humans and, while promoting its own survival and replication in the infected host cells, may acidentally cause MCC development, especially in immunocompromised individuals.

ONCOGENIC MECHANISM

Tumor-Specific Pattern of Large Tumor Antigen Gene Mutations

Much of the MCPyV research in the past decade has been focused on how the MCPyV encoded proteins contribute to tumor development. So does this new cancer virus drive cellular transformation in a way similar to other oncogenic viruses such as human papillomavirus, or does it have its own tricks? In the first study by Feng et al., MCPyV DNA was found to be integrated in 6 out of 11 MCC tumor samples examined (1). By comparing the MCPyV DNA sequences integrated in metastatic tumors isolated from different patients, the team quickly realized that the viral DNA integrates monoclonally into the MCC cellular genomes (**Figure 2**), hinting that the integration events precede monoclonal expansion of cancer cells during the initial phase of tumor development (1). This early observation suggests that, like many other tumor viruses, viral integration is

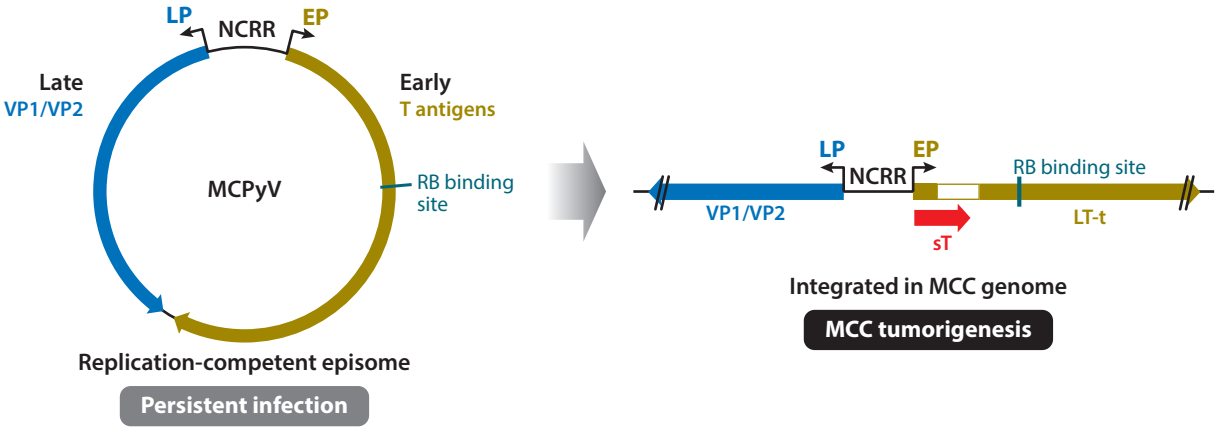

Figure 2

Abortive MCPyV genome integration during MCC development. MCPyV replicates as an episome in persistently infected cells. In MCC tumors, the viral genome is clonally integrated into the cancer genome. The integrated viral genome expresses wild-type sT and LT truncation mutants retaining the N-terminal RB-binding motif but deleting the C-terminal domains needed for viral DNA replication. The double slash indicates the junction of host DNA and the integrated viral genome. Abbreviations: EP, early promoter; LP, late promoter; LT, large tumor antigen; LT-t, tumor-derived LT truncation mutant; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus; NCRR, noncoding regulatory region; RB, retinoblastoma; sT, small tumor antigen; VP, virus protein.

a crucial event in MCPyV-driven MCC tumorigenesis (**Figure 2**). Subsequent studies from many groups confirmed that the MCPyV genome is clonally integrated into the cancer genome in at least ~80% of MCC cases (43–46).

Just like in other polyomaviruses, MCPyV LT contains all the conserved domains involved in manipulating the host cell cycle and replicating the viral genome. The N-terminal region of LT contains the conserved region 1 (CR1) that is functionally similar to the cell transforming region of adenovirus E1A protein, the DnaJ domain that binds heat shock proteins, and an LxCxE binding motif for the tumor suppressor retinoblastoma (RB) protein (47) (**Figure 3**). The C-terminal region of LT contains the Ori binding domain (OBD) and helicase/ATPase domains needed for stimulating viral DNA replication (22, 48) (**Figure 3**). By analyzing the LT transcripts expressed from the MCPyV genome integrated into MCC tumors, Shuda et al. discovered that the tumor-derived LT truncation mutant (LT-t) sequences possess premature stop codon mutations or deletions that the C-terminal OBD and helicase domains needed for productive viral replication but preserve the RB-binding motif as well as the other functional domains within the LT N terminus (47) (**Figures 2** and **3**). Therefore, the integrated viruses retain the ability to inhibit tumor suppressor RB protein and modulate the host cell cycle but can no longer support viral DNA replication (47) (**Figure 2**). In contrast, MCPyV DNA isolated from nontumor sources (wild-type viruses) does not show this LT mutation signature (47).

The sT protein shares the CR1 and DnaJ domains with LT but has a unique C terminus carrying two protein phosphatase 2A (PP2A) binding motifs (**Figure 3**). Integration of the MCPyV genome in MCC typically preserves the expression of the native sT antigen (47, 49–51) (**Figure 2**). Short hairpin RNA–mediated knockdown of MCPyV LT/sT antigens induces growth arrest and cell death in MCPyV-positive MCC cell lines (49) and leads to tumor regression in vivo (52). These findings demonstrate that expression of MCPyV LT and sT antigens is required to support the growth of MCC tumor cells in vitro and in the xeno-transplantation model.

Together, these seminal studies provide direct evidence to support the causative role for MCPyV in tumorigenic development of most MCC tumors (49, 52). It is clear that two

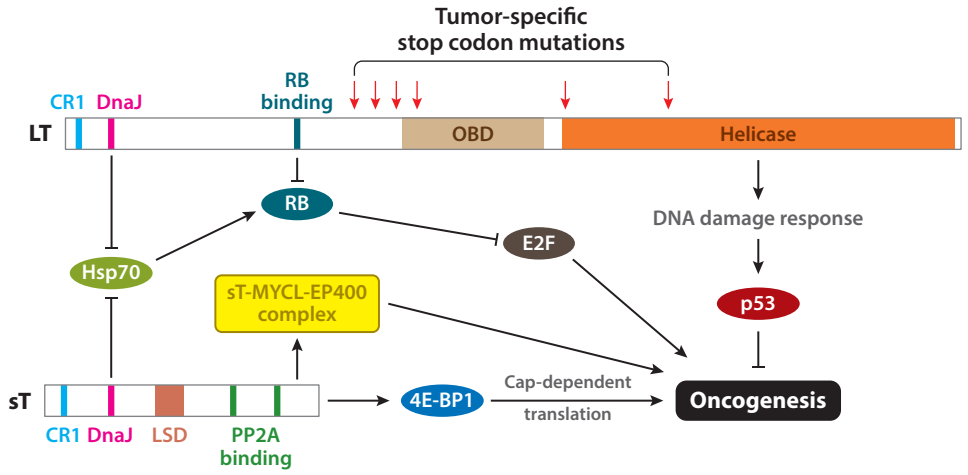

Figure 3

Key cellular factors and pathways targeted by MCPyV oncoproteins to promote oncogenesis. The schematic depicts the domain structures of MCPyV LT and sT antigens. The two viral oncoproteins stimulate cellular growth and transformation through modulating the function of various cellular targets. The C terminus of LT that activates p53 is deleted in the tumor-specific LT-t mutants, thus releasing the antitumor break to allow oncogenic progression. Small red arrows mark the tumor-specific premature stop codon mutations or deletions resulting from MCPyV integration events in MCC. Arrows signify that the domain promotes the factor it points to, while perpendicular bars indicate inhibition. Abbreviations: 4E-BP1, eukaryotic translation initiation factor 4E binding protein 1; CR1, conserved region 1; DnaJ, chaperone protein DnaJ; E2F, the E2 factor; Hsp70, heat shock protein 70; LSD, large tumor–stabilization domain; LT, large tumor antigen; LT-t, tumor-derived LT truncation mutants; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus; OBD, Ori binding domain; p53, tumor protein p53; PP2A, protein phosphatase 2A; RB, retinoblastoma; sT, small tumor antigen. Figure adapted with permission from Reference 18.

independent MCPyV mutagenic events, namely the monoclonal integration of viral DNA into the host genome and the unique tumor-specific MCPyV LT truncation, are required to initiate the development of MCPyV-positive MCC tumors. As the emerging tumor cells proliferate, the persistent replicative state enforced by the RB suppression activity of the integrated viral genomes may allow these cells to acquire and accumulate additional genetic mutations needed to develop into full-blown MCC.

Large Tumor Antigen–Retinoblastoma Interaction

RB inactivation is a critical step in MCC tumorigenesis. While most of the MCPyV-negative MCCs carry genetic deletions/mutations that inactivate the cellular *RB1* gene (40, 53, 54), MCPyV-positive MCCs usually encode the wild-type *RB1* gene (40, 42). However, all known MCC-derived LT-t truncation mutants preserve the RB-binding motif (47), which allows them to bind the tumor suppressor with high affinity (55). These observations suggest that MCPyV interaction with RB may play an important role in driving the oncogenic progression of MCPyV-positive tumors (47). Indeed, MCC cell growth inhibition induced by MCPyV LT knockdown can be rescued only by introducing LT molecules maintaining an intact LxCxE RB-binding site (52). Not surprisingly, the vast majority of gene expression changes induced by the tumor-derived LT-t, such as those implicated in cellular growth control, are dependent on its LxCxE RB-binding motif (56).

Although SV40 LT has the potential to inhibit all three members of the pocket protein family, RB1, p107, and p130, MCPyV LT preferentially interacts with RB1 and discriminates against the other two pocket proteins (57). Accordingly, the growth inhibition induced by MCPyV LT knockdown in MCC cells could be rescued only by knockdown of RB1 but not of p107 or p130. Furthermore, homozygous deletion of *RB1* gene in an MCPyV-positive MCC cell line allows the tumor cells to grow independently of MCPyV LT expression (57). These findings corroborate that inactivation of RB1 is the predominant function of MCPyV LT that promotes cell growth during MCPyV-driven tumorigenesis (57).

A Complex Merkel Cell Polyomavirus Interaction with the p53 Pathway

Unlike the LT proteins encoded by other polyomaviruses, MCPyV LT does not appear to directly bind the tumor suppressor p53 (51, 55). But does MCPyV modulate the p53 pathway in any other ways? To address this question, two groups independently examined the full-length LT protein (51, 58). They discovered that the C-terminal region of MCPyV LT protein expressed in human cells could inhibit their growth (51, 58). MCPyV LT, especially its C-terminal OBD and helicase domain, causes damage to host DNA and induces DNA damage responses (DDRs) (58). This LT function activates the p53 pathway to trigger cell cycle arrest and inhibit cellular proliferation (58). These antitumorigenic effects could be rescued by a dominant-negative p53 inhibitor, suggesting that LT's growth inhibition function is, at least partially, mediated through activating the p53 pathway (58) (**Figure 3**). However, MCPyV proviruses integrated in malignant MCC cells encode LT truncation mutants that almost invariably delete this DDR-activating domain (47) (**Figure 3**). Compared with the full-length LT protein, the LT C terminus truncation mutants are more effective at stimulating cell growth (51), suggesting that virus-induced host DDR could function as a barrier to malignant progression (**Figure 3**). Therefore, during MCPyV-driven tumorigenic development, elimination of the DDR-inducing, growth-inhibitory function intrinsic to the C-terminal domain of MCPyV LT may release the tumor-suppressing brake to allow tumor development (58).

Houben and colleagues examined the p53 signaling pathways in a set of MCPyV-positive and MCPyV-negative MCC lines (59). They discovered that most of the MCPyV-positive MCC lines express wild-type p53 protein with poor activity; however, neither MCPyV sT nor the tumor-specific LT-t antigen has the capacity to inhibit p53 (59). Nevertheless, inhibition of the ubiquitin ligase mouse double minute 2 (MDM2; also called human double minute 2), a major inhibitor of p53 activity, leads to p53 activation and consequential cell cycle arrest and apoptosis (59). This study therefore suggests that p53 could be explored as a promising therapeutic target for treating p53 wild-type MCCs. It was further demonstrated that, by binding to RB, the truncated MCPyV LT stimulates the expression of ARF, an inhibitor of MDM2, and hence indirectly activates p53 (60). However, the sT-associated MYCL-EP400 complex can functionally inhibit p53 by transactivating the expression of MDM2 as well as CK1α, an activator of MDM4 that also negatively regulates p53 (60) (**Figure 3**). Because MCPyV-positive MCC cells usually express a high level of MDM4, dual treatment with MDM2 and MDM4 inhibitors was explored to synergistically activate p53 and induce apoptosis of MCPyV-positive MCC cells both in vitro and in mouse xenograft models (60).

Small Tumor Antigen Is a Dominant Oncogenic Driver in Merkel Cell Carcinoma Carcinogenesis

Another key difference between MCPyV and other polyomaviruses is present in sT. Contrary to sT of other polyomaviruses, MCPyV sT is directly involved in MCPyV-induced cellular

transformation (50). sT is expressed in the majority of MCC tumors and, compared with LT antigen, is more frequently detected in MCPyV-positive MCC tumors (50). When both MCPyV LT-t and sT are knocked down in MCC cells, overexpression of the LT-t does not fully rescue the growth inhibitory effect (52). These observations imply that sT may play an important role in driving MCPyV-associated tumorigenesis. Indeed, sT expression is required to support the growth of MCPyV-positive MCC cell lines (50). In an anchorage-independent focus formation assay, expression of sT alone is sufficient to transform rodent fibroblasts (50). This sT transforming potential could be partly attributed to its ability to promote hyperphosphorylation of eukaryotic translation initiation factor 4E binding protein 1, a crucial downstream target of the PI3K/AKT/mTOR signaling pathway, which contributes to hyperactivated cap-dependent translation of cellular mRNA (50) (**Figure 3**). Other polyomavirus sTs can disrupt the AKT signaling pathway through PP2A inhibition to stimulate cellular proliferation (61, 62). While MCPyV sT is able to bind PP2A, its transforming activity does not appear to involve PP2A inhibition (50, 63).

Targeted expression of sT in the epidermis of transgenic mice leads to compromised differentiation, enhanced hyperplasia, and proliferation, culminating in a robust epithelial transformation phenotype (64–66). Inducible sT expression in embryonic mice enhances progenitor Merkel cell proliferation and promotes tumor formation in the homozygous p53 null genetic background (67). sT also encodes an LT-stabilization domain (LSD), which allows it to increase LT's protein level (68, 69) (**Figure 3**). sT molecules with mutated LSD could no longer drive epithelial transformation in vivo, implying that the in vivo transforming function of sT could be partially mediated through enhancing LT-t protein level in MCC cells (64).

Both in vitro and in vivo studies establish MCPyV sT as a key oncogenic driver in MCC carcinogenesis. To further investigate the functional mechanisms by which sT contributes to this process, the DeCaprio group (70) investigated sT's effect in cells using both transcriptomic and proteomic approaches. Their transcriptome-wide analysis revealed that inducible expression of sT in IMR90 human diploid fibroblasts dramatically alters the levels of metabolite transport genes, especially those implicated in glycolysis (70). Inhibition of one such gene, monocarboxylate lactate transporter SLC16A1, was able to suppress the growth of MCC cells and dampen MCPyV-induced transformation, suggesting that the sT transforming function could be partially attributed to its ability to perturb aerobic glycolysis in normal human cells (70). Further proteomic analysis of sT binding proteins in MCC cells revealed that sT binds specifically to both MYC homolog MYCL (L-MYC) and MYC-associated factor X, recruiting the heterodimer to the EP400 chromatin remodeler complex (71). By combining chromatin immunoprecipitation and sequencing with RNA sequencing, it was discovered that the sT-MYCL-EP400 complex binds and transactivates expression of specific target genes required for maintaining the viability of MCPyV-positive MCC cell lines (71). In IMR90 stably expressing a dominant negative form of p53, hTERT, MYCL, and the truncated LT, sT was able to support anchorage-independent growth in soft agar in a manner that relies on its ability to bind the EP400 complex (71). This finding further suggests that the sT-MYCL-EP400 complex may contribute to cell transformation and MCPyV oncogenic potential (71).

MERKEL CELL POLYOMAVIRUS INFECTION

MCC typically presents as a neuroendocrine carcinoma of the skin. Historically, the tumors have been thought to arise from Merkel cells, a nonproliferative population of neuroendocrine-derived mechanoreceptor cells that can form synapse-like contacts with nerve terminals from the basal layer of the epidermis (72, 73). The discovery of MCPyV sheds significant light on the viral etiology of MCC (1). MCPyV infection, however, is highly prevalent and predominantly asymptomatic

in humans (31, 32, 74, 75). These findings therefore raise the questions of how MCPyV normally infects humans and in what circumstances it causes MCC development.

Entry into the Host Cells

An important part of the MCPyV life cycle that is essential for understanding viral tropism is its entry into the host cell. The MCPyV genome is encapsidated in an icosahedral viral capsid consisting of the structural proteins VP1 and VP2 (1, 28). Because cell culture systems for MCPyV production were not available until recently, recombinant MCPyV pseudoviruses consisting of VP1/VP2 capsids harboring a green fluorescent protein (GFP) or luciferase reporter plasmid were often used to study virus entry into host cells (26). Early studies showed that MCPyV enters its target cells in a slow and asynchronous fashion (27). After entering the host cell, MCPyV must travel to the nucleus so that the virus can use the host cellular replication machinery to replicate its DNA. Cell surface glycoproteins and glycolipids are critical for mediating viral penetration of the cell membrane and channeling the virions through intracellular organelles before they ultimately reach the nucleus. Similar to papillomaviruses, initial attachment of MCPyV to its host cell is mediated by VP1 binding sulfated glycosaminoglycans (GAGs), specifically heparan sulfate (HS) proteoglycans (26). Like in other polyomaviruses, MCPyV VP1 further engages sialylated glycans in the secondary entry step after primary attachment through GAGs (26, 76, 77). Through analyzing the cellular determinants of MCPyV entry into the highly transducible lung carcinoma A549 cells, it was found that MCPyV enters cells via caveolar/lipid raft-mediated endocytosis (78). In this process, the viruses are internalized in small endocytic pits, routed through the endosomal pathway, and transported to the endoplasmic reticulum (ER). However, the molecular events that deliver the encapsidated viral DNA from the ER to the site of transcription and replication remained unexplored.

Host Cellular Tropism

While the discovery of cellular factors that mediate MCPyV attachment and entry was exciting (26, 77), it does not afford many clues for MCPyV host cellular tropism. Although the identified MCPyV binding receptors, such as sialic acid and HS, are ubiquitously expressed, MCPyV infects and replicates poorly in a wide range of cell types that express these cell surface receptors (27, 79, 80). These findings indicate that viral entry is not the limiting factor for MCPyV to target specific cell types. Because of the neuroendocrine markers expressed in MCC, it has been speculated that the malignancy arises from Merkel cells, which therefore could be the primary targets of productive MCPyV infection. However, Merkel cells are of epidermal origin (81) and reside in the epidermis (3), whereas MCC tumors usually develop within the dermis or subcutis (82). An additional conundrum is that Merkel cells are postmitotic and do not have robust proliferative potential (83). They therefore could not account for the millions of MCPyV virions constantly shed from the surface of healthy human skin (74, 84).

Because skin is likely the major site of MCPyV propagation in humans (31, 32, 74, 84), Liu and colleagues examined the MCPyV infectability of the total cell population isolated from human foreskin using both recombinant MCPyV virions carrying the native viral genome and MCPyV pseudoviruses harboring a GFP reporter construct (85). The infectious entry of MCPyV was visualized by GFP signal in the pseudovirus-treated cell population; immunofluorescent staining of cell type–specific markers was performed to further identify the skin cells that support productive viral transcription and replication in the MCPyV virion-treated sample (85). After

examining epidermal keratinocytes, dermal fibroblasts, Merkel cells, and many other types of cells in the skin, it was discovered that only human dermal fibroblasts (HDFs) are permissive to MCPyV entry, transcription, and replication (85). HDFs treated with epidermal growth factor and basic fibroblast growth factor were particularly effective in supporting MCPyV infection (85). The fact that these growth factors are typically upregulated at wounded skin sites suggests that the wounding process may induce a tissue microenvironment conducive to MCPyV infection and replication (85, 86) (**Figure 4**).

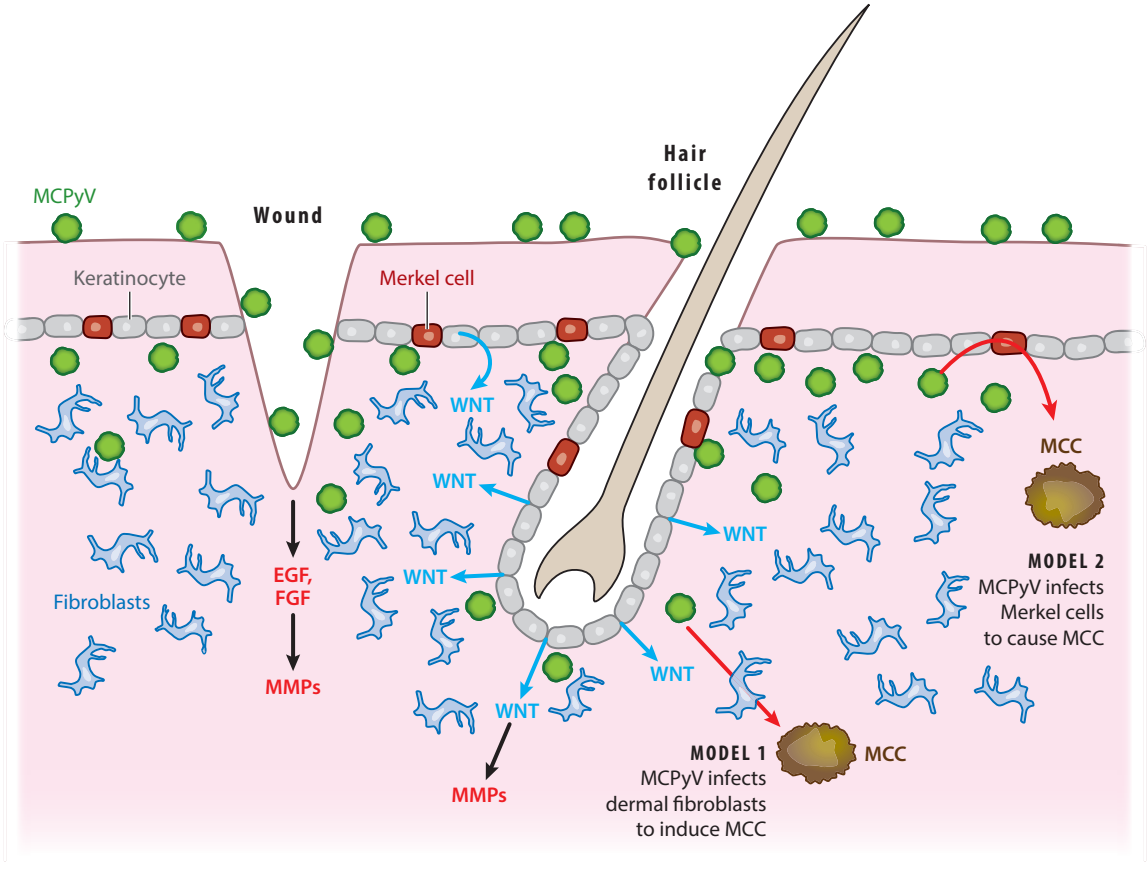

Figure 4

Molecular mechanisms underpinning MCPyV infection and MCC development. HDFs are a permissive target cell of MCPyV infection in the human skin. Upregulation of MMP genes by WNT/β-catenin signaling and growth factors, such as EGF and FGF accumulated at wounded skin sites, can promote MCPyV infection. Also shown are the two proposed models linking MCPyV infection to MCC tumorigenesis. Under yet-to-be-discovered conditions, viral oncogenes expressed in infected HDFs may induce gene expression patterns typical of MCC to trigger tumor development (Model 1). Alternatively, because MCCs also express genes that are normally transcribed in benign Merkel cells, MCPyV infection may induce Merkel cells to undergo malignant transformation. According to this model, MCPyV actively replicating in the dermal fibroblasts may inadvertently enter Merkel cell precursor cells residing in the immediate vicinity. While these bystander precursor cells may be able to support MCPyV replication, as they differentiate into Merkel cells, they may acquire a dead-end replication environment in favor of viral integration, which ultimately leads to cellular transformation (Model 2). Abbreviations: EGF, epidermal growth factor; FGF, fibroblast growth factor; HDF, human dermal fibroblast; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus; MMP, matrix metalloproteinase; WNT, wingless-related integration site. Figure adapted with permission from Reference 18.

In a chemical screening, it was discovered that activators of the WNT/β-catenin signaling pathway could stimulate MCPyV transduction of HDFs (85). In human skin, WNT signals are released from keratinocytes located in the basal layer of the epidermis or within the outer root sheath of hair follicles to stimulate the proliferation of surrounding dermal fibroblasts (87) (**Figure 4**). These observations suggest that WNT signaling activated in these skin locations may prime the skin fibroblasts for MCPyV infection. Indeed, when tested in human skin slices cultured ex vivo to mimic the natural environment of the human skin, MCPyV was found to preferentially infect the skin fibroblasts either underlying the basal layer of the epidermis or encircling hair follicles (85) (**Figure 4**). This finding establishes that active WNT signaling released by the keratinocyte compartment of human skin stimulates MCPyV infection (85) (**Figure 4**). This effect is mediated by inducing WNT/β-catenin signaling pathway downstream genes such as matrix metalloproteinases (*MMP*), which can disrupt the extracellular matrix of the skin fibroblasts to stimulate MCPyV infection (85) (**Figure 4**). In human skin, damage induced by both UV/ionizing radiation exposure and aging can activate *MMP* expression (88–91). Therefore, these major risk factors for MCPyV-associated MCC may stimulate viral infection and promote tumor development through this mechanism (85).

MCPyV-infected HDFs demonstrate robust expression of early and late viral genes, as well as viral DNA replication (85), making them the first in vitro infection model for productive MCPyV infection (85). However, in addition to human skin, MCPyV DNA has also been detected in respiratory, urine, and peripheral blood samples (92, 93). It is therefore reasonable to speculate that MCPyV may establish an infectious reservoir in one of these tissues. These ideas await testing in an in vivo model for MCPyV infection once it becomes available.

The Cell Origin of Merkel Cell Carcinoma

The relation between the cells that MCPyV infects and those that it transforms to cause MCC remains an intriguing question. MCCs have traditionally been thought to arise from Merkel cells because these tumors contain neurosecretory granules indicative of neurocrest origin (94) and also express a unique marker of Merkel cells, cytokeratin 20 (95, 96). It has also been proposed that MCC tumors may derive from the B cell lineage because they express markers of pro-/pre-B cells (97). Identification of skin fibroblasts as the host cells permissive for MCPyV infection is consistent with the observation that most MCC tumors are dermal in origin (82). This finding provides new alternative hypotheses regarding the cell type(s) that may give rise to MCC (85). One possibility is that MCPyV infection of skin fibroblasts could, under conditions not yet discovered, stimulate their transformation and cause deranged gene expression patterns typically observed in other cell types, including B cells and Merkel cells (**Figure 4**). The potential of skin fibroblasts to be reprogrammed to MCC cells is supported by the recent finding that expression of MCPyV LT-t in these cells can induce the neuroendocrine-like growth characteristics of MCC (98). Alternatively, MCPyV actively replicating in the dermal fibroblasts may unintentionally enter the precursor cells of Merkel cells residing in their immediate vicinity, either within the basal layer of the epidermis or inside hair follicles (99). The nonpermissive propagation environment created as these cells develop into Merkel cells may force the replication-defective MCPyV genome to integrate into the host cell genome, eventually leading to oncogenesis (**Figure 4**). Both of these hypotheses—that MCC originates from dermal fibroblasts or through infection of nonproductive bystander Merkel cells—remain to be tested when MCPyV in vivo infection models become available.

Finally, a recent study showed that when MCPyV-positive MCC cells with all T antigen isoforms knocked down were cocultured with keratinocytes, expression of the Merkel cell lineage

MCC markers, such as SOX2 and ATOH1, was ablated (100). Instead, the cells began to express neuronal cell lineage markers and differentiate into neuron-like cells (100). Because the majority of MCCs develop in the dermis and some also express neuron-specific enolase, it is conceivable that MCPyV may provoke the development of Merkel cell–like cancer cells by transforming the currently unknown neuronal precursor cells within the dermis (100).

MERKEL CELL POLYOMAVIRUS PROPAGATION

Many aspects of MCPyV basic virology remain poorly understood. Studies of molecular events in the MCPyV life cycle have been hampered by the fact that MCPyV propagates poorly in the majority of established cell lines tested, and until recently there was no cell culture model available for studying MCPyV infection (27, 79, 101). As discussed below, progress in understanding the molecular regulation of MCPyV replication and transcription was mostly made through transfection of plasmid vectors carrying individual viral gene coding sequences and Ori/promoters into cancer cell lines.

Replication

Like other HPyVs, the MCPyV NCRR contains the origin of replication that controls the initiation of viral DNA replication (**Figure 5**). The OBD and helicase/ATPase domains of LT recognize Ori and assemble a double-hexameric complex that unwinds the Ori to initiate cellular polymerase-mediated replication of the viral genome (21, 22, 48, 102). As described above, tumor-derived LT-t often carries truncating mutations that delete the helicase domain, thereby rendering the virus replication defective in MCC cells (47). Additionally, a tumor-derived MCPyV strain also carries single nucleotide mutations in the Ori that block its replication (22). These observations demonstrate that, during MCPyV tumorigenic development, there is a strong selective pressure to eliminate viral DNA replication activity after the MCPyV genome is integrated into the host DNA. It is conceivable that continuous unwinding of integrated viral Ori by wild-type LT could result in replication fork collisions and double-strand breaks in the host DNA; disrupting LT's OBD and helicase domains would alleviate this genotoxic stress and allow tumorigenesis to occur.

Besides MCPyV LT, other viral and cellular factors are required to achieve robust viral DNA replication. Co-expression of MCPyV sT can significantly stimulate MCPyV replication efficiency (22, 101), likely owing to its two highly conserved iron-sulfur clusters, which could potentially act as a molecular plowshare to unwind the DNA duplex and stimulate LT helicase activity (103). It was further discovered that cellular factors such as bromodomain protein 4 (BRD4) are recruited by MCPyV LT to support viral DNA replication (102). In C33A cells transfected with MCPyV LT/Ori constructs, BRD4 colocalizes with the MCPyV LT/Ori complex and serves as a molecular scaffold that functionally recruits replication factor C to replicate the unwinding viral DNA (102). In addition, during MCPyV replication, components of the ataxia telangiectasia mutated (ATM)- and ataxia telangiectasia and Rad3-related (ATR)-mediated DDR pathways are activated and recruited to MCPyV DNA replication foci (80). While the activities of these DDR pathway factors are important for promoting viral replication, the underpinning mechanism remains poorly understood.

Highly productive MCPyV DNA replication is achieved in MCPyV-infected HDFs, in which LT complexed with the replicated viral genomes is detected as conspicuous nuclear foci (85). Thus, this system allows validation of the observations made in transfected cells. Importantly, the cellular factors such as BRD4 and components of the ATM/ATR DDR pathways are all found to be enriched in the MCPyV LT/viral genome replication foci (80, 85). Therefore, the HDF

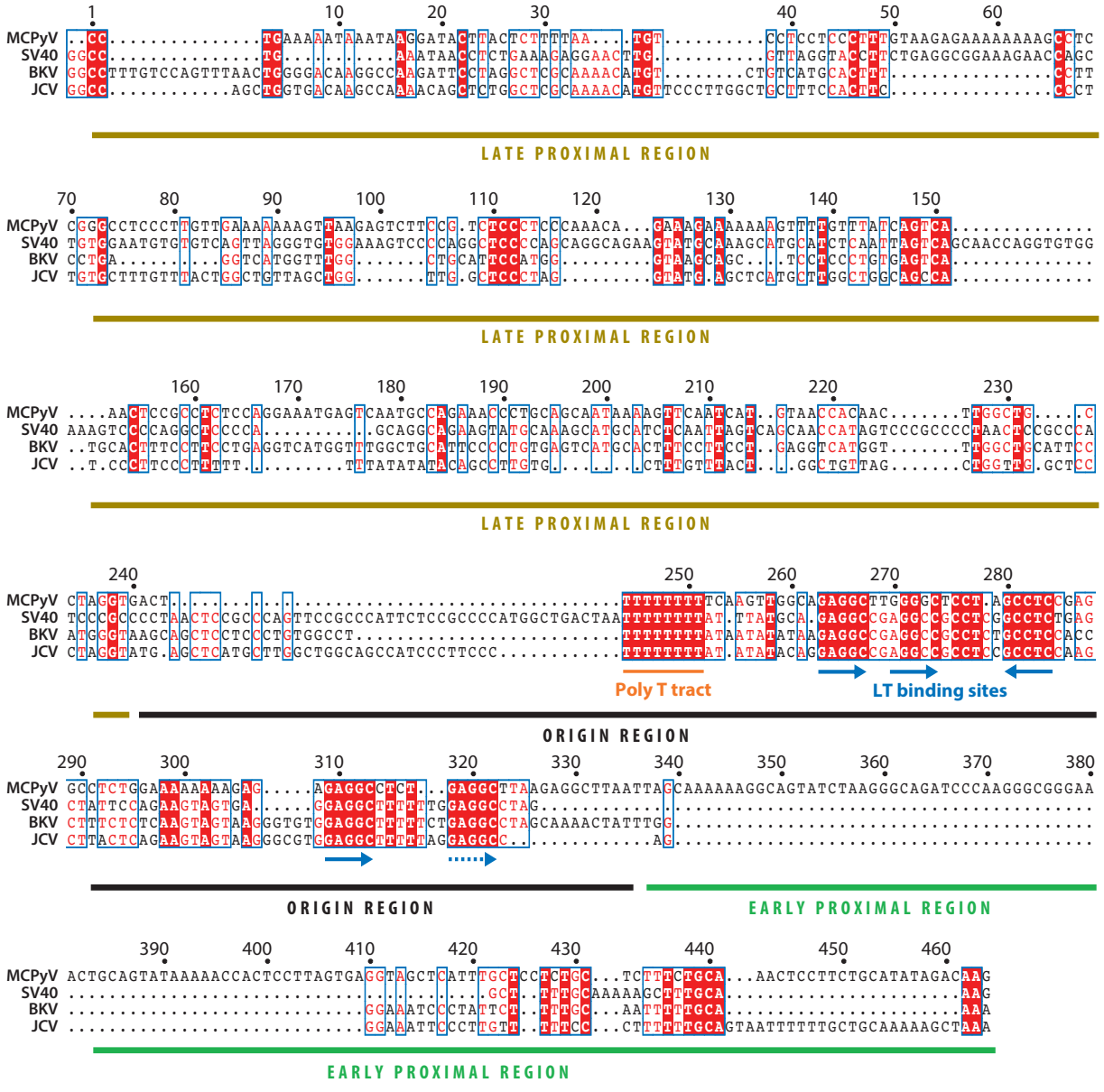

Figure 5

Comparison of the NCRRs of MCPyV, SV40, BKV, and JCV. The DNA sequences for the NCRR of MCPyV (NC_010277), SV40 (NC_001669), BKV (NC_001538), and JCV (NC_001699) were aligned using the MUSCLE program and presented using ESPript 3.0 software. The origin, early proximal, and late proximal regions are defined using the MCPyV NCRR sequence as the reference, and the numbering is relative to the MCPyV sequence. The poly T tract region is highlighted in orange. Functional pentanucleotide MCPyV LT binding sites conserved among all four polyomaviruses are marked with blue arrows, whereas a conserved LT binding site known to be dispensable for MCPyV Ori replication (22) is marked with a dotted blue arrow. Sequences identical in all four polyomaviruses are highlighted in red, whereas those conserved in three out of the four sequences are marked as red letters inside blue boxes. Abbreviations: BKV, BK virus; JCV, JC virus; LT, large tumor antigen; MCPyV, Merkel cell polyomavirus; NCRR, noncoding regulatory region; SV40, simian virus 40.

infection model affords a brand-new platform for studying the mechanistic roles of the viral-host interactions in MCPyV DNA replication.

Transcription

In MCPyV-infected cells, transcription of viral genes allows production of viral proteins to sustain persistent infection. Viral oncogenes expressed in this process may also induce transformation of infected cells to cancer cells. In MCC tumors, viral oncogenes transcribed from the integrated viral genome are essential to support cancer cell growth (49, 52, 104). Therefore, understanding the mechanism of MCPyV transcription that drives both MCPyV infection and MCC tumorigenesis is a subject of the utmost significance. However, very little is known about the mechanisms that regulate MCPyV transcription during either viral infection or MCC development.

The MCPyV NCRR contains the bidirectional promoters that regulate the transcription of both early and late genes of MCPyV (**Figure 5**). Although MCPyV is phylogenetically distant from other polyomaviruses such as SV40, BK virus, and JC virus, its NCRR still carries the sequence elements highly conserved in other polyomaviruses such as a poly T tract and inverted repeats, as well as the characteristic pentanucleotide GAGGC LT binding sites (105) (**Figure 5**). However, MCPyV early and late proximal promoter regions are quite different from those of other HPyVs and SV40, suggesting that distinct cellular factors and molecular mechanisms may control MCPyV transcription (**Figure 5**).

Few studies have investigated how the viral intrinsic components and host cellular factors regulate MCPyV transcription. For example, the miRNA encoded by MCPyV, mir-M1, has been shown to negatively regulate expression of MCPyV genes within the early region that contains a mir-M1 complementary sequence (24, 106). While wild-type MCPyV genomes transfected into the neuroectodermal tumor cell line PFSK-1 cells can persist as extrachromosomal episomes for several months, the MCPyV mir-M1 knockout mutant is lost at an escalated rate during this process, suggesting that mir-M1 is needed for MCPyV to establish and maintain long-term persistent infection (106). As shown using an NCRR bidirectional reporter construct, MCPyV LT can activate the transcriptional activity of the MCPyV early promoter but has little effect on the late promoter (107). However, MCPyV transcription appears to be significantly controlled by host cell properties. When recircularized MCPyV DNA was transfected into 21 cell lines and primary cell cultures, only three showed early and late gene expression (79). While MCPyV can promiscuously enter many different types of cells, robust MCPyV early and late gene expression occurs only in a few cell types such as HDFs and PFSK-1 but is completely silenced in nonpermissive cells such as HFKs and many other types of cells (85, 108, 109). Therefore, viral transcription is the key determinant for the highly restricted MCPyV host cell tropism.

As is the case with SV40, histones are encapsidated into MCPyV virions (28). After MCPyV genomes are transfected into PFSK-1 cells that support MCPyV gene expression, transactivational histone marks such as histone H3 lysine 4 trimethylation are detected in the NCRR (106). These observations indicate that MCPyV DNA is packaged into histone-bound nucleosomes likely carrying epigenetic modifications that can control its distinct transcriptional activities in permissive versus nonpermissive cells. MCPyV DNA could also be differentially methylated in these cell types to account for the varying degree of transcriptional activities. In addition, it is possible that cell type–specific transcriptional activators of MCPyV enhancers/promoters may be uniquely present in permissive cells to support viral transcription. All of these possibilities remain to be tested in future studies in order to fully understand the mechanism of MCPyV transcription that defines its host cell tropism, viral life cycle, and possibly even MCC oncogenesis.

CONCLUSIONS AND FUTURE PERSPECTIVE

The discovery of MCPyV as the first polyomavirus to be clearly associated with human cancer provides an exciting opportunity to examine how a seemingly harmless infection of a specific group of human cells could turn into a highly lethal malignancy (1, 2). As discussed in this review, MCPyV infection is prevalent in the healthy human population (31, 32, 74, 84). In nonmalignant human cells, the virus replicates and maintains an episomal genome (110). During the course of its persistent infection, conditions such as host immune suppression and/or unchecked viral replication may trigger integration of viral genomes into the host DNA. As these cells continue to proliferate, the ones carrying LT truncation mutations that eliminate viral replicative potential but retain the capacity to repress the host tumor suppressor function are favorably selected for. Both the tumor-specific LT-t and sT antigens expressed from the integrated viral genome function as the key viral oncogenes to support uncontrolled cellular proliferation that ultimately inflicts oncogenesis. Dampening host antiviral immune responses by viral effectors is likely important for achieving harmonious virus-host coexistence dynamics, enabling MCPyV to persist in a chronic and asymptomatic infection; it may also inadvertently contribute to tumorigenesis by allowing virally induced precancerous lesions to expand. Thus, elucidation of strategies used by MCPyV to manipulate the host antiviral and anticancer defense mechanisms for promoting its own propagation and driving cellular transformation will likely offer important clues for understanding MCPyV oncogenic mechanisms. The MCPyV oncogenes LT-t and sT not only are constantly expressed as foreign viral antigens in MCC tumors but also actively promote the proliferation of the tumor cells. These key characteristics make them attractive candidates for developing novel virus-targeted therapies to treat MCPyV-associated cancers. The recent discovery of skin fibroblasts as the permissive cells of MCPyV infection within the human host will be instrumental for conducting future research to elucidate the molecular mechanism underpinning the infectious life cycle and tumorigenic potential of this important oncogenic HPyV.

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