Chapter 4
Merkel Cell Polyomavirus: A New DNA Virus Associated with Human Cancer

Margo MacDonald and Jianxin You

Abstract Merkel cell polyomavirus (MCPyV or MCV) is a novel human polyomavirus that has been discovered in Merkel cell carcinoma (MCC), a highly aggressive skin cancer. MCPyV infection is widespread in the general population. MCPyV-associated MCC is one of the most aggressive skin cancers, killing more patients than other well-known cancers such as cutaneous T-cell lymphoma and chronic myelogenous leukemia (CML). Currently, however, there is no effective drug for curing this cancer. The incidence of MCC has tripled over the past two decades. With the widespread infection of MCPyV and the increase in MCC diagnoses, it is critical to better understand the biology of MCPyV and its oncogenic potential. In this chapter, we summarize recent discoveries regarding MCPyV molecular virology, host cellular tropism, mechanisms of MCPyV oncoprotein-mediated oncogenesis, and current therapeutic strategies for MCPyV-associated MCC. We also present epidemiological evidence for MCPyV infection in HIV patients and links between MCPyV and non-MCC human cancers.

Keywords Merkel cell polyomavirus • Merkel cell carcinoma • Oncogenesis • HIV patients • Host cellular tropism

4.1 Introduction

Merkel cell polyomavirus (MCPyV), a member of the *Polyomaviridae* family, was first isolated from Merkel cell carcinoma (MCC) in 2008 using digital transcriptome subtraction [1, 2]. Merkel cell carcinoma (MCC) typically presents as a neuroendocrine carcinoma of the skin. Historically, MCC has been thought to arise from Merkel cells, a unique cell type of the skin bearing both epithelial and

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neurosecretory characteristics [3]. The incidence rates of MCC have tripled in the last decades, and it is incredibly lethal, with a disease-associated mortality of 46% [4]. The major risk factors for MCC include advanced age, immunosuppression, and prolonged exposure to sunlight and ultraviolet (UV) radiation [5, 6]. Since the initial discovery of MCPyV in MCC tumors, a compelling line of evidence has established it as the causative agent of MCC. As many as 80% of all MCC tumors have clonally integrated MCPyV genomes [1, 7]. Integration of MCPyV genome into the host genome appears to occur before clonal expansion of the tumor, while persistent expression of the viral tumor antigens is required to maintain MCC tumor growth [1, 8]. This evidence strongly supports an important oncogenic role of MCPyV in MCC tumors. However, MCPyV infection of the skin is ubiquitous and largely asymptomatic in the general population [9–11]. Therefore, there is a growing interest in understanding the basic molecular virology of MCPyV and its role in oncogenesis. In this chapter, we present our current knowledge on the first polyomavirus linked to human cancer.

4.2 MCPyV Genome and Encoded Proteins

MCPyV, like other members of the polyomavirus family, is a small, non-enveloped, icosahedral, double-stranded circular DNA virus [12]. The 5.3 kb viral genome is composed of a multiply spliced early "tumor antigen" region, a late region, and a noncoding regulatory region (NCRR) which divides the two coding regions (Fig. 4.1). The NCRR contains the viral origin of replication (Ori) flanked by the promoters that drive early and late gene expression.

The early region of MCPyV encodes large tumor antigen (LT), small tumor antigen (sT), 57-kilodalton tumor antigen (57kT), and the overprinting gene

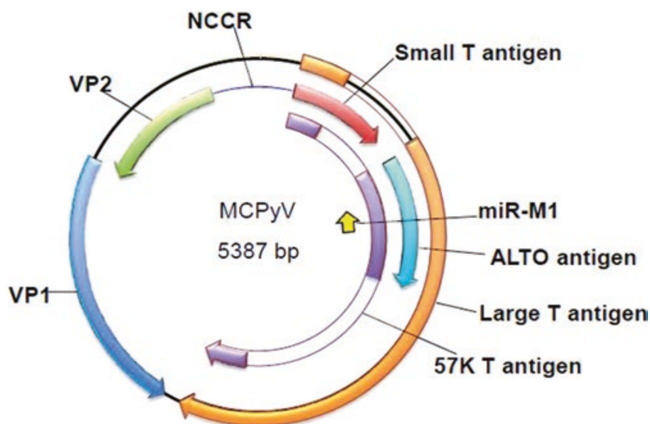

Fig. 4.1 MCPyV genome. This schematic diagram shows the noncoding regulatory region (*NCRR*), early genes, late genes, and a microRNA (*miR-M1*) encoded by the MCPyV genome

alternate LT ORF (ALTO) (Fig. 4.1). The MCPyV LT antigen is a multifunctional protein that plays important roles in host cell-cycle regulation as well as viral genome replication (reviewed in [13]). The N-terminal region of LT contains a conserved region 1 (CR1), a DnaJ domain (for binding heat-shock proteins), and an LxCxE motif that interacts with retinoblastoma protein (RB) to stimulate host cell proliferation (Fig. 4.2) [14]. The C-terminal region of LT contains an Ori binding domain (OBD) necessary for LT binding to the viral Ori and a helicase domain that stimulates replication of the viral genome (Fig. 4.2) [15, 16]. The sT protein shares the LT N-terminal region, including the CR1 and DnaJ domains, but has a unique C-terminus carrying a protein phosphatase 2A (PP2A) binding site [17]. Unlike other polyomavirus sTs, MCPyV sT appears to play a central role in inducing oncogenesis [18]. MCPyV sT has been shown to stimulate cellular proliferation by inducing hyper-phosphorylation of the eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) independent of PP2A binding [18]. It also binds the ubiquitin ligase SCF^{Fbw7} and disrupts proteasomal degradation of both LT and certain cellular cell-cycle regulators [19]. The unique C-terminal domain of MCPyV sT also contains highly conserved iron-sulfur clusters that are important for stimulating LT-mediated viral replication [20]. In contrast to LT and sT, the functions and physiological significance of both 57kT and ALTO remain to be elucidated [12, 21, 22].

The late region of MCPyV encodes VP1 and VP2 (Fig. 4.1), which function as major and minor subunits of the viral capsid, respectively. VP1 and VP2 form capsids around the MCPyV genome. While the major capsid protein VP1 is necessary and sufficient for producing pseudovirions, the minor protein VP2 may confer specificity in host cell targeting [11, 23–26].

Like many polyomaviruses, MCPyV encodes a microRNA, termed miR-M1 (Fig. 4.1), which has been shown to downregulate expression of LT [27, 28]. This regulation of LT was shown to be important for long-term MCPyV episome maintenance in cell culture and potentially for establishing persistent infection in vivo [27, 28].

4.3 Mechanisms of MCPyV Oncoprotein-Mediated Oncogenesis

Like papillomavirus-induced cancers, MCPyV-associated MCC tumors typically carry the viral genome integrated into the host genome [1, 29, 30]. MCPyV-associated MCC tumors demonstrate a clonal integration pattern of the viral genome, suggesting that the integration event occurs prior to the initiation of oncogenesis and expansion of tumor cells. These tumors typically express both of the major viral tumor antigens, LT and sT [8, 31]. However, the MCC tumors carrying the integrated viral genome do not support a productive viral life cycle. Both LT and sT play unique and important roles in driving MCC oncogenesis.

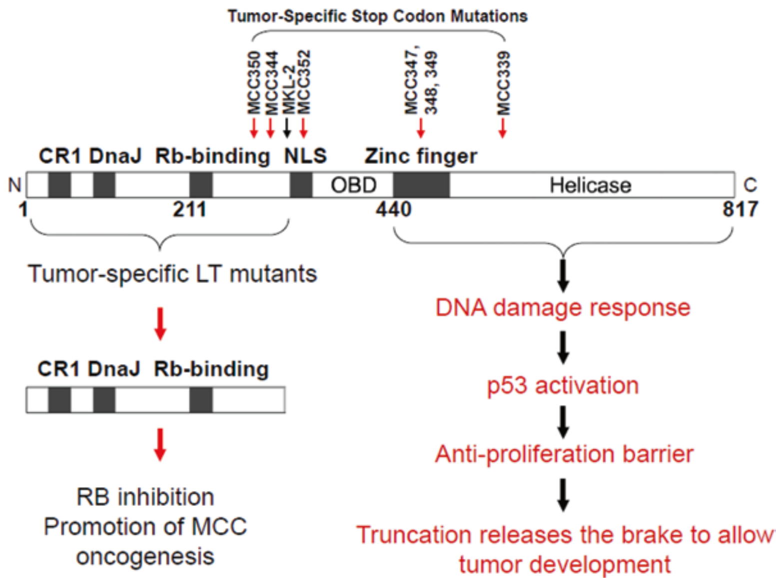

Fig. 4.2 Selective deletion of the C-terminus of MCPyV LT is a critical event during the oncogenic progression of MCPyV-associated cancers. Shown are the domain structures of MCPyV LT antigen and mutations found in MCPyV-associated MCCs that introduce premature stop codons to delete the LT C-terminus. The LT C-terminus can induce DNA damage response to activate the p53 tumor suppressor. This growth inhibitory property may function as an anticancer brake to inhibit cell proliferation and transformation. Deletion of the replication domains in the C-terminus of LT releases this antitumor brake to allow oncogenic progression. The truncated tumor-specific LT mutants retain RB tumor-suppressor inhibiting activities to drive cellular transformation

A common feature of MCPyV genomes integrated into the MCC genome is the selection for mutations in the LT coding sequence that introduce premature stop codons which delete the LT C-terminal Ori binding and helicase domains (Fig. 4.2) [14]. Therefore, the MCPyV LT protein is typically expressed in a truncated form in MCC tumors [14]. In contrast, these tumor-specific mutations do not disrupt the expression of sT. The N-terminal portion of LT still expressed in these tumors is referred to as LTT (tumor-derived LT). LTT retains the CR1, DnaJ, and RB-binding motifs, allowing these tumor-specific LT mutants to robustly disrupt the host cell cycle (Fig. 4.2) [32].

The selection for premature stop mutations in MCPyV LT is remarkably common in MCPyV-associated tumors, suggesting that deletion of the replication domains in the C-terminus of LT is required for tumorigenesis. One selective pressure that may exist is the elimination of viral DNA replication activity after the genome has been integrated into the host DNA [14]. It is conceivable that continuous LT-mediated replication from the integrated viral Ori 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. In addition, other functional activities of the C-terminal domain may need to be negatively selected for during

tumorigenesis. For example, expression of just the OBD and helicase domains of MCPyV LT induces a host cellular DNA damage response (DDR). This activation stimulates p53 activity and can arrest the host cell cycle [33]. This growth inhibitory property of the MCPyV LT C-terminus may function as a barrier to oncogenic progression (Fig. 4.2) [33, 34]. Since replicative stress, DNA damage responses, and cell-cycle arrest all pose challenges to oncogenesis, together they provide a strong rationale for the selection of truncated LT proteins which retain RB tumor-suppressor inhibiting activities while avoiding potentially antagonistic activity conferred by the C-terminal domain of LT.

Compared to MCPyV LT, sT plays a more dominant role in MCPyV-induced carcinogenesis [18]. Contrary to other polyomaviruses, expression of MCPyV LT alone is not sufficient to transform cells [18]. MCPyV sT, however, has been suggested to transform immortalized rat fibroblasts in cell culture even when expressed alone [18]. MCPyV sT also demonstrates robust transforming activity in vivo [35]. sT's oncogenic activity is mostly mediated through induction of the hyperphosphorylated and inactivated state of 4E-BP1, leading to dysregulation of cap-dependent translation that accelerates cell proliferation and malignant transformation [18]. In addition, sT inhibits the E3 ubiquitin ligase SCF^{Fbw7} to prevent proteasomal degradation of MCPyV LT and key cellular proliferative proteins like c-Myc and cyclin E [19]. Unlike LT, sT is commonly expressed in MCPyV-associated tumors, and almost no mutations have been found in the sT-coding regions integrated into the genome of MCC tumors [13], again highlighting the important role this protein plays in MCPyV-associated cancers.

MCPyV-positive MCC cells are dependent on MCPyV LT/sT oncoproteins. Persistent expression of these oncogenes from the integrated viral genome is required to sustain growth of MCPyV-associated tumors, in both in vitro and xenograft models [8, 31]. Knockdown of LT/sT antigens induces growth arrest and cell death in all MCPyV-positive MCC cell lines tested [8, 31] and leads to tumor regression in xenotransplantation models [32].

4.4 Genetic Basis of MCPyV-Associated MCC

Recent studies have begun to delineate the differences in the causes of MCPyV-positive and MCPyV-negative MCCs. Genetic studies have shown that UV radiation is the primary cause of MCPyV-negative MCCs, which constitute about 20% of all MCC cases [36–38]. Compared to MCPyV-positive MCCs, MCPyV-negative tumors demonstrate much higher mutational burdens, which are associated with a prominent UV-induced DNA damage signature [36–38]. Both MCPyV-positive and MCPyV-negative MCC tumors are commonly found on sun-exposed regions of the body, such as the head, neck, and limbs [37]. However, the lower number of genetic mutations found in the genomes of MCPyV-positive MCCs compared to MCPyV-negative tumors, along with the lack of a definitive UV mutation signature in MCPyV-positive MCCs, indicates that UV plays a primary etiologic role in

MCPyV-negative MCC tumorigenesis [36]. In MCPyV-positive MCCs, UV may simply promote tumor growth through immunosuppressive effects on the tumor microenvironment or through inducing the mutations needed for MCPyV integration and generation of the truncated viral LT antigen [36].

Compared to MCPyV-positive MCCs, MCPyV-negative tumors also contain a much higher number of cancer-promoting mutations [36–38]. Some of the common mutations frequently observed in MCPyV-negative MCCs include mutations in *RB1*, *TP53*, and *PIK3CA*, along with mutations in host DDR and chromatin modulation pathways [36–38]. Inactivating mutations in the NOTCH signaling pathway were also detected, supporting a tumor-suppressor role for this pathway in MCC [38]. The lower levels of cancer-promoting mutations observed in MCPyV-positive MCCs confirm that MCPyV oncogenes are the primary oncogenic drivers for these tumors [36–38]. Activating mutations of *HRAS* were among the very few frequently observed in MCPyV-positive tumors, suggesting that these genetic mutations may cooperate with the viral oncogenes to drive MCC tumorigenic progression [36, 38]. In several studies, inactivating mutations in *RB1* were observed in MCPyV-negative tumors, but not in MCPyV-positive tumors [36–38]. This is consistent with the fact that the truncated MCPyV LT antigen interacts with and inactivates RB1, suggesting that RB1 disruption is required for all MCC tumorigenesis [37].

Potentially due to their higher mutational burden, MCPyV-negative MCCs typically display a more aggressive subtype, with patients having an increased risk of disease progression and death [39]. MCPyV-negative tumors are also more likely to recur after treatment than MCPyV-positive tumors [39]. There are a variety of possible reasons for the more aggressive behavior observed in MCPyV-negative subtype of MCCs, including the fact that fewer oncoproteins are expressed as targets for T-cell-infiltrating lymphocytes (TILs), their advanced stage at presentation, and a higher number of mutations in oncogenic pathways [39].

4.5 MCPyV Host Cellular Tropism and the Origin of MCC

4.5.1 MCPyV Entry into the Host Cells

An important part of the MCPyV life cycle that is particularly useful for the development of antivirals and vaccines is viral entry into the host cell. MCPyV dsDNA genome is encapsidated in an icosahedral shell of viral capsid consisting of the structural proteins VP1 and VP2 at a ratio of 5:2 [1, 25]. For most polyomaviruses, the major capsid protein VP1 determines antigenicity and receptor specificity. It initiates viral entry into host cells and has a significant impact on attachment, tissue tropism, and viral pathogenicity [40]. In line with findings from other polyomaviruses, MCPyV's entry into host cells is mediated by binding of the major capsid protein VP1 pentamer to cellular receptors [41]. The minor capsid protein VP2 is essential for infectious MCPyV entry in some cell types, but others could be

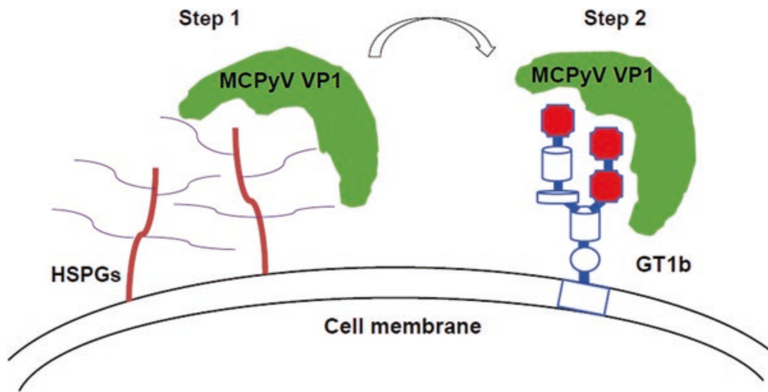

Fig. 4.3 MCPyV entry into host cells. MCPyV viral entry is a two-step process mediated primarily by the major capsid protein VP1. The primary binding partner for MCPyV VP1 is the glycosaminoglycan (GAG) heparan sulfate. This GAG is found on the host cell membrane in the form of heparan sulfate proteoglycans (*HSPGs*). While this interaction is all that is necessary for VP1 binding to occur, a secondary interaction with the sialylated ganglioside GT1b is required for entry. MCPyV interacts with the sialic acids in both arms of GT1b, which are shown in red. MCPyV minor capsid protein VP2 (not shown) has been suggested to play a role in post-attachment entry

transduced with MCPyV pseudovirions deficient in VP2, indicating that in cell types where VP2 is necessary for MCPyV entry, there is some barrier that it helps to overcome [25, 42]. Virion assembly, packaging of DNA, and attachment to target cells were not significantly affected by knockdown of VP2, indicating that the role of VP2 is in post-attachment entry [25]. While most polyomaviruses also contain another minor capsid protein VP3, this minor capsid protein is not detectable in either MCPyV-infected cells or native MCPyV virions [25]. Phylogenetic analysis suggests that MCPyV belongs to a member of a divergent clade of polyomaviruses lacking the conserved VP3 N-terminus [25].

The functional receptors of most human polyomaviruses are sialylated glycans [43]. Sialic acids are found mostly in glycoproteins and gangliosides [43]. The ganglioside GT1b, which has sialic acids on both arms, was the first proposed to be the receptor for MCPyV VP1 (Fig. 4.3) [44]. VP1 interacts with the sialic acids on both branches of the GT1b carbohydrate chain (Fig. 4.3) [44]. In a later study, where MCPyV reporter vectors and native MCPyV virions were used to transduce human cells, it was discovered that the initial attachment receptors of MCPyV VP1 are sulfated glycosaminoglycans (GAGs), specifically heparan sulfate (HS) proteoglycans [26]. While VP1 can bind to GAGs such as HS and chondroitin sulfate (CS), only the N-sulfated and 6-O-sulfated forms of HS mediate infectious entry, and both CS and other forms of HS were dispensable for this process [26]. In addition, Neu et al. used X-ray structures to show that a shallow binding site on the apical surface of the VP1 capsomer recognizes the linear sialylated disaccharide Neu5Ac-α2,3-Gal, which is present in GT1b [41]. Previous studies were not able to show GD1a binding with VP1, yet this study showed VP1 interactions with GT1b, GD1a, 3SLN,

and DSL oligosaccharides, all of which contain the Neu5Ac motif [41, 44]. This indicates that GT1b, and possibly these other sialylated glycans, are the post-attachment co-receptors for VP1 that enable the secondary entry step after primary attachment through GAGs. More importantly, mutagenesis studies revealed that the VP1 sialic acid binding site plays a role in post-attachment infection, not initial attachment [41]. Together, current knowledge supports a two-step attachment and entry process for MCPyV, with sulfated GAGs being the initial attachment receptors for VP1. The primary binding, mediated mostly by HS, is followed by secondary interactions with a sialylated glycan post-attachment co-receptor (Fig. 4.3) [26, 40, 42, 43, 45]. These glycans are not required for initial attachment of MCPyV virions, but they are necessary for viral entry into the cell (Fig. 4.3) [26, 41, 43].

MCPyV enters its target cells in a slow and asynchronous motion [45]. After entry, MCPyV must travel through the cytoplasm to the nucleus in order to use the host cell replication machinery. Host cell surface glycoproteins and glycolipids play a role in both the entry stage of viral infection and channeling the virions to specific intracellular membrane-bound compartments and ultimately to the nucleus [43]. However, the molecular events that occur after MCPyV penetrates the cell membrane and allow delivery of the encapsidated viral DNA to the host nucleus have not been elucidated. This is largely due to a lack of cell culture model for MCPyV infection [45].

4.5.2 MCPyV Host Cellular Tropism

While the MCPyV binding factors, such as sialic acid and heparan sulfate, which mediate attachment and entry, have been actively discovered [26, 41], much remains to be elucidated with respect to MCPyV natural infection and MCPyV host cellular tropism. It is unclear how MCPyV targets specific cell types given both sialic acid and heparan sulfate are ubiquitous. Studies of basic MCPyV virology have been hampered by the facts that MCPyV replicates poorly in the majority of cell lines tested thus far and its natural host cell up until very recently had not been described. The lack of a robust cell culture system for MCPyV infection has limited our understanding of this important tumor virus.

Multiple lines of evidence point toward the skin being the major site of MCPyV replication in humans. First, various deep sequencing studies have provided evidence of persistent and asymptomatic infection of MCPyV in adult skin [46, 47]. Additionally, the cell types which support MCPyV replication have been either epithelial or fibroblast in origin [15, 23, 48]. Finally, MCC is a tumor of the dermis, and the presumed cells of origin for MCC, Merkel cells, are a resident of the epidermis. Following this line of reasoning, our group examined the ability of various skin cell types to support MCPyV infection and discovered that human dermal fibroblasts (HDFs) are a natural host cell of MCPyV [49]. We demonstrated that both epidermal growth factor (EGF) and fibroblast growth factor (FGF) were required to promote efficient MCPyV infection of dermal fibroblasts; these factors may stimulate

expression of cellular factors necessary for infection, producing an environment conducive to MCPyV infection and replication [49]. Interestingly, these growth factors are stimulated upon wounding [50], suggesting that wounding processes may facilitate MCPyV infection in the human skin. We also found that induction of matrix metalloproteinases (MMPs) mediated by the WNT/β-catenin signaling pathway is critical for MCPyV infection of HDFs. WNT signaling is crucial for the formation of hair follicles [51]. Interestingly, we showed that MCPyV could efficiently infect the HDFs surrounding hair follicles [49]. This finding is in line with the observation that MCPyV is frequently detected in eyebrow hair bulbs [52]. Remarkably, several MCC risk factors, including UV exposure and aging, are known to upregulate MMPs [53–58], once again linking risk of MCC incidence with MCPyV infection.

4.5.3 Origin of MCC

The relationship between the cells that MCPyV infects and those that it transforms to cause MCC remains a central question for MCPyV research. The establishment of dermal fibroblasts as a natural host cell for MCPyV may help resolve a long-standing puzzle in the MCC field regarding the cells of origin for MCC [59]. Historically, MCC has been thought to arise from Merkel cells due to its expression of cytokeratin 20, a unique marker of Merkel cells. However, this assumption has been challenged by a number of recent studies. First, Merkel cells are postmitotic and do not have robust proliferative potential, making them less likely to support MCPyV infection, replication, and associated tumorigenesis [60, 61]. Additionally, Merkel cells are of epidermal origin, while MCC tumors are thought to derive from the dermis [62–64]. Because MCC tumors also express markers common to pro-/pre-B cells, such as paired box gene 5 (PAX5) and terminal deoxynucleotidyl transferase (TdT), it has been suggested that MCC tumors may derive from the B-cell lineage [65]. The finding that dermal fibroblasts support MCPyV infection provides new alternative hypotheses [49]. For example, MCPyV infection of dermal fibroblasts can, over time, induce their transformation and upregulate genes commonly expressed in other cell types, including B cells and Merkel cells. This hypothesis is in line with the finding that MCC tumors are dermal in origin. Alternatively, Merkel cells residing at the boundary of the epidermis and dermis may be infected as a nonproductive bystander of dermal fibroblast infection. Along this line, the fact that Merkel cells do not support the full MCPyV life cycle may predispose this infection toward genome integration, which eventually lead to oncogenesis. Both of these models – dermal fibroblast origin or infection of bystander Merkel cells – remain to be tested in vivo using animal and skin explant models.

4.6 Current Therapeutic Strategies for MCPyV-Associated MCC

4.6.1 Surgery, Radiation Therapy, and Chemotherapy

Early-stage, localized MCCs are usually treated with surgical excision [66]. Adjuvant radiation therapy applied after the initial surgery has been shown to improve local and regional recurrence rates and therefore has also been recommended for primary tumors [66]. However, MCC frequently undergoes metastasis, increasing the probability that tumors may arise in areas that are harder to reach and to fully eradicate with radiotherapy [67]. Thus, chemotherapy has been used to treat advanced stage MCC. Although MCC tumors are responsive to chemotherapy in the short term, the duration of the response is usually transient, and many tumors often develop resistance to chemotherapy [66, 68, 69]. Additionally, chemotherapy has little overall survival benefit for MCC tumors due to its immunosuppressive effect, which counteracts the cellular immune reaction to MCC tumors. Currently, there are very few viable options for patients with advanced MCCs [69].

4.6.2 Immune Checkpoint Inhibitors and Immunotherapy

MCC patients with robust immune responses and higher level of intratumoral TILs generally showed better prognoses and increased rates of regression [70–73]. Intratumoral CD8+ and CD3+ lymphocytes, which predict better survival, are typically more commonly found in MCPyV-positive MCCs [74]. This tight correlation between prognosis and immune function suggests that immunotherapies may have great potential for treating metastatic MCCs. Methods of increasing interferon production, such as stimulation by the targeted delivery of the IL-12 gene using vaccine and electroporation, are currently being investigated [75]. A promising immunotherapy strategy for MCC treatment targets the programmed cell death receptor 1/programmed cell death ligand 1 (PD-1/PD-L1) checkpoint. PD-L1 is often overexpressed in MCC tumors, especially in MCPyV-positive cases [37]. MCPyV-specific T cells also express elevated levels of PD-1 [76]. Interaction of PD-L1 with the PD-1 receptor on the surface of MCPyV-specific T cells activates an immune checkpoint pathway, which inhibits the antitumor immune response [77, 78]. Therefore, anti-PD-1 monoclonal antibody treatment has become an attractive treatment option for MCC [79]. A response rate of 56% was observed in patients treated with an anti-PD-1 antibody called pembrolizumab [77]. However, these responses were not lasting, ranging in duration from 2.2 to 9.7 months [77]. Studies with this drug and another anti-PD-1 antibody called avelumab both showed more success following fewer first-line treatments, suggesting that they should be used as a first-line therapy for advanced MCC rather than the last in a long line of treatments [75]. While these and some other studies have shown improvements in patients with various

immunotherapies, the success has for the most part been minimal, further highlighting the need for new treatments for this cancer.

4.6.3 MCPyV DNA Vaccine

One potential treatment option to explore for MCC is a MCPyV DNA vaccine. As described above, it has been repeatedly shown that CD8+ T-cell tumor infiltration in MCC is associated with better prognosis, decreased likelihood of metastasis, and prolonged survival [70, 74]. Therefore, DNA vaccines capable of generating potent antigen-specific CD8+ T-cell immunity are a promising option for MCC treatment [80]. DNA vaccines are an attractive therapeutic option due to their safety, simplicity, stability, and the possibility to be administered multiple times [80].

MCPyV LT is truncated by stop codons in nearly all MCPyV-positive MCC tumors, losing its C-terminal domain responsible for viral replication, while retaining the N-terminal RB-interacting domain for driving cancer development [8]. Because the MCPyV LT amino terminus plays an important role in oncogenesis and is expressed in all MCPyV-positive tumors, it is an ideal vaccine target. In addition, as a foreign antigen, MCPyV LT avoids the issue of immune tolerance that could impede the development of antitumor immunity. Stop codons introduced by MCPyV integration into MCC tumor genome typically truncate LT at amino acid 258 (aa258) or beyond, so the Hung laboratory created a DNA vaccine to specifically target the MCPyV LT aa1-258 region [80]. When tested in mice injected with the B16/LT murine melanoma cell line stably expressing LT, this vaccine demonstrated both protective and therapeutic effects against LT-expressing tumors in vivo [80]. Compared to mice vaccinated with control empty vector, MCPyV LT-vaccinated mice injected with B16/LT cells exhibited smaller tumors and better survival, and the tumor-bearing mice given the MCPyV LT vaccine as a treatment showed smaller tumor volume and longer survival [80]. These antitumor effects appear to be mediated by CD4+ T-cell induction, while no significant CD8+ T-cell induction was observed [80].

Due to CD8+ T cells' association with better prognosis and disease clearance [70, 74], the Hung group went on to construct a DNA vaccine tailored to eliciting LT-specific CD8+ T-cell responses [81]. This vaccine encodes LT fused to a damage-associated molecular pattern protein, calreticulin (CRT), which has been shown to promote induction of CD8+ T cells when fused to other antigens [80, 81]. The new vaccine, named CRT/LT, was also tested on the B16/LT mice and showed prolonged survival after tumor challenge compared to mice vaccinated with the original MCPyV LT vaccine [81]. Compared to MCPyV LT vaccine or control empty vector, this vaccine also resulted in the best survival when given to tumor-bearing mice [81]. Confirming that this better performance was due to CD8+ T-cell induction, CD8+-depleted mice were not protected by the CRT/LT vaccine [81]. The results indicate that CD8+ T cells were the main mediator of the antitumor effects of the CRT/LT vaccine [81].

A MCPyV DNA vaccine was also created to target the sT antigen, which is a key driver of MCC oncogenesis [82]. MCPyV sT shares the same N-terminus with LT but contains a different C-terminus with a PP2A binding site that is important for virus-induced transformation in other PyVs [17, 82]. When tested against a murine tumor model that expresses MCPyV sT antigen, this vaccine demonstrated successful protection and treatment, leading to increased survival and decreased tumor volume in vivo [83]. As in the case of CRT/LT vaccine, CD8+ T-cell induction was essential for the sT vaccine antitumor effect, which was diminished upon CD8+ T-cell depletion [83]. These preliminary results are promising, but testing in an actual MCC cell line and a MCC animal model would be needed to confirm the efficacy of these MCPyV-targeted vaccines for the control of MCC.

4.7 MCPyV Infection in HIV Patients

Immunosuppression is one of the most important risk factors for the development of MCPyV-associated MCC skin cancer, with immunocompromised individuals making up about 10% of the MCC patient population [84]. This relationship is likely linked to the causative role played by MCPyV in MCC tumorigenesis. A significant portion of these patients experiences immunosuppression as a result of HIV/AIDS infection. HIV-infected individuals have a 13.4-fold increased risk of developing MCC compared to the general population [85]. While this association between HIV infection and MCC has been observed for some time, various recent studies have started to validate the link between HIV and MCPyV infection. The elevated MCPyV prevalence in HIV patients was confirmed by a study looking at MCPyV status in HIV-positive men [86]. This study showed that 59.0% of HIV-positive men had MCPyV DNA in their forehead swabs, compared to only 49.4% of HIV-negative men [86]. However, the level of viral DNA loads in HIV-positive and HIV-negative men did not differ significantly [86]. Another study confirmed that there is no difference in MCPyV viral load between HIV-positive and HIV-negative populations of women [87]. Nonetheless, within the HIV-positive subset of patients, men with poorly controlled HIV infection had higher viral loads compared to those with well-controlled infection [86].

The majority of healthy adults (45–85%, increasing with age) are positive for MCPyV immunoglobulin G (IgG). Using VLP-based enzyme-linked immunosorbent assay (ELISA) to measure MCPyV IgG titers, it was recently shown that levels of MCPyV IgG were higher in HIV/AIDS patients than in either non-AIDS/HIV patients or uninfected controls [88]. Again, MCPyV viral loads did not differ significantly between the tested populations, and there was not much of a difference between uninfected controls and HIV patients without severe immunosuppression [88].

MCPyV detection in the skin is frequent, but the virus is rarely detected in the blood [89]. One study found that only 5.5% of the general population had MCPyV-positive blood serum, while MCPyV DNA was found in the sera of 39.1% of

untreated HIV-positive patients [89]. For those in each population whose sera were MCPyV positive, the copy number did not differ significantly between the HIV-positive and HIV-negative groups [89].

MCPyV DNA is usually found in the skin, and MCC typically arises in sun-exposed areas of the body [84, 90]. However, in HIV-positive individuals, MCC often arises in sites not exposed to the sun [84, 90, 91]. One HIV-positive patient even had an oral MCC tumor that tested positive for MCPyV DNA [90]. In some other HIV-positive patients, MCPyV DNA has been detected not only on the skin but also in oral and anogenital mucosa as well as in plucked eyebrow hairs [91].

MCC in HIV-positive individuals is also unusual in the sense that it typically has a much earlier onset in HIV/AIDS patients, with a mean age of diagnosis of 49 years – 20 years younger than the average for immunocompetent patients [84, 91]. In addition, MCCs in AIDS patients are characterized by aggressive clinical course with higher-grade lesions, more advanced tumor stage, and shortened survival [84]. These differences suggest that viral oncogenesis is more rapid and aggressive in patients with HIV-induced immunosuppression [91]. One reason could be that MCPyV infectivity may be exacerbated by these patients' impaired immune response [84]. In addition, the elevated MCPyV DNA loads associated with HIV-induced immunosuppression could explain the increased likelihood of MCC development observed in HIV-infected individuals [91]. Also, the increased viral infection in HIV-positive individuals could make integration of MCPyV into the host cell genome more likely and therefore increase the risk of tumorigenesis [91].

In summary, significantly increased risk of developing MCPyV-associated MCC has been observed among immunocompromised individuals, including HIV-infected patients [84]. This data suggests that screening for early detection of MCC in HIV-positive patients and MCPyV antiviral therapy could both be beneficial to the survival of these patients [91].

4.8 Epidemiological Evidence for MCPyV in Non-MCC Cancers

While MCPyV has an established correlation with MCC, with 80% of this cancer being MCPyV positive, its potential association with a variety of other cancers has been a common topic of exploration recently. There is some evidence suggesting that, in addition to MCC, MCPyV may be associated with extrapulmonary small cell carcinoma (ESCC), cervical cancer, other types of skin cancer, lung cancer, and even some types of leukemia.

One of these cancers, ESCC, was investigated because it shows histological similarities to both small cell lung cancer (SCLC) and MCC, although ESCC is negative for the CK20 marker [92]. ESCC tumors were tested for MCPyV DNA through the use of qPCR, and 19% of the tumors were MCPyV positive [92]. While this

prevalence is not high, it is significant enough to suggest that MCPyV may be the driver of a small number of ESCC cases. On the other hand, this same study found no MCPyV DNA in any SCLC samples tested [92].

Among the lung cancers investigated, non-small cell lung cancer (NSCLC) has shown the most evidence supporting an association with MCPyV. In one study, 18% of NSCLC patients had MCPyV DNA present in their tumors, and viral infection was significantly correlated with poorer cancer prognosis within subgroups [93]. Another study found MCPyV DNA in various types of NSCLC in Japanese patients, including squamous cell carcinomas, adenocarcinomas, and others, with some tumors expressing LT RNA transcripts [94]. Prevalence was low in this study as well, but the virus's presence suggests that MCPyV is at least partially associated with NSCLC pathogenesis in some patients. A study of MCPyV in NSCLC in Chilean patients also found a small but not insignificant portion of patients with MCPyV-positive tumors, with 4.7% of patients' cancer testing positive for the virus [95].

While these recent studies suggest that lung cancer may be associated with MCPyV, others show contradictory results. One study looked for MCPyV and other human PyV antibodies in lung cancer samples from patients in China but found no association between MCPyV or other human PyV antibodies and incident lung cancer [96]. Another study examined PyV infection and the risk of lung cancer in never smokers but also found no association. MCPyV seropositivity was detected in 59.3% of lung cancer samples and 61.6% of controls, indicating that there is no difference in MCPyV infection rates in populations with and without lung cancer [97].

In addition to lung cancer, there is contradictory evidence regarding whether MCPyV is associated with various nonmelanoma skin cancers. One paper suggested that 36% of immunocompetent cutaneous squamous cell carcinoma (SCC) patients that participated in their study had one or more samples test positive for MCPyV, while MCPyV positivity in SCCs overall was found to be only 15% [98]. However, most other studies found no correlation between MCPyV and common nonmelanoma skin cancers such as SCC and basal cell carcinoma (BCC). For example, in a study examining SCC and BCC samples from Japanese patients for MCPyV DNA, only 13% of SCCs were found to be MCPyV positive, and none of the BCC samples tested were [99]. One case study tested the SCC tumors of a patient who had both SCC and MCC but only found MCPyV LT in the MCC tumor [100]. Another study detected MCPyV DNA in both BCC and SCC samples but observed a low immunohistochemical detection rate of MCPyV and a lack of MCC-specific MCPyV mutations in the samples [101]. Similarly, Reisinger et al. tested BCC and SCC samples for MCPyV LT and found that none of the samples contained this protein [102]. These results indicate that frequent MCPyV detection in these cancers could simply be due to the ubiquitous spread of MCPyV in the general population, not a result of a causative relationship between MCPyV and these cancers.

However, MCPyV DNA was found in the SCC lesions of a patient with both MCC and SCC, along with HPV coinfection in both lesions, indicating a potential for co-oncogenesis between the two viruses [103]. It was suggested that a low viral

copy number in SCC cases has led to difficulties with immunohistochemical detection and may be the reason why many studies do not detect MCPyV in these cancers [103]. To help elucidate whether MCPyV is truly associated with SCC and BCC, larger epidemiological studies are likely necessary.

Additional studies have also investigated the role of MCPyV in non-cutaneous squamous cell carcinomas. A study of esophageal SCCs in Northern Iran detected MCPyV DNA at a low viral copy number in both cancerous and noncancerous esophageal samples but did not find a statistically significant difference in detection rates [104]. Imajoh et al. investigated MCPyV in cervical SCCs and cervical adenocarcinomas (ACs) in Japanese women. They detected MCPyV DNA in 19% of cervical SCCs and 25% of cervical ACs [105]. MCPyV LT was detected in virus-positive tumors [105].

There is also evidence that MCPyV may be associated with some blood cancers. For example, 50% of follicular mycosis fungoides, a lymphoma of the skin, contained MCPyV DNA [106]. However, MCPyV LT was not detected in these samples [106]. In addition, the complete DNA sequence of MCPyV was found in a patient with acute myeloid leukemia [107]. Since there is an established epidemiological link between chronic lymphocytic leukemia (CLL) and MCC, one group investigated the potential role of MCPyV in CLL oncogenesis [108]. They discovered that 13% of T cells in CLL patients tested positive for MCPyV, while none of the patients' B cells did, suggesting that MCPyV may have tropism for T cells [108]. Another study detected MCPyV in 27.1% of CLL patients and even observed LT expression and deletions in some of these patients, suggesting that MCPyV may play a role in a subset of CLL cases [109].

Despite its association with immunosuppression, no correlation between Kaposi's sarcoma and MCPyV has been demonstrated [110, 111]. On the other hand, some recent studies have shown possible correlations between MCPyV and various rare cancers. One group investigated porocarcinoma, a rare malignant neoplasm that arises from the intraepidermal ductal portion of the eccrine sweat glands [112]. MCPyV was found in 68% of primary porocarcinomas, compared to 30% of healthy controls, suggesting that MCPyV may play a role in oncogenesis of this cancer [112]. Another study investigated epidermodysplasia verruciformis-associated (EV-associated) skin neoplasms and detected MCPyV in the in situ carcinomas of all congenital EV patients tested, revealing a strong association between the disease and MCPyV [113].

In summary, most of the recent studies suggest a possible link between MCPyV and various non-MCC cancers. However, there is conflicting information regarding whether MCPyV truly is involved in the pathogenesis of other cancers beside MCC. Therefore, larger epidemiological studies and more definitive data are necessary to further elucidate MCPyV's role in tumorigenesis outside of MCC.

4.9 Conclusions and Future Perspectives

Accumulating evidence demonstrates a role for MCPyV in the development of MCC, making MCPyV the first polyomavirus to be clearly associated with human cancer [1, 12]. MCPyV infection is prevalent in the general population. During the course of its persistent infection, integration of viral genomes into the host genome induces LT truncation mutations that antagonize the tumor-suppressor function. Proliferation of cells carrying integrated viral genomes expressing LT truncation mutations and the sT viral oncogene is selected for during viral oncogenesis. Immune downregulation by viral proteins likely allows MCPyV to establish a persistent infection; it may also play a key role in allowing virally induced early-stage MCC tumors to persist and expand. MCPyV oncogenes are not only persistently expressed as foreign viral antigens in MCC tumors but also required for the growth of the tumors cells. These key features make them ideal targets for developing novel immunotherapy to treat MCC tumors. Elucidation of the mechanism by which MCPyV escapes host immune surveillance and modulates the host immune system to drive cellular transformation will offer important leads for developing viral-targeted therapeutic strategies to treat MCPyV-associated cancers. The recent discovery of HDFs as a target of MCPyV infection in human skin provides an exciting opportunity to study the infectious life cycle of this important oncogenic human polyomavirus [49]. Identification of the target cells of MCPyV natural infection will also facilitate establishment of better animal models to fully elucidate the MCPyV infectious life cycle and MCPyV-induced tumorigenesis in vivo.

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