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Merkel Cell Polyomavirus Infection and Merkel Cell Carcinoma

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Summary

Merkel cell polyomavirus is the only polyomavirus discovered to date that is associated with a human cancer. MCPyV infection is highly prevalent in the general population. Nearly all healthy adults asymptotically shed MCPyV from their skin. However, in elderly and immunosuppressed individuals, the infection can lead to a lethal form of skin cancer, Merkel cell carcinoma. In the last few years, new findings have established links between MCPyV infection, host immune response, and Merkel cell carcinoma development. This review discusses these recent discoveries on how MCPyV interacts with host cells to achieve persistent infection and, in the immunocompromised population, contributes to MCC development.

Introduction

Merkel cell polyomavirus (MCPyV or MCV) has been linked to the development of a human cancer, Merkel cell carcinoma (MCC) [1**]. MCC, a neuroendocrine carcinoma of the skin, is one of the most aggressive skin cancers, with a disease-associated mortality of 46% [2]. MCC kills more patients than some other well-known cancers such as cutaneous T-cell lymphoma and chronic myelogenous leukemia [3,4].

Excessive exposure to sunlight and ultraviolet (UV) radiation, immune suppression, and advanced age are the major risk factors for MCC skin cancers [5,6]. MCC is therefore more frequently observed among people with fair skin, the elderly, and organ transplant or AIDS patients, with a strong predilection for the sun-exposed skin of the head and neck [5,6]. Clonal integration of MCPyV DNA into the host genome is a causal factor for the development of the majority of MCC tumors [7**].

MCPyV is also an abundant virus frequently detected on healthy human skin, suggesting that its infection is widespread in the general population [8–10]. Additional studies confirmed that exposure to the virus occurs in 80% or more of the general population [8*, 11,12]. Primary infection occurs during childhood. Once acquired, MCPyV becomes a life-long component of the skin flora [13]. The strong association of MCC tumor development

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with immunosuppression suggests a viral causative link. The discovery of MCPyV in MCC tumors further supports a viral etiology for MCC [1**].

MCPyV is a small DNA virus with a circular, double-stranded DNA (dsDNA) genome of ~5kb [14]. The viral genome is divided into three major regions: the non-coding regulatory region (NCRR), which contains the viral origin of replication and transcriptional regulatory elements; the early coding region; and the late coding region [14,15]. The early region encodes large T (LT) antigen, small T (sT) antigen, the 57kT antigen, and a protein called alternative LT Open reading frame (ALTO) [14,16**,17**]. The late region encodes the capsid proteins, VP1 and VP2 [18]. During the normal life cycle, the polyomavirus genome is maintained as a circular dsDNA episome (Fig. 1).

MCPyV genome and viral T antigens are detected in at least 80% of MCC cases, and expression of the T antigens is restricted to the tumor cells and not the surrounding healthy tissues [1,19–23]. Genetic analysis of MCPyV genome in these MCC tumors revealed that it is clonally integrated into the host cell genome [1,24]. The viral integration event appears to take place before the clonal expansion of tumor cell proliferation [1,25–27]. Persistent expression of MCPyV T antigens from the integrated viral genome is required for MCC tumor cells to survive [20*]. Together, these findings provide strong support for a causal role of MCPyV in the development of MCC skin cancer.

The incidence of MCC has tripled over the past twenty years as the aging population with prolonged sun exposure increases[28]. With the high prevalence of MCPyV infection and an increasing number of MCC diagnoses, there is a need to better understand the biology and oncogenic potentials of MCPyV. In this review, we summarize recent discoveries of the molecular mechanisms underlying MCPyV infection and associated oncogenesis.

MCPyV oncogenes and cellular transformation

A vital question in MCPyV research has been how MCPyV-encoded proteins contribute to viral activities and MCC development. Most of our knowledge of MCPyV oncogenic mechanisms was gained from studies of the viral oncogenes, LT and sT antigens, both of which have been implicated in MCPyV-induced tumorigenesis (See reviews [29**–31] for more detailed discussion on this subject). LT carries a J-domain (binds heat-shock proteins), retinoblastoma (RB) binding motif (inhibits RB family members), and the C-terminal origin-binding and helicase/ATPase domains (required for viral DNA replication) [16**] (Fig. 2A). Much of the oncogenic function of LT antigen has been attributed to its high binding affinity for RB, which causes sequestration and inactivation of this tumor suppressor [32] (Fig. 2A). The RB binding function of MCPyV LT is required for sustained growth of MCPyV-positive MCC tumor both *in vitro* and in xenograft models [33]. MCPyV sT shares an N-terminal 78 amino acid region (harboring the J-domain) with LT and carries a unique C-terminal domain (Fig. 2A). Unlike other polyomaviruses, MCPyV sT is the major viral oncogene that contributes to virally-induced cellular transformation [22]. Expression of sT alone is sufficient to transform immortalized rodent fibroblasts [22] and also demonstrates transforming activity *in vivo* [34*]. sT accomplishes this function partly through maintaining the hyper-phosphorylated and inactivated state of the eukaryotic translation

initiation factor 4E-binding protein 1 (4E-BP1), leading to dysregulation of cap-dependent translation that accelerates cell proliferation and malignant transformation [22]. sT also targets the cellular ubiquitin ligase SCF^{Fbw7} to inhibit proteasomal degradation of MCPyV LT and other cell-cycle regulators such as c-Myc and cyclin E, thus contributing to viral replication and host cell transformation [35*]. Unlike LT, nearly no mutations have been found in the sT-coding regions integrated into the genome of MCC tumors (Fig. 1), further confirming that the native MCPyV sT is needed to drive MCPyV-induced carcinogenesis.

MCPyV genomes discovered in many MCC tumors were frequently found to be integrated into the cellular genome, which resulted in tumor-specific truncation mutations that deleted the LT origin-binding and helicase domains needed for productive viral replication [16**]. Such integration events preserve expression of the native sT antigen and LT truncation mutants encoding the N-terminal J-domain and RB binding motif, but disrupt the C-terminal regions of LT necessary for completion of the lytic viral replication cycle [1**,27]. Disruption of LT-mediated replication may protect MCPyV-infected cells from cell death induced by collisions of cellular DNA replication forks with the ones initiated from the viral origin [16**]. The clonal integration pattern of MCPyV suggests that viral infection and nonproductive integration of the MCPyV genomic DNA into the host cell genome occur prior to expansion of the MCC tumor cells [1**,7**,36].

Truncation of the LT C-terminal origin-binding and helicase domains observed in integrated viral genomes found in MCC tumors also suggests that an unknown activity located in this region may antagonize tumor formation, and that there is a selective pressure to remove it during tumor development. Our group showed that expression of the LT C-terminal origin-binding and helicase domains causes DNA damage and stimulates host DNA damage responses [37*]. This in turn leads to p53 activation and inhibition of cellular proliferation, which functions as a barrier to malignant progression [37*] (Fig. 2A). Suppression of cellular proliferation by a C-terminal region of MCPyV LT was also reported by Cheng *et al.*, who mapped the MCPyV LT growth inhibitory properties to the C-terminal 100 residues of LT [38*]. We further demonstrated that phosphorylation of the MCPyV LT C-terminus by Ataxia telangiectasia mutated (ATM) kinase induces apoptosis and inhibits host proliferation, contributing to the C-terminal anti-tumorigenic properties of LT [39]. Together, these studies explain, in part, why deletion of the MCPyV LT C-terminus is necessary for the oncogenic progression of MCPyV-associated cancers (Fig. 2A).

Target cells of MCPyV infection

Despite the well-defined function of the viral oncogenes in cellular transformation described above, how MCPyV infection leads to MCC is not fully understood. Many aspects of the MCPyV infectious life cycle also remain unknown. This is largely because MCPyV replicates poorly in the majority of cell lines tested thus far [40–44] and the host cells naturally infected by MCPyV were previously unknown. Mechanistic studies to fully investigate MCPyV molecular biology and oncogenic mechanisms have thus been hampered by the lack of knowledge of the natural host cell tropism of MCPyV and the technical difficulties of propagating the virus in cell culture. In a recent study, our group identified human dermal fibroblasts as natural host cells that support productive MCPyV infection

[45**]. We discovered that induction of matrix metalloproteinase (MMP) genes by the WNT/ β -catenin signaling pathway, along with other growth factors, stimulates MCPyV infection. This suggests that MCC risk factors, such as UV radiation and aging, which are known to stimulate WNT signaling and MMP expression, may promote viral infection and thereby drive MCC tumorigenesis (Fig. 2B) [45**].

The cells of origin in MCC

Historically, MCC tumors were thought to arise from Merkel cells, which are mechanoreceptor cells located in the basal layer of the epidermis that share the immunohistochemical marker Cytokeratin 20 (CK20) with MCC tumors (Table 1). However, new evidence suggests that MCC does not derive from mature Merkel cells [46]. For example, Merkel cells are post-mitotic and do not have proliferative activity, making them less likely to have tumorigenic potential [47,48]. In addition, Merkel cells are derived from the epidermal lineage [49,50], whereas MCC tumors mostly develop within the dermis and subcutis, without apparent connection to the epidermis [51]. These studies suggest that Merkel cells may not be the cells of origin for MCC. Because MCCs express Paired box gene 5 (PAX5) and terminal deoxynucleotidyl transferase (TdT), which are only expressed in pro/pre- and pre-B cells under physiological circumstances (Table 1), it has been speculated that MCC tumors may arise from pro-/pre-B cells, although this theory remains to be tested experimentally [52**]. In light of these recent findings, the true origin of MCC tumors remains unknown.

Identification of human dermal fibroblasts as the target cells of MCPyV infection presents new opportunities to understand how MCPyV-infected cells ultimately give rise to MCC tumors (Table 1). This finding is consistent with the fact that most MCC tumors develop in the human dermis [51]. It is possible that MCPyV infection of dermal fibroblasts induces their transformation and activates gene expression patterns of MCC tumors during the process of tumorigenesis (Fig. 3, Model 1). On the other hand, MCCs also express genes that are normally transcribed in benign Merkel cells [53*], suggesting that MCPyV infection may induce benign Merkel cells or progenitor cells to undergo malignant transformation. Along this line, MCPyV actively replicating in the dermal fibroblasts located at the boundary between epidermis and dermis may shed viral particles that inadvertently enter Merkel cells or their precursor cells present in the immediate vicinity within the basal layer of the epidermis (Fig. 3, Model 2) [45**]. MCPyV infection of bystander Merkel cells may cause MCC simply because Merkel cells represent a dead-end replication environment that favors viral integration and transformation. These scenarios remain to be tested *in vivo* using animal and human skin explant models.

MCPyV infection, host immune responses, and MCC

A complete understanding of how MCPyV drives MCC tumor development hinges on deciphering the interaction between MCPyV and the host immune system. The role of MCPyV in MCC development, along with the fact that immune-suppressed individuals are more likely to develop this cancer, indicate that the host immune response plays a key role in MCC tumorigenesis [54]. Additional evidence such as complete spontaneous tumor

regression and tumor reactive T cells all support the role of the immune system in preventing and controlling MCC [55]. Furthermore, MCC patients with robust immune response generally have better prognoses, and high intratumoral CD8+ and CD4+ lymphocyte infiltration predicts better survival [56–58]. However, more than 90% of the MCC patients have normal immune function [59], and yet fail to clear the MCC tumors that express the non-self MCPyV oncoproteins. MCC tumors continue to develop despite the production of T cells recognizing MCPyV capsid proteins and oncoproteins that are immunogenic and persistently expressed in the tumors [60]. These facts argue that MCPyV-induced MCCs are capable of escaping immunological destruction. Indeed, the potential roles of MCPyV proteins in modulation of the immunological response of host cells have been demonstrated in recent studies.

One potential way in which MCPyV has been observed to avoid detection by the immune system is through downregulation of major histocompatibility complex class 1 (MHC-1). MHC-1 is necessary for presenting peptides from intracellular proteins to CD8+ T cells, making its downregulation an efficient mechanism for immune escape. In one study, 84% of MCC tumors showed MHC-1 downregulation, and MHC-1 expression was lower in MCPyV-positive tumors than in those that were MCPyV negative [61**]. Another way in which MCPyV evades the immune response is through downregulation of Toll-like Receptor 9 (TLR9), a critical sensor of the host innate immune response that recognizes viral and bacterial dsDNA [62]. This MCPyV function has been suggested to allow infected cells to escape host immune surveillance and may be critical for repressing host innate immune responses during MCPyV-associated tumorigenesis [62]. MCPyV sT also down-regulates the expression of cellular genes associated with NF- κ B pathways, which may subvert the host immune response to allow establishment of MCPyV persistent infection in the host cells [63*].

Additional work is needed to further elucidate the various mechanisms by which MCPyV escapes immune surveillance. MCPyV viral oncoproteins are not only specific and important for the growth of MCC cancer cells, but also foreign to the immune system. These key features of the viral oncogenes make them the idea targets of immunotherapy treatment for MCC. Once identified, the immune modulatory features of the viral proteins could also be subverted to induce antitumor immune response against MCPyV-positive tumor cells.

Conclusions

MCPyV is the only one of all 13 known human polyomaviruses that has been associated with cancer. MCPyV infection occurs at a young age and establishes a persistent infection. In time, either due to prolonged sunlight exposure, viral genome replication in dermal cells, or infection of bystander Merkel cells, a dead-end host, MCPyV genomes become integrated into the host genome. Proliferation of cells carrying integrated viral genomes selects for LT truncation mutations that promote efficient transformation. Induction of MMP by aging and prolonged UV exposure may favor a model of infection of bystander Merkel cells. Finally, immune downregulation by viral proteins allows MCPyV to establish a persistent infection; it may also play a role in supporting MCC tumors to persist and expand. Although MCC is a highly aggressive carcinoma, currently there is no effective therapeutic regime available for

treating advanced stage MCC. Recent identification of human dermal fibroblasts as the major target of MCPyV infection in the human skin provides an exciting opportunity for studying the productive life cycle of this important oncogenic human polyomavirus. Identification of the target cells of MCPyV natural infection will also facilitate establishment of new animal models to investigate MCPyV biology and oncogenic mechanisms *in vivo*. Persistent expression of MCPyV oncoproteins in MCC tumors and the dynamic interaction of viral proteins with the host immune system allow MCPyV to escape host immune surveillance. New insights into these immunomodulatory mechanisms are critical for the development of more effective virus-targeted immunotherapies for treating this lethal cancer.

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Highlights

- MCPyV infection is highly prevalent in the general population, but in elderly and immunosuppressed individuals, causes a lethal form of skin cancer, Merkel cell carcinoma
- MCPyV LT and sT antigens are the key oncogenes that drive MCPyV-induced tumorigenesis
- Human dermal fibroblasts have been recently discovered to be the natural host cells that support productive MCPyV infection in human skin
- Induction of matrix metalloproteinase genes by growth factors and WNT signaling stimulates MCPyV infection
- Elucidation of the mechanisms by which MCPyV escapes immune surveillance are critical for the development of more effective virus-targeted immunotherapies for treating the highly lethal Merkel cell carcinoma

Figure 1. An intact MCPyV sT antigen-coding sequence is found in nearly all of the viral genomes integrated into MCC tumors

Analysis of 30 sequences of MCPyV-positive MCC obtained from published studies [1**, 16**, 25, 42, 64, 65] show that all of them encode a wild type sT gene. The break points represent the break sites of the integrated MCPyV genomes. The first stop mutations are the first stop codons found in the T antigen sequences of the integrated MCPyV genomes.

Figure 2. Molecular and cellular events contributing to MCPyV infection and MCC tumorigenesis

A. MCPyV oncoproteins drive cellular transformation through interaction with host proteins. Shown are the domain structures of MCPyV LT and sT as well as the cellular targets that they interact with to drive cancer development. CR1: conserved region 1; OBD: Origin Binding Domain; LSD: LT-stabilization domain. **B. Molecular signaling contributing to MCPyV infection.** Induction of MMP genes by the WNT/ β -catenin signaling pathway and other growth factors, such as EGF and FGF, stimulates MCPyV

infection. MMPs may contribute to MCPyV infection by disrupting the extracellular matrix of the host cells. In the physiological skin environment, skin damage induced by UV/ionizing radiation (IR) and wounding processes can activate both WNT signaling and MMP expression. Aging skin also shows dramatically increased MMP expression. Therefore, these major MCC risk factors may promote viral infection to drive MCC tumorigenesis.

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Figure 3. The working models for MCPyV infection and MCC development

Human dermal fibroblasts are the major target cells of MCPyV infection in the human skin. Shown are two working models for understanding how MCPyV-infected cells may give rise to MCC tumors. MCPyV infection of dermal fibroblasts could induce their transformation (Model 1). Alternatively, benign Merkel cells or progenitor cells located in the immediate vicinity of the infected dermal fibroblasts could be infected as accidental bystanders. MCPyV infection of Merkel cells may lead to MCC because these cells represent a dead-end replication environment that promotes viral integration and transformation (Model 2).

Table 1

Potential cells of origin for MCC and their shared markers/features with MCC

	Markers/features	References
Merkel cell carcinoma	CK20+, NSE+, PAX5+, NSP+, TdT+, TTF-1–, LCA–, S100–	[1–4]
Merkel cells	CK20+, Electron microscope morphology	[5,6]
Pro/pre-B and pre-B cells	PAX5+, TdT+	[7,8]
Dermal fibroblasts	Major target of MCPyV infection, support productive viral transcription and replication	[9]

CK20: Cytokeratin 20; NSE: neuron-specific enolase; NFP: neurofilament protein; PAX5: Paired box gene 5; TdT: terminal deoxynucleotidyl transferase; LCA: leukocyte common antigen; TTF-1: thyroid transcription factor 1.

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