Merkel Cell Polyomavirus and Human Merkel Cell Carcinoma

Wei Liu and Jianxin You

1 Introduction

Merkel cell polyomavirus (MCPyV) is a member of the Polyomaviridae. It was first identified in Merkel cell carcinoma (MCC) using digital transcriptome subtraction methodology (Feng et al. 2008). MCPyV is also the first polyomavirus proven to be associated with human cancer. MCPyV-associated MCC typically presents as a type of neuroendocrine cancer. In 1972, it was first described by Dr. Cyril Toker, who named it "trabecular carcinoma of the skin" (Toker 1972). MCC is one of the most aggressive skin cancers, with disease-associated mortality of nearly 46% (Becker 2010; Harms 2017; Agelli et al. 2010), which exceeds the mortality rate of melanoma. It kills more patients than some well-known cancers such as cutaneous T-cell lymphoma and chronic myelogenous leukemia (Lemos and Nghiem 2007; Bhatia et al. 2011).

About 80% of MCC cases can be directly linked to MCPyV infection (Feng et al. 2008; Sihto et al. 2009). Immunosuppression caused by aging (Fitzgerald et al. 2015; Bichakjian et al. 2007), HIV infection (Engels et al. 2002), and organ transplant (Clarke et al. 2015) has been shown to stimulate the development of MCPyV-positive MCC. Sunlight exposure and ultraviolet (UV) radiation are also important risk factors for MCC development (Lunder and Stern 1998; Heath et al. 2008).

Epidemiological surveys for MCPyV seropositivity (Tolstov et al. 2009; Kean et al. 2009) and sequencing analyses (Foulongne et al. 2012) have shown that MCPyV is an abundant virus frequently shed from healthy human skin, suggesting that MCPyV infection is widespread in the general population (Schowalter et al.

W. Liu · J. You (✉)
Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
e-mail: jianyou@pennmedicine.upenn.edu

T.-C. Wu et al. (eds.), *Viruses and Human Cancer*, Recent Results in Cancer Research 217, https://doi.org/10.1007/978-3-030-57362-1_12

2010). Most of the primary MCPyV infection occurs during early childhood. Once acquired, the virus becomes a permanent component of the skin flora (Chen et al. 2011). Integration of MCPyV genome into the host genome has been shown to occur before the clonal expansion of the tumor, in which continued expression of the viral oncogenes drives MCC tumor growth. These findings provide key evidence to support the oncogenic role of MCPyV in MCC tumor development (Feng et al. 2008; Shuda et al. 2008). The incidence of MCC has tripled over the past twenty years (Hodgson 2005; Stang et al. 2018) and increased by >95% in the US since 2000 (Paulson et al. 2017). With the high prevalence of MCPyV infection and the increasing amount of MCC diagnosis (Hodgson 2005), there is a growing concern for MCC (Hodgson 2005). Understanding MCPyV biology and its oncogenic mechanism will provide insights for developing novel prevention and treatment strategies for MCC. In this chapter, we present the recent advancement in MCPyV virology and associated MCC tumors.

2 The Life Cycle of MCPyV

2.1 MCPyV Genome Structure

MCPyV is a small, non-enveloped, icosahedral, double-stranded circular DNA virus (Feng et al. 2008). The 5.4 kb viral genome encodes seven gene products under the control of early and late promoters (Fig. 1). A non-coding regulatory

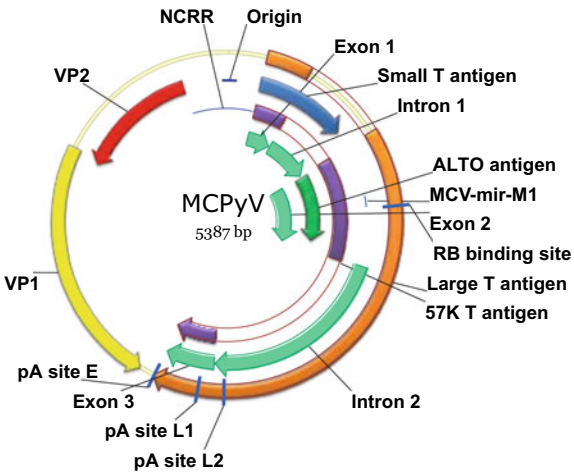

Fig. 1 MCPyV genome. This schematic diagram shows the non-coding regulatory region (NCRR), early genes, late genes, and a microRNA (miR-M1) encoded by the MCPyV genome. Alternate LT ORF (ALTO), Early gene poly A site (pA site E), Late gene poly A site 1 (pA site L1), and Late gene poly A site 2 (pA site L2)

region (NCRR) divides the genome into early and late regions (Gjoerup and Chang 2010). NCRR contains the viral origin (Ori) of replication and bidirectional promoters for viral transcription (Harrison et al. 2011; Kwun et al. 2009).

The MCPyV early region encodes large tumor antigen (LT antigen), small tumor antigen (sT antigen), 57-kilodalton tumor antigen (57KT antigen), and alternate LT ORF (ALTO) (Feng et al. 2008; Schowalter et al. 2010; Carter et al. 2013). Among these early proteins, LT antigen is the largest viral coding protein. It is encoded by T antigen exon 1, exon 2, intron 2 and exon 3 (Shuda et al. 2008). LT antigen not only regulates the viral genome replication but also controls the host cell cycle progression. The N-terminus of the protein contains a conserved region 1 (CR1), a DnaJ domain (for binding heat-shock proteins), and a LxCxE motif that is responsible for binding retinoblastoma protein (RB), which regulates the cell cycle (Feng et al. 2008; Shuda et al. 2008). The C-terminal region of LT antigen contains an Origin Binding Domain (OBD), which binds the MCPyV Ori GAGGC pentameric sequences (Harrison et al. 2011; Kwun et al. 2009), and a helicase domain, which unwinds double-strand MCPyV DNA to initiate replication (Li et al. 2013). The MCPyV sT antigen is encoded by T antigen exon 1 and intron 1 (Shuda et al. 2008). Therefore, it shares the LT N-terminal CR1 and DnaJ domains but carries a unique C-terminal protein phosphatase 2A (PP2A) binding site (Kwun et al. 2015) as well as two highly conserved iron-sulfur clusters, [2Fe–2S] and [4Fe–4S] (Tsang et al. 2016). The MCPyV 57KT antigen is encoded by T antigen exon 1, exon 2 and exon 3 (Cheng et al. 2013). Therefore, it does not have an OBD domain and a complete helicase domain. The MCPyV ALTO protein is translated from an overprinting ORF that is +1 frameshifted relative to the T antigen exon 2 (Carter et al. 2013). In contrast to LT and sT antigens, the function of 57KT antigen and ALTO remains poorly understood.

The MCPyV late region encodes structural proteins VP1 and VP2 (Fig. 1) (Schowalter et al. 2010; Schowalter and Buck 2013), which are the major and minor subunits of the viral capsid, respectively. VP1 and VP2 form the capsids that encapsidate the MCPyV genome (Schowalter and Buck 2013; Schowalter et al. 2011). The major capsid protein VP1 is indispensable and sufficient for producing pseudovirions, whereas the minor protein VP2 confers specificity in host cell targeting (Schowalter and Buck 2013). MCPyV minor capsid protein VP3 is not detectable in either MCPyV-infected cells or native MCPyV virions. Phylogenetic analysis suggests that MCPyV belongs to a unique clade of polyomaviruses that does not encode the conserved VP3 N-terminus (Schowalter and Buck 2013).

Besides the early and late genes, MCPyV also encodes a microRNA, miR-M1 (Seo et al. 2009) (Fig. 1), which has been shown to down-regulate the expression of LT. miR-M1 also appears to be important for long-term MCPyV episome maintenance in cell culture as well as for persistent infection in vivo (Theiss et al. 2015).

2.2 MCPyV Entry into Host Cells

The particle size of MCPyV virions is about 50 nM. The viral capsid is composed of VP1 and VP2 at a ratio of 5:2. Like most polyomaviruses, the major capsid protein VP1 determines antigenicity and receptor specificity. The entry of MCPyV virions into the host cell is mediated by VP1 binding to cellular receptors. Although VP2 knockout does not affect virion assembly, viral DNA packaging, or cell attachment, it reduces native MCPyV infectivity by more than 100-fold (Schowalter and Buck 2013).

MCPyV enters into its target cells through a gradual and asynchronous motion. The initial attachment receptors for MCPyV VP1 are sulfated glycosaminoglycans (GAGs), specifically the N-sulfated and/or 6-O-sulfated forms of heparan sulfate (Schowalter et al. 2011). It has been shown by X-ray structure analysis 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 ganglioside GT1b containing sialic acids on both arms (Erickson et al. 2009). The study also revealed VP1's interaction with the Neu5Ac motif of GD1a, 3SLN, and DSL oligosaccharides (Neu et al. 2012). This finding indicates that, during MCPyV infectious entry, sialylated glycans serve as post-attachment co-receptors after MCPyV primary attachment through GAGs. MCPyV penetrates cells through caveolar/lipid raft-mediated endocytosis (Becker et al. 2019). The virus internalizes in small endocytic pits, which deliver the virus to endosomes (Becker et al. 2019). From there, the virus moves to the endoplasmic reticulum by taking advantage of microtubule transport, acidification of endosomes, and a functional redox environment. The virus was found to gain a membrane envelope within endosomes, a phenomenon that has not been observed for other viruses (Becker et al. 2019).

2.3 MCPyV Replication

Both MCPyV LT and sT antigens play an important role in replicating viral DNA. After binding to the viral replication Ori through its OBD domain, LT unwinds the Ori using its helicase domain to initiate viral DNA replication (Kwun et al. 2009; Diaz et al. 2014). Several LT phosphorylation sites have been discovered through mass spectrometry analysis (Diaz et al. 2014). Mutagenesis and functional analysis revealed that phosphorylation of LT at these sites dynamically regulates viral replication by controlling Ori recognition, adjusting LT-Ori affinity, as well as initiating viral DNA unwinding (Diaz et al. 2014). As discussed below, MCPyV LT contributes to viral genome replication by recruiting cellular proteins as well. MCPyV sT is also required for efficient viral DNA replication. It does so mostly through increasing LT protein stability (Kwun et al. 2013). It was discovered that LT is normally targeted for proteasomal degradation by the cellular SCF^{Fbw7} E3 ligase. sT can bind and inhibit this E3 ligase to prevent LT degradation (Kwun et al. 2013).

Several host proteins involved in MCPyV replication have been discovered. Vam6p, a vacuolar sorting protein associated with MCPyV LT, is the first cellular factor shown to have an effect on MCPyV replication (Feng et al. 2011). Mutation of the Vam6p binding site on LT enhances MCPyV replication, whereas overexpression of exogenous Vam6p reduces MCPyV virion production by more than 90% (Feng et al. 2011). These studies suggest that Vam6p can inhibit MCPyV replication through its interaction with LT antigen (Feng et al. 2011). Bromodomain protein 4 (BRD4) is another cellular protein that interacts with MCPyV LT antigen and plays a vital role in viral DNA replication. BRD4 colocalizes with the MCPyV LT/replication origin complexes (MCPyV replication center) in the nucleus and recruits replication factor C (RFC) to the viral replication sites (Wang et al. 2012). *BRD4* knockdown inhibits MCPyV replication, which can be rescued by adding purified recombinant BRD4 protein in vitro. Human DNA damage response (DDR) factors are important for MCPyV DNA replication as well. Components of the Ataxia telangiectasia-mutated (ATM)- and Ataxia-telangiectasia-mutated and Rad3-related (ATR)-mediated DDR pathways accumulate in MCPyV replication centers inside the cells infected with recombinant MCPyV virions (Li et al. 2013). This DDR factor recruitment does not happen when a replication-defective LT mutant or an MCPyV Ori mutant was introduced instead of their wild-type counterparts (Li et al. 2013). Components of promyelocytic leukemia nuclear bodies (PML-NB) are another set of host factors that control MCPyV DNA replication. Notably, MCPyV replication was increased in cells depleted of Sp100, one of the key factors of PML-NBs. This observation suggests that Sp100 is a negative regulator of MCPyV DNA replication (Neumann et al. 2016).

2.4 Assembly and Release

The assembly and release processes of MCPyV are largely unexplored. Based on VP1 protein localization during virus infection (Schowalter et al. 2011; Liu et al. 2016), it has been suggested that the virus packages in the nucleus and induces cell lysis events so that it can be released from the infected cells (Liu et al. 2016).

2.5 MCPyV Host Cellular Tropism

Although the MCPyV binding factors, such as heparan sulfate and sialic acid that mediates viral attachment and entry, are ubiquitously expressed, MCPyV infects and replicates poorly in the majority of cell lines tested in a number of studies (Schowalter et al. 2011; Neu et al. 2012). The cells naturally infected by MCPyV have not been discovered until very recently. Several lines of evidence implicate the skin as the major site of MCPyV productive infection in humans. First, multiple deep sequencing studies have detected persistent and asymptomatic infection of MCPyV in adult skin (Foulongne et al. 2012; Schowalter et al. 2010). In addition, cell culture experiments suggest that the cell types conducive for MCPyV

replication are either epithelial or fibroblast in origin (Kwun et al. 2009; Feng et al. 2011; Wang et al. 2012). Finally, MCC is a tumor derived from the dermis and the presumed cells of origin for MCC, Merkel cells, reside in the epidermis. Following this line of reasoning, different types of cells in human skin were surveyed for MCPyV infectability. It was discovered that among all of the skin cell types tested, only human dermal fibroblasts (HDFs) could support robust MCPyV propagation (Liu et al. 2016). It was also found that epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) are essential to support MCPyV infection in HDFs, likely by inducing cellular factors to promote a cellular environment beneficial to MCPyV infection (Liu et al. 2016). Interestingly, in human skin, EGF and FGF are typically stimulated during the wounding and healing process (Quan et al. 2009), suggesting that wounding of human skin may spur MCPyV infection. It was further discovered that the expression of matrix metalloproteinases (MMPs), which can be stimulated by the Wnt/β-catenin signaling pathway, is important for MCPyV infection of HDFs (Liu et al. 2016). Several MCC risk factors, including UV exposure and aging, can upregulate MMPs (Quan et al. 2009; Cho et al. 2009; Fisher et al. 1996; Gill and Parks 2008; Quan and Fisher 2015; Varani et al. 2006), suggesting they may promote MCPyV infection to stimulate MCC development.

Despite the discovery of productive MCPyV replication in HDFs, much remains to be elucidated with respect to MCPyV natural infection and host cellular tropism. For instance, both MCPyV attachment receptors, sialic acid and heparan sulfate are ubiquitously expressed. It is unclear how MCPyV is able to effectively enter HDFs but not many other cell types (Schowalter et al. 2012). MCPyV DNA has also been detected in respiratory, urine, and blood samples (Spurgeon and Lambert 2013). Therefore, the range of tissues in which MCPyV establishes persistent infection remains unclear.

2.6 MCPyV Species Tropism

Mechanistic studies aiming to fully clarify the oncogenic mechanisms of MCPyV have been hampered by the lack of MCPyV infection animal models. To overcome this hurdle, recombinant MCPyV virions and several MCPyV chimeric viruses were used to test the infectivity of dermal fibroblasts isolated from a variety of model animals, including chimpanzee (*Pan troglodytes*), mouse (*Mus musculus*), rabbit (*Oryctolagus cuniculus*), rat (*Rattus norvegicus*), rhesus macaque (*Macaca mulatta*), common woolly monkey (*Lagothrix lagotricha),* patas monkey (*Erythrocebus patas*), red-chested mustached tamarin (*Saguinus labiatus*) and tree shrew (*Tupaia belangeri*). Interestingly, among all of the cells tested, only chimpanzee dermal fibroblasts supported strong MCPyV gene expression and viral replication, and they did so to a much greater extent when compared to HDFs. Therefore, among all of the tested small mammals and non-human primates, chimpanzee represents the only animal type that can support native MCPyV infection (Liu et al. 2018). Since chimpanzee is not available to be used as an

animal model for MCPyV research, additional studies are needed to establish more suitable animal models. Chimeric viruses that can overcome species-specific restriction should be constructed to support these studies.

3 The MCPyV Tumorigenic Mechanisms

MCPyV DNA is frequently integrated into MCC genome (Feng et al. 2008; Liu et al. 2016; Krump and You 2018). The MCPyV genome in MCC tumor cells is invariably truncated by the integration event such that it is replication-incompetent, yet the cell growth-promoting functions of viral genes called tumor antigens are preserved (Shuda et al. 2008). MCPyV-positive MCCs typically express intact sT and a tumor-specific truncation mutant of LT that preserves the N-terminal half of LT, referred to as LTT (tumor-derived LT) antigen (Feng et al. 2008; Shuda et al. 2008; Sastre-Garau et al. 2009; Borchert et al. 2014; Houben et al. 2012). MCPyV-positive MCCs harbor very few genetic mutations (Harms et al. 2015; Goh et al. 2016), suggesting that the expression of these viral oncogenes is sufficient to drive tumor development. Indeed, sT and LTT have demonstrated robust oncogenic potential to promote tumorigenesis (Spurgeon and Lambert 2013; Grundhoff and Fischer 2015; Wendzicki et al. 2015; Shuda et al. 2011; Verhaegen et al. 2014). MCPyV-positive MCC cells are addicted to sT/LTT oncogenes and require their continued expression from integrated viral genome to survive (Houben et al. 2010; Shuda et al. 2014). Knockdown of sT/LTT antigens induces growth arrest and cell death in MCPyV-positive MCC cells (Houben et al. 2010; Shuda et al. 2014) and leads to tumor regression in xeno-transplantation (Houben et al. 2012). These key findings demonstrated the important impact of viral oncogene expression in the development of MCPyV-associated MCCs.

A common characteristic of MCPyV genomes integrated into the MCC genome is the selection for mutations in the LT antigen coding sequence that introduce premature stop codons, which delete the LT C-terminal helicase domains (Shuda et al. 2008). The resulting tumor-specific LTT antigen retains the CR1, DnaJ, and RB-binding motifs, allowing the LTT molecules to efficiently disrupt the host cell cycle (Shuda et al. 2008). Phosphorylation of serine 220 of MCPyV LTT is required this viral oncogene to inactivate RB in MCC cells (Schrama et al. 2016). This RB-inhibiting function of MCPyV LTT antigen has also been shown to stimulate cell proliferation by upregulating cyclin E and CDK2 (Richards et al. 2015).

Unlike MCPyV LT, intact MCPyV sT is consistently expressed in MCPyV-positive MCC tumors. Nearly no mutations have been detected in the sT-coding regions integrated into MCC genome (Shuda et al. 2008; Starrett 2017), corroborating a key functional role for this viral oncogene in the development of MCPyV-positive tumors. MCPyV sT has been shown to transform immortalized rat fibroblasts in cell culture (Shuda et al. 2011). Its transforming activity has also been demonstrated in transgenic mouse models (Verhaegen et al. 2017; Verhaegen et al.

2015; Shuda et al. 2015). In line with these observations, co-expression of MCPyV sT antigen with Atonal bHLH transcription factor 1 (ATOH1) induces cell aggregates with morphology and marker expression pattern mimicking MCC (Verhaegen et al. 2017). The oncogenic activity of MCPyV sT antigen is mostly supported by its ability to induce hyperphosphorylated, and thus inactivated, 4E-BP1, causing dysfunction of cap-dependent translation to stimulate cell proliferation and transformation (Shuda et al. 2011; Velasquez et al. 2016; Shuda et al. 2015; Sun et al. 2011). As described above, MCPyV sT antigen can inhibit the E3 ubiquitin ligase SCF^{Fbw7}. This sT function prevents the proteasomal degradation of MCPyV LT antigen as well as several important cellular proliferative proteins, such as c-Myc and cyclin E, which are normally targeted by SCF^{Fbw7} (Kwun et al. 2013). From a large-scale co-immunoprecipitation and proteomic study, MCPyV sT antigen was found to be associated with the MYCL-EP400 complex, which together bind promoters of specific cellular genes to stimulate their expression and cellular transformation (Cheng et al. 2017). In line with this finding, a transcriptome analysis of normal human fibroblasts with inducible expression of MCPyV sT revealed its ability to dynamically change cellular gene expression (Berrios et al. 2016). sT expression leads to upregulation of glycolytic genes, including the monocarboxylate lactate transporter SLC16A1 (MCT1) (Berrios et al. 2016). Additional functional analysis suggested that these gene expression changes lead to elevated aerobic glycolysis, which may also contribute to the MCPyV-dependent cellular transformation (Berrios et al. 2016). In addition, MCPyV sT modulates cellular microtubule network, motility, and migration through upregulation of microtubule- and actin-associated proteins as well as the cellular sheddases, A disintegrin and metalloproteinase (ADAM) 10 and 17. Together, these cellular factors contribute to sT-induced cell dissociation and motility, a feature that may support MCPyV-mediated cellular transformation and metastasis (Nwogu et al. 2018; Stakaityte 2018; Knight et al. 2015).

Clonal integration of MCPyV DNA into the host genome is a key causative factor for MCC development (Houben et al. 2009; Chang and Moore 2012). However, the molecular mechanism that contributes to viral integration remains poorly understood. As described above, both LTT and sT expressed from the integrated viral genome demonstrate strong potential for modulating cellular proteins to drive cell proliferation. The function of these viral oncogenes offers a strong growth advantage for selecting the precancerous cells with the integrated viral genome expressing these viral oncogenes. Another selective pressure may be presented by the loss of viral DNA replication activity caused by the deletion of the LT C-terminal OBD and helicase domains after the integration of the viral DNA into the host genome. As continuous LT-mediated replication from the integrated viral Ori could result in replication fork collisions and double-strand breaks in the host DNA, disrupting the OBD and helicase domains of LT antigen would relieve this genotoxic stress. Finally, other functional activities of the LT antigen C-terminal domain may also need to be negatively selected during tumorigenesis. For example, expression of the C-terminal helicase-containing region of MCPyV LT induces a host cellular DNA damage response, leading to p53

activation, upregulation of its downstream target genes, and cell cycle arrest (Li et al. 2013). Compared to the N-terminal MCPyV LT region normally preserved and expressed in MCC tumors, full-length MCPyV LT shows a significantly decreased potential to support cellular proliferation, focus formation, and anchorage-independent cell growth (Li et al. 2013). It was further discovered that activated ATM phosphorylates the MCPyV LT C-terminal residue serine 816, which functions to promote apoptosis (Li et al. 2015). In line with these observations, an additional study showed that expression of the MCPyV LT antigen C-terminal 100 residues was sufficient to cause growth inhibition in many different cell types (Cheng et al. 2013). Together, the growth-inhibitory activities of the MCPyV LT C-terminal domain revealed in these studies suggest that the truncation mutations that remove the MCPyV LT C-terminal region found in MCC is not only needed to prevent replication of the integrated viral genome but also essential for overcoming the anti-tumorigenic properties intrinsic to the MCPyV LT C-terminus (Li et al. 2013; Cheng et al. 2013; Li et al. 2015). MCPyV viral genome integration, therefore, promotes MCC tumorigenesis by overcoming the obstacles to oncogenesis presented by replicative stress, DNA damage responses, and cell cycle arrest.

Several recent studies have attempted to establish transgenic mouse models for MCPyV oncogenes. Although expression of MCPyV sT and LT can induce hyperplasia and benign lesions in the epidermis of the transgenic mice, they failed to induce lesions that fully recapitulate MCC pathogenesis (Verhaegen et al. 2014; Spurgeon et al. 2015). Therefore, better MCPyV animal models are needed to investigate MCPyV-induced MCC development in vivo.

4 Therapeutic Strategy Targeting MCPyV Infection

An FDA-approved MEK inhibitor, Trametinib, inhibits MCPyV infection in cultured HDFs, making it the first drug capable of blocking the viral infection (Liu et al. 2016). Trametinib could potently reduce the MCPyV viral load in immunocompromised patients. Therefore, it has the potential of preventing the development of MCC tumors in these individuals (Liu et al. 2016).

5 Human Merkel Cell Carcinoma

5.1 MCC Histopathologic Features

MCC is a rare and aggressive cutaneous malignancy of neuroendocrine origin. Based on its histopathologic patterns, it was first named by Dr. Toker as “trabecular carcinoma of the skin” (Toker 1972). Additional names for MCC include the Toker tumor, primary cutaneous neuroendocrine tumor, primary small cell carcinoma of the skin, and malignant trichodiscoma (Schwartz and Lambert 2005). It is the

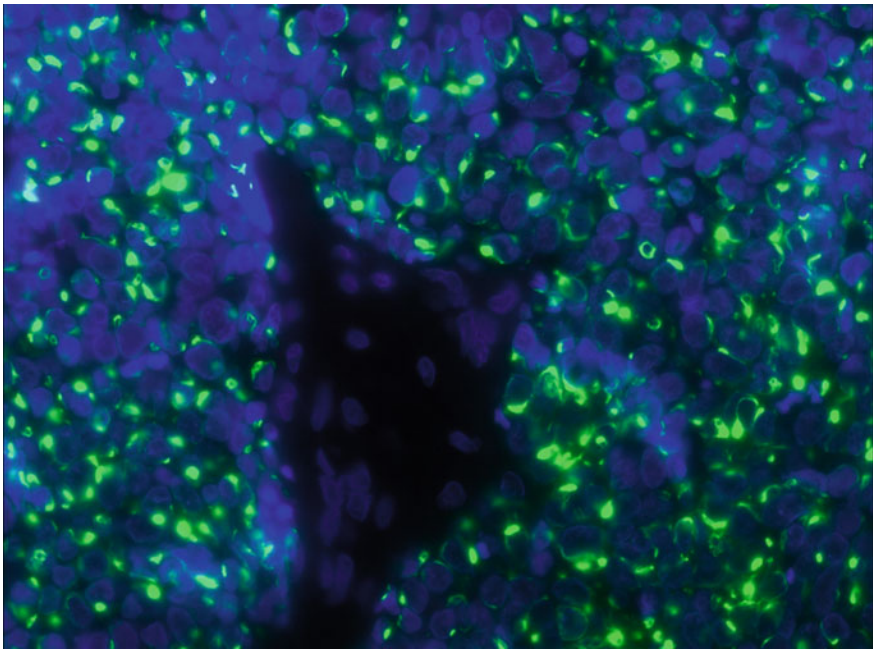

Fig. 2 Cytokeratin 20 (CK20) staining of MCC. Perinuclear dot-like cytokeratin 20 staining (Green). Nuclei (Blue)

second most common cause of skin cancer death after melanoma (Albores-Saavedra et al. 2010). Because of the similarity in histopathologic patterns, MCC was sometimes misdiagnosed as the malignant small blue cell tumors (Xue and Thakuria 2019). The advent of immunohistochemistry staining for Cytokeratin 20 (CK20) has greatly improved the diagnostic accuracy of MCC (Fig. 2) (Scott and Helm 1999).

5.2 Origin of MCC

The origin of tumors remains a central question for MCC research. Historically, MCC tumors were thought to arise from Merkel cells, which are mechanoreceptor cells located in the basal layer of the epidermis and also in hair follicles of the skin (Winkelmann and Breathnach 1973) that share the immunohistochemical marker CK20 with MCC tumors (Table 1). However, Merkel cells are known to be derived from the epidermal lineage (Morrison et al. 2009; Van Keymeulen et al. 2009), whereas MCCs mostly develop within the dermis and subcuitis (Calder and Smoller 2010). In addition, Merkel cells are post-mitotic and have lost the proliferative

Table 1 Prospective cells of origin for MCC and the markers and key characteristics they shared with MCC (Liu et al. 2016)

	Markers/characteristics
Merkel cell carcinoma	CK20+, NSE+, PAX5+, NFP+, TdT+, TTF-1−, LCA−, S100−
Human dermal fibroblasts	Major skin cell types that support productive MCPyV infection, transcription, and replication
Merkel cells	CK20+, electron microscope morphology
Pro/pre-B and pre-B cells	PAX5+, TdT+

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

activity, thus are less likely to develop into tumorigenic MCC cells (Vaigot et al. 1987; Moll et al. 1996). These evidence suggest that Merkel cells are not likely to be the cells of origin for MCC.

Identification of HDFs as the target cells of productive MCPyV infection (Liu et al. 2016) is in line with the clinical observation that most MCCs develop in the dermis (Calder and Smoller 2010). It also suggests that MCPyV infection of HDFs in the dermis may ultimately give rise to MCC tumors. For example, it is possible that over-amplification of MCPyV in HDFs may stimulate viral genome integration into the host DNA to induce cellular transformation. Alternatively, MCPyV actively replicating in HDFs located at the boundary between epidermis and dermis may release viral particles that inadvertently infect bystander Merkel cells or their precursor cells present in the immediate vicinity within the basal layer of the epidermis. The dead-end replication environment present in Merkel cells may favor viral integration and cellular transformation.

Recent studies also showed that MCC tumors express Paired box gene 5 (PAX5) and terminal deoxynucleotidyl transferase (TdT), which, under physiological conditions, are specifically expressed in pro/pre-B and pre-B cells (https://pubmed.ncbi.nlm.nih.gov/23576560/). Based on this finding, it was speculated that MCC tumors might originate from pro-B/pre-B cells, although this theory remains to be examined experimentally.

5.3 MCPyV-Positive and -Negative MCCs

The majority of MCC tumors are associated with MCPyV infection, while the remaining can be attributed to UV-induced mutation (Harms et al. 2015; Goh et al. 2016; Starrett 2017; Cohen et al. 2016). In the Northern hemisphere, approximately 80% of MCC tumors carry monoclonally integrated MCPyV genome (Feng et al. 2008; Sihto et al. 2009). However, the percentage of MCPyV-positive MCC is significantly lower in other geographic areas such as Australia (∼30%) (Garneski et al. 2009). The fact that MCPyV-positive MCC tumors typically carry an integrated viral genome is reminiscent of papillomavirus-induced cancers (Feng et al. 2008).

However, unlike papillomavirus-associated malignancies, MCPyV-positive MCC tumors grow swiftly with no clear precancerous stage. MCPyV-positive MCC tumors are believed to be derived from monoclonal expansion and have a very low mutation burden (Harms et al. 2015; Goh et al. 2016; Starrett et al. 2017; Cohen et al. 2016). These findings suggest that MCPyV-induced MCC tumors may develop rapidly after MCPyV genome integration.

Whole-genome sequencing has begun to shed light on the differences in the causes of MCPyV-positive and -negative MCC tumors. These genetic studies revealed that UV radiation is the primary cause of MCPyV-negative MCC tumors, which accounts for about 20% of all MCC cases (Harms et al. 2015; Goh et al. 2016; Starrett 2017; Cohen et al. 2016). Compared to MCPyV-positive MCCs, MCPyV-negative tumors revealed a prominent UV-mediated DNA damage signature and displayed a dramatically higher mutation burden (Harms et al. 2015; Goh et al. 2016; Starrett et al. 2017; Cohen et al. 2016). Common cancer activating mutations often observed in MCPyV-negative MCC tumors include mutations in RB1, p53, PIK3CA as well as key components of the host DNA damage response, Notch signaling, and chromatin remodeling pathways (Harms et al. 2015; Goh et al. 2016; Starrett 2017; Cohen et al. 2016). Much lower levels of cancer-promoting mutations were observed in MCPyV-positive MCC tumors, supporting that the MCPyV sT and LTT oncogenes expressed from the integrated viral genomes are the predominant oncogenic drivers for these tumors (Harms et al. 2015; Goh et al. 2016; Starrett et al. 2017; Cohen et al. 2016).

6 Current Therapeutic Strategies for MCC

6.1 Surgery and Radiation Therapy

Early-stage, localized MCC tumors are mostly treated with wide-section surgery and radiation. However, MCC frequently undergoes metastasis, which increases the chance that tumors may be developed in body sites that are harder to reach and fully eradicated with radiotherapy (Bichakjian et al. 2007; Allen et al. 1999). Therefore, chemotherapy has been applied to treat advanced-stage MCCs. Despite the early MCC response to chemotherapy, the duration of the response is usually short-lived and many tumors often develop chemoresistance (Brummer et al. 2016; Saini and Miles 2015; Cassler et al. 2016). Because chemotherapy also has an immunosuppressive effect, which counteracts the cellular immune response to MCC tumors, it offers little overall survival benefit for MCC tumors. Currently, there are very few feasible options for patients with advanced MCCs (Cassler et al. 2016).

6.2 Immunotherapy

MCPyV antigens or ultraviolet-mutation-associated neoantigens expressed in MCC tumors represent ideal targets for anti-tumor immunotherapy. Robust intratumoral

CD8+ T-cell infiltration has been associated with 100% MCC-specific survival, independent of tumor stage (Paulson et al. 2011). This strong correlation between immune function and prognosis reveals the potential for using immunotherapies to treat metastatic MCCs.

6.2.1 Immune Checkpoint Inhibitor Therapy

Targeting the programmed cell death receptor 1/programmed cell death ligand 1 (PD-1/PD-L1) checkpoint has become an attractive treatment option for MCC (Mantripragada and Birnbaum 2015). Both MCPyV-positive and -negative MCCs have been treated with anti-PD-L1 (Kaufman et al. 2016) or anti-PD-1 therapy (Nghiem et al. 2016). In one of the studies, 88 patients with advanced MCCs were treated with the anti-PD-L1 antibody and followed for at least 12 months. An objective response rate of 33.0% was confirmed with 11.4% of the patients showing durable and complete responses. A one-year progression-free survival (PFS) rate of 30% and overall survival (OS) rates of 52% were achieved (Kaufman et al. 2018). In another study with a median follow-up time of 14.9 months (Nghiem 2019), the treatment of 50 patients with the anti-PD-1 antibody, pembrolizumab, resulted in a 56% objective response rate (ORR), including 24% of the patients showing complete response and 32% partial response. Since these studies, the anti-PD-L1 antibody Avelumab and the anti-PD-1 antibody pembrolizumab have been approved by FDA as new treatments for metastatic MCC. Several clinical trials are ongoing to assess the safety and efficacy of anti-PD-1 and anti-PD-L1 immuno-checkpoint therapies for MCC. These early studies using PD-1/PD-L1 immune checkpoint blockade therapies showed promising results but a significant portion of MCC patients does not respond to the treatment (Nghiem et al. 2016; Becker et al. 2017; Terheyden and Becker 2017; Winkler et al. 2017; D'Angelo 2018).

6.2.2 Adoptive Cell Transfer Therapy

MCPyV-encoded T antigens are continuously expressed in MCC to support tumor growth; therefore, they represent an appealing target for viral oncoprotein-directed T-cell therapy. Tumor-infiltrating CD8+ T cells are associated with improved survival of MCC patients. However, CD8+ T-cell infiltration is present in less than 18% of MCC tumors (Miller et al. 2017), suggesting that MCC may benefit from adoptive T-cell transfer therapy. In several recent studies, naturally processed epitopes of MCPyV LT antigen were identified and the T antigen-specific T-cell receptors (TCRs) isolated (Miller et al. 2017; Iyer et al. 2011; Lyngaa et al. 2014). These studies also revealed that intratumoral infiltration of MCPyV-specific T cells is associated with significantly improved MCC-specific survival, demonstrating the therapeutic benefit of MCPyV-specific T cells (Miller et al. 2017). Indeed, tumor-bearing animals treated with engineered T cells expressing MCPyV T antigen-specific TCR leads to tumor regression (Gavvovidis et al. 2018). Recently, Chapuis group treated two patients with advanced MCC tumors with autologous MCPyV-specific CD8+ T cells followed by immune checkpoint

inhibitors (Paulson et al. 2018). In both cases, significant tumor regressions were associated with increased CD8+ T-cell infiltration into the regressing tumors (Paulson et al. 2018). However, tumors relapsed and escaped T-cell treatment during the late stage. Single-cell RNA sequencing suggests that treatment failure could be caused by HLA-loss (Paulson et al. 2018). Therefore, genetically engineered T cells with chimeric antigen receptor (CAR), which can recognize cancer cells in an HLA-independent manner, is an alternative approach for overcoming the problem of HLA-loss during MCC treatment.

6.3 DNA Cancer Vaccine

One of the earliest therapeutic approaches explored for treating MCC tumors is an MCPyV DNA vaccine. MCPyV LTT and sT consistently expressed in MCC are attractive foreign antigen targets for vaccine development. In 2012, the Hung laboratory developed a DNA vaccine to specifically target the MCPyV LTT region. When tested in mice injected with the B16/LT murine melanoma cell line stably expressing LTT, this vaccine showed protection against the LTT-expressing tumors in vivo (Zeng et al. 2012). These anti-tumor effects of the DNA vaccine appear to be mediated by CD4 + T-cell stimulation, natural killer cells and CD8+ T cells (Zeng et al. 2012). Because CD8+ T cells are associated with a better outcome, the Hung group went on to produce another DNA vaccine specifically designed to promote MCPyV LT-specific CD8+ T-cell responses. This vaccine encodes LTT antigen fused to a damage-associated molecular pattern protein, calreticulin (CRT), which has the ability to induce CD8+ T cells when fused to other foreign antigens (Zeng et al. 2012; Gomez et al. 2012). This new vaccine, named CRT/LT, showed prolonged survival after tumor challenge in the B16/LT mice model compared to mice vaccinated with the previous MCPyV LT vaccine. It was further demonstrated that this better performance was mediated by the induction of MCPyV LT-specific CD8+ T cells (Gomez et al. 2012). Another MCPyV DNA vaccine developed in the Hung group targeted the sT antigen, which is the main driver of MCC oncogenesis. The DNA vaccine pcDNA3-MCC/sT generated a significant number of sT antigenic peptide-specific CD8+ T cells and demonstrated markedly enhanced protection and treatment, leading to increased survival and decreased tumor volume in vivo (Gomez et al. 2013). These encouraging preliminary results provide a great platform for the development of MCPyV-targeted vaccines for MCC treatment.

6.4 Targeted Therapies

Targeted therapies are necessary for patients with advanced-stage MCCs that don't respond well to immunotherapy. Currently, multiple types of targeted therapies have been evaluated in MCC cell lines as well as in xenograft models, with some of them entering early phase clinical trials. Anti-apoptosis gene *BCL-2* is frequently unregulated in MCC and has become a major target for MCC therapies (Verhaegen

et al. 2014). BCL-2 antisense oligonucleotides have been shown to inhibit tumor growth in MCC xenograft models (Schlagbauer-Wadl et al. 2000). However, they were not able to induce an objective response in a phase II trial (Shah et al. 2009). On the other hand, ABT-263, a small-molecule inhibitor of the BCL-2 family members (BCL-2, BCL-XL and BCL-W) could effectively induce apoptosis in most of the MCC cell lines tested (Verhaegen et al. 2014). MCC cell lines are also responsive to inhibitors of PI3K and mTOR pathways (Chteinberg et al. 2018; Kannan et al. 2016; Lin et al. 2015; Nardi et al. 2012; Hafner et al. 2012). In addition, most of the MCPyV-positive MCCs maintain wild-type p53 (Park et al. 2019). Not surprisingly, inhibitors of MDM2 (Mouse double minute 2 homolog or HDM2), which targets p53 for degradation, have been found to be effective in triggering p53-dependent apoptosis and cell cycle arrest in the majority of MCPyV-positive MCC tumors tested (Houben et al. 2013). A clinical trial is ongoing to evaluate a novel MDM2 small molecular inhibitor, KRT-232, for the effectiveness in treating patients with wild-type p53 MCC tumors but have failed anti-PD-1 or PD-L1 immunotherapy (NCT03787602). Since somatostatin receptors are highly expressed in MCC tumors, somatostatin analogues have been explored for their potential to be used in MCC molecular imaging and treatment (Orlova et al. 2018; Sollini et al. 2016; Buder et al. 2014). PEN-221, an inhibitor of somatostatin receptor 2 (SSTR2), is being evaluated in a clinical trial to target MCC and other advanced cancers with highly expressed SSTR2 (NCT02936323). Finally, antiapoptotic factor survivin is highly up-regulated in MCPyV-positive MCCs when compared to MCPyV-negative MCCs (Arora et al. 2012). Consistent with the fact that survivin expression is essential to support the survival of these tumor cells, YM155, a small-molecule inhibitor of survivin, has yielded promising results in inhibiting the growth of MCPyV-positive MCC cell lines both in cell culture and in xenograft models (Arora et al. 2012; Dresang et al. 2013).

7 Remaining Questions and Future Perspectives

MCPyV offers a unique opportunity to explore the oncogenic mechanism of a DNA tumor virus. Although the small viral genome of 5.4 kb DNA encodes just seven gene products, MCPyV successfully infects the skin of most humans and can establish long-lasting infections (Tolstov et al. 2009; Schowalter et al. 2010). While most of the MCPyV infections remain asymptomatic throughout the life of the infected hosts, in rare cases, it can cause extremely lethal skin cancer, MCC (Feng et al. 2008; Gjoerup and Chang 2010; Liu et al. 2016).

Despite recent advancements, much remain to be learned about MCPyV and its role in the development of MCC tumors. For instance, the cells of origin for MCC are currently unknown and the mechanisms by which MCPyV infection leads to cancer also remain enigmatic. MCPyV maintains persistent and latent infection in more than 80% of the general population (Tolstov et al. 2009; Foulongne et al. 2012; Schowalter et al. 2010) but tends to cause MCC in immunocompromised

individuals (Heath et al. 2008). This observation suggests that the virus has evolved to exist in a dynamic state of mutual antagonism with the host cells and that changes to host immune status for which MCPyV is not adapted can result in cellular transformation and malignancy. However, the mechanism by which MCPyV escapes host immune eradication and establishes persistent infection remains unexplored. Few studies have examined the immunomodulatory effects of MCPyV-encoded proteins, and none have done so in the context of natural MCPyV infection. It is also unclear how changes to host conditions, such as the decline of immune competency, increase the chance of MCPyV-associated tumorigenesis. Although the uncontrolled proliferation of MCPyV may make viral genome integration more likely, the events that precede MCPyV integration into the host genome have not been elucidated.

Until recently, it was impossible to study biologically relevant host responses to MCPyV as the host cell of MCPyV was unknown. The discovery of HDFs as the host cells supporting productive MCPyV infection allows the development of a physiologically relevant in vitro model system (Liu et al. 2016), thus offering many new opportunities to explore the virus-host interactions in the setting of productive infection. No effective chemotherapies for metastatic MCC are currently available. Recent MCC immunotherapy successes suggest that overcoming MCC immune non-responsiveness is likely to yield improved patient outcomes. The continued discovery of the host restriction mechanisms that normally prevent MCPyV infection and viral oncogenesis could unveil more effective strategies for preventing and treating MCPyV-associated human cancers.

References

Agelli M et al (2010) The etiology and epidemiology of Merkel cell carcinoma. Curr Probl Cancer 34(1):14–37

Albores-Saavedra J et al (2010) Merkel cell carcinoma demographics, morphology, and survival based on 3870 cases: a population based study. J Cutan Pathol 37(1):20–27

Allen PJ et al (1999) Surgical management of Merkel cell carcinoma. Ann Surg 229(1):97–105

Arora R, et al (2012) Survivin is a therapeutic target in Merkel cell carcinoma. Sci Transl Med 4 (133):133ra56

Becker JC (2010) Merkel cell carcinoma. Ann Oncol 21(Suppl 7):vii81-5

Becker JC et al (2017) Merkel cell carcinoma. Nat Rev Dis Primers 3:17077

Becker M, et al (2019) Infectious entry of Merkel cell polyomavirus. J Virol

Berrios C et al (2016) Merkel cell polyomavirus small T antigen promotes pro-glycolytic metabolic perturbations required for transformation. PLoS Pathog 12(11):e1006020

Bhatia S et al (2011) Immunobiology of Merkel cell carcinoma: implications for immunotherapy of a polyomavirus-associated cancer. Curr Oncol Rep 13(6):488–497

Bichakjian CK et al (2007) Merkel cell carcinoma: critical review with guidelines for multidisciplinary management. Cancer 110(1):1–12

Borchert S et al (2014) High-affinity Rb binding, p53 inhibition, subcellular localization, and transformation by wild-type or tumor-derived shortened Merkel cell polyomavirus large T antigens. J Virol 88(6):3144–3160

Brummer GC et al (2016) Merkel cell carcinoma: current issues regarding diagnosis, management, and emerging treatment strategies. Am J Clin Dermatol 17(1):49–62

Buder K et al (2014) Somatostatin receptor expression in Merkel cell carcinoma as target for molecular imaging. BMC Cancer 14:268
Calder KB, Smoller BR (2010) New insights into Merkel cell carcinoma. Adv Anat Pathol 17 (3):155–161
Carter JJ et al (2013) Identification of an overprinting gene in Merkel cell polyomavirus provides evolutionary insight into the birth of viral genes. Proc Natl Acad Sci U S A 110(31):12744–12749
Cassler NM et al (2016) Merkel cell carcinoma therapeutic update. Curr Treat Options Oncol 17 (7):36
Chang Y, Moore PS (2012) Merkel cell carcinoma: a virus-induced human cancer. Annu Rev Pathol 7:123–144
Chen T et al (2011) Serological evidence of Merkel cell polyomavirus primary infections in childhood. J Clin Virol 50(2):125–129
Cheng J et al (2013) Merkel cell polyomavirus large T antigen has growth-promoting and inhibitory activities. J Virol 87(11):6118–6126
Cheng J et al (2017) Merkel cell polyomavirus recruits MYCL to the EP400 complex to promote oncogenesis. PLoS Pathog 13(10):e1006668
Cho S et al (2009) Effects of infrared radiation and heat on human skin aging in vivo. J Investig Dermatol Symp Proc 14(1):15–19
Chteinberg E et al (2018) Phosphatidylinositol 3-kinase p110delta expression in Merkel cell carcinoma. Oncotarget 9(51):29565–29573
Clarke CA, et al (2015) Risk of Merkel cell carcinoma after solid organ transplantation. J Natl Cancer Inst 107(2)
Cohen PR et al (2016) Genomic portfolio of Merkel cell carcinoma as determined by comprehensive genomic profiling: implications for targeted therapeutics. Oncotarget 7 (17):23454–23467
D'Angelo SP, et al (2018) Efficacy and safety of first-line avelumab treatment in patients with stage IV metastatic Merkel cell carcinoma: a preplanned interim analysis of a clinical trial. JAMA Oncol
Diaz J et al (2014) Phosphorylation of large T antigen regulates Merkel cell polyomavirus replication. Cancers (Basel) 6(3):1464–1486
Dresang LR et al (2013) Response of Merkel cell polyomavirus-positive Merkel cell carcinoma xenografts to a survivin inhibitor. PLoS ONE 8(11):e80543
Engels EA et al (2002) Merkel cell carcinoma and HIV infection. Lancet 359(9305):497–498
Erickson KD et al (2009) Ganglioside GT1b is a putative host cell receptor for the Merkel cell polyomavirus. J Virol 83(19):10275–10279
Feng H et al (2008) Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science 319(5866):1096–1100
Feng H et al (2011) Cellular and viral factors regulating Merkel cell polyomavirus replication. PLoS ONE 6(7):e22468
Fisher GJ et al (1996) Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature 379(6563):335–339
Fitzgerald TL et al (2015) Dramatic increase in the incidence and mortality from Merkel cell carcinoma in the United States. Am Surg 81(8):802–806
Foulongne V et al (2012) Human skin microbiota: high diversity of DNA viruses identified on the human skin by high throughput sequencing. PLoS ONE 7(6):e38499
Garneski KM et al (2009) Merkel cell polyomavirus is more frequently present in North American than Australian Merkel cell carcinoma tumors. J Invest Dermatol 129(1):246–248
Gavvovidis I et al (2018) Targeting Merkel cell carcinoma by engineered T cells specific to T-antigens of Merkel cell polyomavirus. Clin Cancer Res 24(15):3644–3655
Gill SE, Parks WC (2008) Metalloproteinases and their inhibitors: regulators of wound healing. Int J Biochem Cell Biol 40(6–7):1334–1347

Gjoerup O, Chang Y (2010) Chapter 1—update on human polyomaviruses and cancer. In: George FVW, George K (eds.) Advances in cancer research, pp. 1–51. Academic Press
Goh G et al (2016) Mutational landscape of MCPyV-positive and MCPyV-negative Merkel cell carcinomas with implications for immunotherapy. Oncotarget 7(3):3403–3415
Gomez BP et al (2012) Strategy for eliciting antigen-specific CD8+ T cell-mediated immune response against a cryptic CTL epitope of Merkel cell polyomavirus large T antigen. Cell Biosci 2(1):36
Gomez B et al (2013) Creation of a Merkel cell polyomavirus small T antigen-expressing murine tumor model and a DNA vaccine targeting small T antigen. Cell Biosci 3(1):29
Grundhoff A, Fischer N (2015) Merkel cell polyomavirus, a highly prevalent virus with tumorigenic potential. Curr Opin Virol 14:129–137
Hafner C et al (2012) Activation of the PI3K/AKT pathway in Merkel cell carcinoma. PLoS ONE 7(2):e31255
Harms PW (2017) Update on Merkel cell carcinoma. Clin Lab Med 37(3):485–501
Harms PW et al (2015) The distinctive mutational spectra of polyomavirus-negative Merkel cell carcinoma. Cancer Res 75(18):3720–3727
Harrison CJ et al (2011) Asymmetric assembly of Merkel cell polyomavirus large T-antigen origin binding domains at the viral origin. J Mol Biol 409(4):529–542
Heath M et al (2008) Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features. J Am Acad Dermatol 58(3):375–381
Hodgson NC (2005) Merkel cell carcinoma: changing incidence trends. J Surg Oncol 89(1):1–4
Houben R et al (2009) Molecular pathogenesis of Merkel cell carcinoma. Exp Dermatol 18 (3):193–198
Houben R et al (2010) Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. J Virol 84(14):7064–7072
Houben R et al (2012) An intact retinoblastoma protein-binding site in Merkel cell polyomavirus large T antigen is required for promoting growth of Merkel cell carcinoma cells. Int J Cancer 130(4):847–856
Houben R et al (2013) Mechanisms of p53 restriction in Merkel cell carcinoma cells are independent of the Merkel cell polyoma virus T antigens. J Invest Dermatol 133(10):2453–2460
Iyer JG et al (2011) Merkel cell polyomavirus-specific CD8(+) and CD4(+) T-cell responses identified in Merkel cell carcinomas and blood. Clin Cancer Res 17(21):6671–6680
Kannan A et al (2016) Dual mTOR inhibitor MLN0128 suppresses Merkel cell carcinoma (MCC) xenograft tumor growth. Oncotarget 7(6):6576–6592
Kaufman HL et al (2016) Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. Lancet Oncol 17 (10):1374–1385
Kaufman HL et al (2018) Updated efficacy of avelumab in patients with previously treated metastatic Merkel cell carcinoma after >/=1 year of follow-up: JAVELIN Merkel 200, a phase 2 clinical trial. J Immunother Cancer 6(1):7
Kean JM et al (2009) Seroepidemiology of human polyomaviruses. PLoS Pathog 5(3):e1000363
Knight LM et al (2015) Merkel cell polyomavirus small T antigen mediates microtubule destabilization to promote cell motility and migration. J Virol 89(1):35–47
Krump NA, You J (2018) Molecular mechanisms of viral oncogenesis in humans. Nat Rev Microbiol 16(11):684–698
Kwun HJ et al (2009) The minimum replication origin of Merkel cell polyomavirus has a unique large T-antigen loading architecture and requires small T-antigen expression for optimal replication. J Virol 83(23):12118–12128
Kwun HJ et al (2013) Merkel cell polyomavirus small T antigen controls viral replication and oncoprotein expression by targeting the cellular ubiquitin ligase SCFFbw7. Cell Host Microbe 14(2):125–135

Kwun HJ et al (2015) Restricted protein phosphatase 2A targeting by Merkel cell polyomavirus small T antigen. J Virol 89(8):4191–4200
Lemos B, Nghiem P (2007) Merkel cell carcinoma: more deaths but still no pathway to blame. J Invest Dermatol 127(9):2100–2103
Li J et al (2013) Merkel cell polyomavirus large T antigen disrupts host genomic integrity and inhibits cellular proliferation. J Virol 87(16):9173–9188
Li J et al (2015) Phosphorylation of Merkel cell polyomavirus large tumor antigen at serine 816 by ATM kinase induces apoptosis in host cells. J Biol Chem 290(3):1874–1884
Lin Z et al (2015) Effect of the dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235 against human Merkel cell carcinoma MKL-1 cells. Oncol Lett 10 (6):3663–3667
Liu W, et al (2016) Identifying the target cells and mechanisms of Merkel cell polyomavirus infection. Cell Host Microbe
Liu W, et al (2018) Merkel cell polyomavirus infection of animal dermal fibroblasts. J Virol 92(4)
Liu W et al (2016a) Identifying the target cells and mechanisms of Merkel cell polyomavirus infection. Cell Host Microbe 19(6):775–787
Liu W et al (2016b) Merkel cell polyomavirus infection and Merkel cell carcinoma. Curr Opin Virol 20:20–27
Lunder EJ, Stern RS (1998) Merkel-cell carcinomas in patients treated with methoxsalen and ultraviolet a radiation. N Engl J Med 339(17):1247–1248
Lyngaa R et al (2014) T-cell responses to oncogenic Merkel cell polyomavirus proteins distinguish patients with Merkel cell carcinoma from healthy donors. Clin Cancer Res 20(7):1768–1778
Mantripragada K, Birnbaum A (2015) Response to anti-PD-1 therapy in metastatic Merkel cell carcinoma metastatic to the heart and pancreas. Cureus 7(12):e403
Miller NJ et al (2017) Tumor-infiltrating Merkel cell polyomavirus-specific T cells are diverse and associated with improved patient survival. Cancer Immunol Res 5(2):137–147
Moll I et al (1996) Proliferative Merkel cells were not detected in human skin. Arch Dermatol Res 288(4):184–187
Morrison KM et al (2009) Mammalian Merkel cells are descended from the epidermal lineage. Dev Biol 336(1):76–83
Nardi V et al (2012) Activation of PI3K signaling in Merkel cell carcinoma. Clin Cancer Res 18 (5):1227–1236
Neu U et al (2012) Structures of Merkel cell polyomavirus VP1 complexes define a sialic acid binding site required for infection. PLoS Pathog 8(7):e1002738
Neumann F et al (2016) Replication of Merkel cell polyomavirus induces reorganization of promyelocytic leukemia nuclear bodies. J Gen Virol 97(11):2926–2938
Nghiem P, et al (2019) Durable tumor regression and overall survival in patients with advanced Merkel cell carcinoma receiving pembrolizumab as first-line therapy. J Clin Oncol JCO1801896
Nghiem PT et al (2016) PD-1 blockade with pembrolizumab in advanced Merkel-cell carcinoma. N Engl J Med 374(26):2542–2552
Nwogu N et al (2018) Cellular sheddases are induced by Merkel cell polyomavirus small tumour antigen to mediate cell dissociation and invasiveness. PLoS Pathog 14(9):e1007276
Orlova KV et al (2018) Somatostatin receptor type 2 expression in Merkel cell carcinoma as a prognostic factor. J Eur Acad Dermatol Venereol 32(6):e236–e237
Park DE et al (2019) Dual inhibition of MDM2 and MDM4 in virus-positive Merkel cell carcinoma enhances the p53 response. Proc Natl Acad Sci U S A 116(3):1027–1032
Paulson KG et al (2011) Transcriptome-wide studies of Merkel cell carcinoma and validation of intratumoral CD8+ lymphocyte invasion as an independent predictor of survival. J Clin Oncol 29(12):1539–1546
Paulson KG, et al (2017) Merkel cell carcinoma: current United States incidence and projected increases based on changing demographics. J Am Acad Dermatol

Paulson KG et al (2018) Acquired cancer resistance to combination immunotherapy from transcriptional loss of class I HLA. Nat Commun 9(1):3868
Quan T, Fisher GJ (2015) Role of age-associated alterations of the dermal extracellular matrix microenvironment in human skin aging: a mini-review. Gerontology 61(5):427–434
Quan T et al (2009) Matrix-degrading metalloproteinases in photoaging. J Investig Dermatol Symp Proc 14(1):20–24
Richards KF et al (2015) Merkel cell polyomavirus T antigens promote cell proliferation and inflammatory cytokine gene expression. J Gen Virol 96(12):3532–3544
Saini AT, Miles BA (2015) Merkel cell carcinoma of the head and neck: pathogenesis, current and emerging treatment options. Onco Targets Ther 8:2157–2167
Sastre-Garau X et al (2009) Merkel cell carcinoma of the skin: pathological and molecular evidence for a causative role of MCV in oncogenesis. J Pathol 218(1):48–56
Schlagbauer-Wadl H et al (2000) Bcl-2 antisense oligonucleotides (G3139) inhibit Merkel cell carcinoma growth in SCID mice. J Invest Dermatol 114(4):725–730
Schowalter RM, Buck CB (2013) The Merkel cell polyomavirus minor capsid protein. PLoS Pathog 9(8):e1003558
Schowalter RM et al (2010) Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. Cell Host Microbe 7(6):509–515
Schowalter RM et al (2011) Glycosaminoglycans and sialylated glycans sequentially facilitate Merkel cell polyomavirus infectious entry. PLoS Pathog 7(7):e1002161
Schowalter RM et al (2012) Entry tropism of BK and Merkel cell polyomaviruses in cell culture. PLoS ONE 7(7):e42181
Schrama D et al (2016) Serine 220 phosphorylation of the Merkel cell polyomavirus large T antigen crucially supports growth of Merkel cell carcinoma cells. Int J Cancer 138(5):1153–1162
Schwartz RA, Lambert WC (2005) The Merkel cell carcinoma: a 50-year retrospect. J Surg Oncol 89(1):5
Scott MP, Helm KF (1999) Cytokeratin 20: a marker for diagnosing Merkel cell carcinoma. Am J Dermatopathol 21(1):16–20
Seo GJ et al (2009) Merkel cell polyomavirus encodes a micro RNA with the ability to autoregulate viral gene expression. Virology 383(2):183–187
Shah MH et al (2009) G3139 (Genasense) in patients with advanced Merkel cell carcinoma. Am J Clin Oncol 32(2):174–179
Shuda M et al (2008) T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. Proc Natl Acad Sci U S A 105(42):16272–16277
Shuda M et al (2011) Human Merkel cell polyomavirus small T antigen is an oncoprotein targeting the 4E-BP1 translation regulator. J Clin Invest 121(9):3623–3634
Shuda M et al (2014) Merkel cell polyomavirus-positive Merkel cell carcinoma requires viral small T-antigen for cell proliferation. J Invest Dermatol 134(5):1479–1481
Shuda M et al (2015a) Merkel cell polyomavirus small T antigen induces cancer and embryonic Merkel cell proliferation in a transgenic mouse model. PLoS ONE 10(11):e0142329
Shuda M et al (2015b) Mitotic 4E-BP1 hyperphosphorylation and cap-dependent translation. Cell Cycle 14(19):3005–3006
Sihto H et al (2009) Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. J Natl Cancer Inst 101(13):938–945
Sollini M et al (2016) Somatostatin receptor positron emission tomography/computed tomography imaging in Merkel cell carcinoma. J Eur Acad Dermatol Venereol 30(9):1507–1511
Spurgeon ME, Lambert PF (2013) Merkel cell polyomavirus: a newly discovered human virus with oncogenic potential. Virology 435(1):118–130
Spurgeon ME et al (2015) Tumorigenic activity of Merkel cell polyomavirus T antigens expressed in the stratified epithelium of mice. Cancer Res 75(6):1068–1079
Stakaityte G, et al (2018) Merkel cell polyomavirus small T antigen drives cell motility via Rho-GTPase-induced filopodium formation. J Virol 92(2)

Stang A et al (2018) The association between geographic location and incidence of Merkel cell carcinoma in comparison to melanoma: an international assessment. Eur J Cancer 94:47–60
Starrett GJ, et al (2017) Merkel cell polyomavirus exhibits dominant control of the tumor genome and transcriptome in virus-associated Merkel cell carcinoma. MBio 8(1)
Sun CH et al (2011) Activation of the PI3K/Akt/mTOR pathway correlates with tumour progression and reduced survival in patients with urothelial carcinoma of the urinary bladder. Histopathology 58(7):1054–1063
Terheyden P, Becker JC (2017) New developments in the biology and the treatment of metastatic Merkel cell carcinoma. Curr Opin Oncol
Theiss JM et al (2015) A comprehensive analysis of replicating Merkel Cell polyomavirus genomes delineates the viral transcription program and suggests a role for mcv-miR-M1 in episomal persistence. PLoS Pathog 11(7):e1004974
Toker C (1972) Trabecular carcinoma of the skin. Arch Dermatol 105(1):107–110
Tolstov YL et al (2009) Human Merkel cell polyomavirus infection II. MCV is a common human infection that can be detected by conformational capsid epitope immunoassays. Int J Cancer 125(6):1250–1256
Tsang SH et al (2016) The oncogenic small tumor antigen of Merkel cell polyomavirus is an iron-sulfur cluster protein that enhances viral DNA replication. J Virol 90(3):1544–1556
Vaigot P et al (1987) The majority of epidermal Merkel cells are non-proliferative: a quantitative immunofluorescence analysis. Acta Derm Venereol 67(6):517–520
Van Keymeulen A et al (2009) Epidermal progenitors give rise to Merkel cells during embryonic development and adult homeostasis. J Cell Biol 187(1):91–100
Varani J et al (2006) Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. Am J Pathol 168(6):1861–1868
Velasquez C et al (2016) Mitotic protein kinase CDK1 phosphorylation of mRNA translation regulator 4E-BP1 Ser83 may contribute to cell transformation. Proc Natl Acad Sci U S A 113 (30):8466–8471
Verhaegen ME, et al (2014) Merkel cell polyomavirus small T antigen is oncogenic in transgenic mice. J Invest Dermatol
Verhaegen ME et al (2014) Merkel cell carcinoma dependence on bcl-2 family members for survival. J Invest Dermatol 134(8):2241–2250
Verhaegen ME et al (2015) Merkel cell polyomavirus small T antigen is oncogenic in transgenic mice. J Invest Dermatol 135(5):1415–1424
Verhaegen ME et al (2017) Merkel cell polyomavirus small T antigen initiates Merkel cell carcinoma-like tumor development in mice. Cancer Res 77(12):3151–3157
Wang X, et al (2012) Bromodomain protein Brd4 plays a key role in Merkel cell polyomavirus DNA replication. PLoS Pathog 8(11):e1003021. PMCID:PMC3493480
Wang X et al (2012) Bromodomain protein Brd4 plays a key role in Merkel cell polyomavirus DNA replication. PLoS Pathog 8(11):e1003021
Wendzicki JA et al (2015) Large T and small T antigens of Merkel cell polyomavirus. Curr Opin Virol 11:38–43
Winkelmann RK, Breathnach AS (1973) The Merkel cell. J Invest Dermatol 60(1):2–15
Winkler JK et al (2017) PD-1 blockade: a therapeutic option for treatment of metastatic Merkel cell carcinoma. Br J Dermatol 176(1):216–219
Xue Y, Thakuria M (2019) Merkel cell carcinoma review. Hematol Oncol Clin North Am 33 (1):39–52
Zeng Q et al (2012) Development of a DNA vaccine targeting Merkel cell polyomavirus. Vaccine 30(7):1322–1329