Published in final edited form as:

Annu Rev Pathol. 2012 ; 7: 123–144. doi:10.1146/annurev-pathol-011110-130227.

Merkel Cell Carcinoma: A Virus-Induced Human Cancer

Yuan Chang^{1,3} and Patrick S. Moore^{2,3}

Yuan Chang: yc70@pitt.edu; Patrick S. Moore: psm9@pitt.edu

¹Department of Pathology, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

²Department of Microbiology and Molecular Genetics, University of Pittsburgh, Pittsburgh, Pennsylvania 15213

³Cancer Virology Program, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania 15213

Abstract

Merkel cell polyomavirus (MCV) is the first polyomavirus directly linked to human cancer, and its recent discovery helps to explain many of the enigmatic features of Merkel cell carcinoma (MCC). MCV is clonally integrated into MCC tumor cells, which then require continued MCV oncoprotein expression to survive. The integrated viral genomes have a tumor-specific pattern of tumor antigen gene mutation that incapacitates viral DNA replication. This human cancer virus provides a new model in which a common, mostly harmless member of the human viral flora can initiate cancer if it acquires a precise set of mutations in a host with specific susceptibility factors, such as age and immune suppression. Identification of this tumor virus has led to new opportunities for early diagnosis and targeted treatment of MCC.

Keywords

polyomavirus; primary cutaneous neuroendocrine cancer; large T antigen; small T antigen; digital transcriptome subtraction

INTRODUCTION

Merkel cell carcinoma (MCC), first described in 1972 by Cyril Toker (1), is an aggressive skin cancer associated with immunosuppression (Figure 1) (2). Although frequently regarded as rare, MCC diagnoses have tripled over the past two decades (3, 4). A new impetus to understand and study this enigmatic cancer followed the 2008 discovery of a new human polyomavirus, Merkel cell polyomavirus (MCV), in ~80% of MCC cases (5).

In this review, we examine MCV, concentrating on its association with the biology and pathology of MCC. Because investigations of MCV have proceeded in the context of and have been facilitated by more than 50 years of research on other polyomaviruses, we also briefly discuss the general features and behavior of these viruses. Recent advances in molecular biology have seen an increased rate in the identification of polyomaviruses. To date, there are nine human polyomaviruses, and continuing improvements in technology is likely to increase this number. Identification of MCV has led to the rapid characterization of

Copyright © 2012 by Annual Reviews. All rights reserved

DISCLOSURE STATEMENT

The authors are named as inventors on several patents related to MCV. These patents have been assigned to the University of Pittsburgh.

viral oncogenes that contribute to MCC and to the development of new diagnostic tests for MCV infection. Additionally, the discovery of MCV in MCC is opening new avenues toward understanding the origin of virus-induced cancers and is illuminating the complex relationship between viruses and their human hosts.

MERKEL CELL CARCINOMA AND MERKEL CELL POLYOMAVIRUS

Brief Review of Polyomaviruses

Polyomaviruses are small, double-stranded DNA viruses with ~5.0–5.4-kb genomes. They are distantly related to papillomaviruses, and these two virus families were previously grouped together as papovaviruses, a taxonomic term that is no longer used. In 1954, Ludvik Gross (6) identified the first member of this family, murine polyomavirus (MPyV), which causes multiple tumor types in inbred mice. In 1960, simian vacuolating virus 40 (SV40), a common, asymptomatic infection of rhesus monkeys was discovered as a contaminant of polio vaccine tissue cultures (7). Although SV40 does not cause tumors in primates, its demonstrated transforming capability in rodents led to concerns regarding its introduction into human populations. Despite intensive follow-up investigations, no convincing evidence that SV40 is a human tumor virus has emerged (for reviews, see References 8 and 9). SV40, nonetheless, has become a workhorse for cancer biology, which has led to the discovery of the tumor-suppressor protein p53 (10, 11), the identification of nuclear localization signals for proteins (12), and the molecular characterization of DNA replication (13, 14).

Human Polyomaviruses

At present, nine human polyomaviruses have been identified (Table 1). The first two human polyomaviruses, JC virus (JCV) and BK virus (BKV), were described in back-to-back *Lancet* articles in 1971 (15, 16). These two polyomaviruses were culture isolated from opportunistic diseases (e.g., progressive multi-focal leukoencephalopathy and posttransplant nephropathy) that occur in immunosuppressed patients and were named after their source patients' initials. Although JCV has been linked to brain tumors and prostate cancers and BKV to colon cancer, a consistent and robust association between these viruses and diseases has not yet been demonstrated, and they are not widely believed to be human cancer viruses. It was not until 2007 that two more human polyomaviruses were found: Karolinska Institute polyomavirus (KIV) and Washington University polyomavirus (WUV) were named after the institutions wherein they were identified (17, 18). Both were detected in samples from children who had respiratory tract infections, but it is not known whether these viruses are responsible for symptomatic infection. Encapsidated viral particles were enriched by DNAase treatment followed by library construction and mass sequencing of cloned complementary DNA.

MCV was the fifth human polyomavirus to be identified. The search for a viral cause of MCC was based on the epidemiology of this unusual tumor (see the section entitled Association of Merkel Cell Carcinoma with Immunosuppression, below). Isolated messenger RNA from four carefully dissected MCC tumor samples was examined by digital transcriptome subtraction by use of high-fidelity sequence databases made available through the Human Genome Project. This process is tailored to distinguish viral sequences from human sequences without physical separation. Deep-sequencing data from sample libraries made from polyadenylated transcripts were first verified for stringent high-fidelity reads; then an *in silico* subtraction, with removal of all identifiable human sequences, was performed. These steps produced a tractable number of candidate transcripts that were subsequently compared with viral sequences at a lower stringency to find sequences having homology to known viruses. Out of ~400,000 MCC transcripts sequenced, one transcript had 54% homology to the tumor antigen (T antigen) of the African lymphotropic green

monkey polyomavirus, which was isolated in 1972 by zur Hausen & Gissmann (19) from a monkey lymphoblastoid cell line. A second MCV transcript from a genome region with low homology to other polyomaviruses was subsequently found in the sequenced libraries.

In the initial description of MCV, several important determinations were made. First, a panel of tissues was tested in a randomized and blind fashion for the presence of the viral genome (5). Of 10 MCC tumors, 8 tested positive for MCV, and 7 had high copy numbers that were later found to range between 1.6 and 48 virus copies per tumor cell (20). Of 84 non-MCC tissues, 9 (11%) tested positive for the viral genome; all were detected at the limits of polymerase chain reaction (PCR) sensitivity. Studies from other laboratories reported similar rates of MCV positivity in MCC tumors. A survey of 819 MCC patient tumors in 27 published studies from North America, Asia, and Europe, conducted through November 2010, revealed 634 tumors (77.4%) to be positive for MCV DNA (E. Mirvish, unpublished data).

Second, when the virus is present in tumors, the integration pattern is clonal in both primary tumors and metastases (5, 21), which provides evidence that the viral infection was present prior to the clonal expansion of the neoplastic cell. Southern blotting of Merkel cell tumors and cell lines is most consistent with the virus integrating as head-to-tail concatemers at a single insertion site in each particular tumor, but the insertion sites mapped from different MCCs appear to be distributed throughout the genome (5, 21–23).

The integration of the viral genome refutes the possibility that MCV is merely a coincidental, passenger infection in MCC, and it supports the contention that virus-associated tumors are biologic accidents (24). Although one study (25) reports the presence of viral particles by electron microscopy in two of five MCCs, robust antibodies developed against the late capsid protein viral protein 1 (VP1) universally show that tumor cells are negative (26, 27).

Following the description of MCV, three human polyomaviruses were discovered by use of rolling-circle amplification (RCA). This technique is based on random primer extension with a DNA-dependent polymerase that can displace an annealed DNA strand such as P29, preferentially amplifying circular DNA (28). Products of amplification are then cloned and sequenced. Using RCA, Schowalter et al. (29) in 2010 found human polyomavirus 6 (HPyV6) and HPyV7 as common skin flora. In the same study, the authors also routinely found MCV in skin samples of healthy donors, which accords with the hypothesis that MCV is part of the normal viral flora of the skin. Also in 2010, an eighth human polyomavirus was identified in virus-associated trichodysplasia spinulosum (VATS) (30). VATS is a rare, nonneoplastic disorder that manifests with hyperkeratotic spinous papules of the face and is observed exclusively in posttransplant and immunosuppressed individuals. On the basis of this association with immunosuppression and ultrastructural detection of polyomavirus-like particles (31), van der Meijden and colleagues (30) applied RCA to VATS spicules to find trichodysplasia spinulosum polyomavirus (TSaPyV). More recently, fluids and tissues from immunosuppressed patients were screened by consensus PCR, and a ninth human polyomavirus (HPyV9) was found in kidney secretions (32). Most of the newly discovered human polyomaviruses appear to be widespread but previously unrecognized human infections.

Of the nine human polyomaviruses, JCV and BKV are the most closely related to SV40. WUV and KIV form another group and are the most similar to each other, among the human polyomaviruses. HPyV6 and HPyV7 form yet another group and phylogenetically are the most closely related to MCV. HPyV9 is the most like African green monkey polyomavirus, and it branches with TSaPyV to cluster in the MPyV group (Figure 2). There appears to be

no inherent correlation between phylogenetic position and tumorigenic potential in the different human, as well as nonhuman, polyomaviruses. The discovery of seven new human polyomaviruses over the past four years shows that additional members of this family of human viruses are likely to be described, and such viruses may be etiologically linked to human disease.

Polyomavirus Genome Organization

The polyomaviruses share common genome features, as illustrated by the genome of MCV in Figure 3. All express overlapping transcripts from a single T antigen locus that comprises approximately half of the viral genome. These genes are expressed immediately after infection and thus are considered early genes. A critical function for these early genes is the initiation of viral DNA replication. Late structural proteins, including VP1, VP2, and VP3, are secondarily induced in an orderly cascade of VP expression. VP1 is the principal component of polyomavirus capsomers, and it assembles with VP2 and VP3 to form the viral capsid structure.

All the early T antigen proteins share a common exon 1 sequence and then diverge as a result of differential splicing. Two T antigen isoforms have been the focus of cancer studies: a large T antigen (LT) and a small T antigen (sT). Middle T antigen, present in some polyomaviruses but not in MCV, led to the elucidation of the importance of phosphatidylinositol 3-kinase activation for transformation (33). Other multiply-spliced T antigen isoforms, such as 17-kT antigen in SV40, have also been described, but their biological functions remain obscure. SV40 and several other polyomaviruses also encode a small agnoprotein that is important for replication (34) but is not found in the MCV genome.

On the basis of SV40 studies, LT is generally thought of as the principal polyomavirus oncoprotein. Expression of SV40 LT fully transforms rodent fibroblasts into cancer cells, and this transforming capability has been attributed, in part, to LT's ability to inactivate retinoblastoma protein (pRB) and p53 tumor-suppressor family members (35). LT localizes to the nucleus and sequesters pRB through an LXCXE motif in its N terminus. Its interaction with p53 maps to a C-terminal site that is proximal to the LT DNA-replication origin-binding domain (OBD) and a helicase domain required for viral DNA replication. To initiate viral DNA replication, LT assembles as a double hexamer on the viral origin, and its helicase activity opens the viral DNA, allowing assembly of the cellular DNA-replication machinery (36).

The sT protein is expressed from the same start site as LT, but rather than splicing out the first intron, the sT message reads through the splice site to encode the rest of the sT protein. Thus, both LT and sT polyomavirus proteins encode a heat shock protein-binding (DnaJ) domain from their common N terminus (37, 38). sT also encodes a protein phosphatase 2A (PP2A) family interaction domain that is not present in LT (39). For SV40, sT does not transform cells when expressed alone but enhances LT-induced cell transformation (40). Both SV40 LT expression and sT expression are required, together with activated H-Ras and telomerase, to transform human fibroblasts by defined oncogene expression (41).

Genome Details of Merkel Cell Polyomavirus

Similar to other polyomaviruses, MCV has a 5,387-bp genome that is divided into early and late coding regions by a noncoding regulatory region (NCRR) (Figure 3). The MCV NCRR contains a 71-bp core sequence that constitutes the viral replication origin, which efficiently sustains T antigen-directed DNA replication (42). This minimum core origin encompasses two subdomains: an AT-rich tract that contributes to DNA melting and an LT-binding sequence that contains eight GAGGC pentanucleotide sequences. In addition to the

minimum core origin, NCRR contains regulatory elements and transcriptional promoters for early and late viral gene expression that are bidirectionally coordinated with viral DNA replication (43).

The MCV early region encodes an LT, an sT, and a 57 kT, all of which share a 78-amino acid (aa) N terminus (Figure 4). The LT is expressed from a transcript that splices after the first exon (78 aa; frame 1) into a C-terminal exon of 738 aa (frame 3). The 432-aa MCV 57-kT antigen (analogous to the SV40 17-kT antigen) derives from a multiply-spliced transcript with three exons (78 aa, 254 aa, and 100 aa). Its second intron deletes a 350-aa region of LT associated with viral replication functions, but 57 kT is otherwise co-C-terminal with the last 100 aa of LT. The MCV sT transcript reads through the initial LT splice site and translates a 186-aa protein. MCC-derived MCVs have tumor-associated missense mutations or deletions in the portion of the T antigen gene encoding LT. These mutations eliminate the C-terminal replication functions of this protein but do not interfere with N-terminal LT domains, including the LXCXE motif that targets cellular retinoblastoma and related pocket proteins, or with expression of the sT protein, which plays a more critical role in cell transformation than does SV40 sT.

The late region encoding VP1, VP2, and VP3 is expressed after the onset of viral DNA replication to self-assemble into ~55-nm-diameter viral particles, which demonstrate the icosahedral symmetry that is characteristic of polyomaviruses (44). MCV structural proteins are relatively dissimilar to those of BKV and JCV, so antibodies against MCV VP generally do not cross-react to these viruses (26).

MERKEL CELL CARCINOMA: PATHOLOGY

In his initial report of five cases, Toker (1) applied the name trabecular carcinoma to these primary cutaneous neoplasms because of the perceived pattern of growth of the tumor cells. Over the next decades, as ultrastructural analysis and immunohistochemical reagents were used to examine these tumors, a wider spectrum of pathologic features were described. Despite the apparent phenotypic complexity of MCC, a more clearly defined profile of MCC has evolved with the identification of MCV.

Morphologic Features of Merkel Cell Carcinoma

MCCs are typically located in the dermis, where the tumor cells form sheets, nests, or less commonly, trabecular and anastomosing aggregates. Invasion of the subcutis is common, whereas extension into overlying epidermis is unusual. Morphologically, MCC tumor cells have round to oval nuclei that have been variably described as vesicular or finely granular. Cells have small nucleoli; significant mitotic activity; and scant, syncytial-appearing cytoplasm. Intratumoral necrosis and inflammatory infiltration vary from case to case. These cytologic features alone, however, are not sufficiently distinctive to permit differentiation of MCC from other primary or metastatic small, round, blue cell tumors that may occur in the skin; such tumors include lymphoma, small cell carcinoma, the Ewing family of tumors, sarcomas with round cell features including rhabdomyosarcoma, and other noncutaneous neuroendocrine carcinomas (see Reference 45 for a review).

Diagnostic Considerations

Electron microscopy played a key early role in defining several unique features of MCC. Most tumor cells were observed to contain membrane-bound, dense-core structures in the cytoplasm that were reminiscent of neurosecretory-type granules (46, 47). The identification of these granules by electron microscopy prompted investigation of MCC through the use of enzymatic and immunohistochemical assays, which confirmed that such tumors can express chromogranin, synaptophysin, and other neuropeptides. Neoplasia arising from normal cells

that contain these granules include various neuroendocrine carcinomas, such as carcinoids, paragangliomas, pheochromocytomas, and pulmonary small cell carcinomas. Whereas the last group of tumors may be associated with paraneoplastic syndromes secondary to physiologically active neuropeptides, MCCs are not known to manifest these symptoms. Nevertheless, because of their ability to elaborate neuropeptides, MCCs are also known as primary cutaneous neuroendocrine carcinomas.

Another consistently recognized ultrastructural feature of MCC tumor cells is the presence of so-called fibrous bodies composed of intermediate filaments localized in a perinuclear distribution (47). As shown by immunohistochemical staining, these unusual structures correspond to skeins of cytokeratin filaments of low molecular weight. It is unknown why this unique arrangement of cytokeratin occurs in Merkel cells, although oligomerization is important for the diffuse cytoplasmic distribution and function of cytokeratin filaments. Such perinuclear fibrous bodies correspond immunohistochemically to low-molecular-weight keratins that are localized in a perinuclear, dot-like pattern and are recognized by several antibodies: AE1/AE3, Cam 5.2, and cytokeratin 20 (CK20) (Figure 5). CK20 positivity, which is considered the most discriminating feature, along with several exclusionary markers, including thyroid transcription factor 1 and leukocyte common antigen, constitutes the primary diagnostic panel for MCCs. Frequently, chromogranin and synaptophysin are sought as confirmatory support for diagnosis, and proliferation markers are used to guide clinical prognosis (see Reference 48 for a review). Expression of both neuroendocrine and epithelial markers, phenotypes confirmed both ultrastructurally and immunohistochemically, has complicated the assignment of histogenesis for MCC.

Immunohistochemical staining for MCV proteins has expanded the list of clinical diagnostic tools used to identify MCCs. MCV-related and MCV-unrelated MCC tumors may have different pathoetiologies and prognoses (49). Viral antigens are readily detected in most MCC tumors with high copy number integrants (20, 50, 51) and are useful for distinguishing MCV-positive MCCs from other tumors (52, 53). MCV antigen expression is restricted to tumor cells in these tumors without requiring staining of intermixed healthy tissues. Antibodies to MCV LT in MCC lesions show robust, diffuse nuclear reactivity in tumor cells (Figure 6*a,b*). Nevertheless, a minority of MCV DNA-positive MCC tumors score negative for LT expression (20, 54). Although this finding may arise from the increased sensitivity of PCR-based DNA detection, compared with immunohistochemical detection of protein in fixed tissues, some tumors express MCV sT in the absence of MCV LT positivity (Figure 6*c,d*). This finding provides evidence that MCV sT may play a critical role in MCV-induced tumorigenesis (55).

As a direct result of the detection of MCV in some but not all MCC tumors, a finer distinction of different morphologies and genetic changes in MCC is now possible. For example, Kuwamoto et al. (56) used multiobserver morphometric analyses to determine that MCV-positive MCC tumor cells have relatively regular, round nuclei and relatively little cytoplasm. In contrast, MCV-negative MCC tumor cells are associated with more irregular nuclei and abundant cytoplasm. Similarly, by using high-definition comparative genomic hybridization array, Paulson et al. (57) found that MCV-positive MCC tumors have fewer genomic deletions.

Pathologic Variants of Merkel Cell Carcinoma

The vast majority of MCCs develop as histologically pure lesions; however, there are cases of primary cutaneous neuroendocrine carcinomas with a mixed phenotype that reveals concurrent involvement with neoplasms of ectodermal and, rarely, mesodermal derivation. Whether these represent collision tumors versus combination tumors is difficult to resolve. In any event, neither MCV DNA nor its encoded proteins have ever been found to be present

in the neuroendocrine carcinoma portions of these variants (25, 50). Further, neuroendocrine carcinomas from various noncutaneous sites, including pulmonary, cervical, and mucosal head and neck neuroendocrine carcinomas, are uniformly negative for MCV (53, 58).

MERKEL CELL POLYOMAVIRUS: MOLECULAR BIOLOGY AND PATHOLOGY

Large T Antigen Mutations and Tumorigenesis

Presently, little is known about the natural biology of MCV. What is the human host cell for this virus? Can this virus infect cells of varied histogenesis? How does the virus get into Merkel cells, and is this cell type non-permissive for viral replication? Initial studies on MCV have understandably concentrated on transforming features in MCC tumors that harbor high copy numbers of the viral genome. Nearly all MCV genomes isolated from tumors have mutations that truncate the C terminus of the LT protein such that this protein's natural ability to activate MCV DNA replication is lost (59). These mutations always occur distal to the pRB-binding site and disrupt the OBD or the helicase domain, but they are otherwise randomly distributed. Thus, LT proteins expressed in different tumors vary in size, depending on the site of their mutations (20). There is strong selective pressure within tumors to eliminate viral replication capacity, and in addition to LT truncation, mutations in VP1 and the viral origin that prevent the formation of active virus have been reported (42, 60).

Loss of replication capacity is a common feature of tumor viruses, including some papillomavirus-induced cancers. Tumor selection of MCV LT mutations is likely to arise from active viral replication that causes cell lysis and death (24). In the case of MCV, the expression of full-length LT protein, once the virus has integrated, leads to unlicensed DNA replication from the newly integrated site, thereby causing replication-fork collisions and DNA-strand breakage (59). For the tumor cell to survive once MCV has integrated, it appears that a second mutation to eliminate LT-initiated DNA replication is needed. Because virus integration and T antigen mutation are uncommon and independent events, the requirement for both events may explain why MCC is rare even though MCV infection is common (Figure 7). In addition to inhibiting viral DNA replication, another explanation for such tumor-derived mutations is that the C terminus of LT may encode a potent, cytotoxic T lymphocyte-recognized epitope. This speculation is reasonable, given that MCC is highly susceptible to immune status, and studies suggest that levels of infiltrating T cells are directly associated with favorable prognosis (61).

For SV40, expression of LT alone leads to the in vitro transformation of susceptible cell lines. Neither wild-type nor tumor-derived MCV LT proteins, in contrast, initiate cellular transformation, as measured by soft agar colony formation, focus formation, or cell growth in low serum (55). However, this finding does not imply that MCV LT does not play a role in MCV tumorigenesis. MCV LT has protein motifs in its N terminus similar to those found in SV40 LT. Its conserved LXCXE domain mediates MCV LT interactions with pRB family members, and mutation of this domain abolishes pRB binding (59). Likewise, the DnaJ domain of MCV LT can bind to Hsc70, as has been observed with SV40 LT (42). All the MCV tumor mutations found so far spare these domains, which suggests that they contribute to MCC tumorigenesis. Consistent with this hypothesis, Houben et al. (62) found that in vitro survival of tumor cells from established MCC-derived cell lines depends on a functional pRB interaction domain.

Using tandem-affinity purification, Liu et al. (63) found that MCV LT also interacts with a novel component of the cellular-vacuolar sorting protein machinery termed Vam6p or VPS39. This interaction is unique to MCV and does not occur in SV40. It is also entirely

unexpected because MCV LT is a nuclear protein and binding to Vam6p causes the cellular protein to relocate to the nucleus. The site of Vam6p interaction has been identified as a domain immediately adjacent to the pRB-binding site on LT; thus, it is spared from tumor-derived LT mutations. Although the consequences of Vam6p sequestration by LT are unknown, it appears that Vam6p can regulate MCV replication. An engineered MCV molecular clone with a mutated LT Vam6p-binding site shows increased replication in 293 cells compared with a wild-type MCV molecular clone (43). It is unknown whether MCV LT binding to Vam6p contributes to MCC tumorigenesis.

Small T Antigen and Tumorigenesis

The MCV sT and LT share the N-terminal region that includes the CR1 and DnaJ domains. The unique MCV sT C terminus interacts with the cellular PP2A protein, as occurs in other polyomaviruses (42, 64). Evidence suggests, however, that there are subtle differences between the activities of MCV and SV40 sT proteins that may contribute to major features of MCV-driven MCC.

Unlike SV40, MCV sT is sufficient to fully transform rodent fibroblasts into independently growing cancer cell lines (55), which is not enhanced by coexpression of either wild-type or tumor-derived forms of MCV LT. Knockdown of MCV sT expression in MCC cell lines causes cells to stop proliferating but does not cause MCC cell death. Mutations to the MCV sT PP2A interaction domain do not have major effects on MCV sT as an oncoprotein, which suggests that PP2AA targeting is not this protein's primary function during cell transformation.

Surveys of signaling pathways targeted by MCV sT also reveal unique differences that are not found in SV40 sT protein. MCV sT activates cap-dependent translation by maintaining hyperphosphorylation of the 4E-BP1 regulatory protein. 4E-BP1 is a downstream target of the Akt-mTOR (mammalian target of rapamycin) signaling pathway: When 4E-BP1 is hyperphosphorylated by mTOR, it releases the protein translation-initiation factor eIF4E, thereby allowing eIF4E to bind to 7-methyl guanosine-capped messenger RNA and to recruit the ribosomal machinery required for translation. MCV sT promotes hyperphosphorylation of 4E-BP1; this dysregulated cap-dependent translation appears to enhance cell transformation. Even though no cooperation has been observed in rodent cell transformation assays between MCV sT and LT proteins, there is strong evidence to suggest that this process does occur. Expression of MCV sT does not rescue MCC cells in which the T antigen locus has been repressed, indicating that other T antigen isoforms also play a role in MCC carcinogenesis.

EPIDEMIOLOGY OF MERKEL CELL CARCINOMAS AND MERKEL CELL POLYOMAVIRUS

Incidence and Trends

Evidence of the incidence and mortality of MCC has been established only over the past few decades as MCC has gained recognition as a distinct entity. Most early studies comprised large case series rather than population-based analyses, and pathologic definition of MCC was often variable and incomplete. Increased awareness of MCC over time has probably contributed in part to the apparent increases in MCC incidence.

With these caveats in mind, early Surveillance, Epidemiology, and End Results (SEER) data collected in the United States between 1984 and 1996 showed an annual age-adjusted incidence of 2.3 cases per million among whites and only 0.1 cases per million among blacks (65). Melanoma was diagnosed in this analysis 65 times as often as MCC, but both

skin cancers showed a correlation between UVB exposure at residence of diagnosis and cancer incidence. Unlike melanoma, whose risk increases at a relatively constant rate with age, MCC incidence sharply increases in elderly populations, and only 5% of the MCC patients in this survey were diagnosed before age 50. Cases among immunosuppressed transplant patients were also noted; taken together, the epidemiology of MCC largely reflects the mnemonic A-E-I-O-U [asymptomatic, expanding rapidly, immune suppression, older than 50, and UV exposure (66)] for clinical diagnosis of MCC. Overall SEER incidence for MCC tripled from 1.5 cases per million in 1986 to 4.4 cases per million in 2001 (4), and long-term survival among these patients was highly dependent on the extent of tumor dissemination; localized MCC cases had a five-year survival rate of 75%, compared with 25% for persons with disseminated disease (3).

Only a few population-based studies of this cancer have been performed outside the United States, mainly in relatively homogeneous European populations such as Finland (67), Denmark (68), and the Netherlands (69). These studies revealed rates and patterns for MCC similar to those observed in the United States. In particular, there also has been a general increase in MCC diagnosis over time in these populations. MCC is a rare (approximately one to four cases per million per year) aggressive neoplasm with high mortality, although the rates of initial dissemination differ among the registries. Whereas racial differences in MCC risk are evident in most studies (presumably due to differences in skin pigmentation), isolated reports have described MCC in populations throughout the world.

Association of Merkel Cell Carcinoma with Immunosuppression

Immunosuppression was recognized as a risk factor for MCC in early case reports on transplantation (70, 71) and AIDS patients (72). Engels et al. (2) compared AIDS and cancer registries to identify immune-related cancers and found that AIDS patients have a 13-fold-increased age- and sex-adjusted risk for developing MCC compared with the general population. Together with the increased risk among the elderly, which may reflect an age-related decline in cell immune surveillance, these clinical findings are consistent with MCC having an infectious etiology. Kaposi's sarcoma is an analogous tumor that afflicts the elderly and immunosuppressed (73).

Prevalence of Merkel Cell Polyomavirus Infection in Populations

Early studies identified low copy numbers of the MCV genome in various tissues from healthy persons and from non-MCC patients. Although most MCC tumors have high copy numbers of the viral genome, MCV DNA is found at trace levels in other tissues, particularly skin, which is consistent with MCV being part of the healthy human flora (29). Serologic studies have confirmed this finding and have revealed that MCV is a common if not ubiquitous human infection. Polyomavirus serology has been based largely on antibodies reacting to the late structural proteins VP1 and, to a lesser extent, VP2. For viruses that are closely related to SV40, such as BKV and JCV, there can be extensive cross-reactivity among viral structural antigens that is best distinguished by competition assays in which each viral antigen is used to preadsorb sera prior to testing. Serologic cross-reactivity to BKV and JCV is likely to explain reports of widespread human infection with the SV40 rhesus macaque virus (9, 74, 75).

Serum reactivity to isolated MCV VP1 was first reported by Kean et al. (76), who found 25–42% prevalence in MCV antibody positivity. Carter et al. (77) used an improved multiplex antibody-binding assay to detect levels of MCV VP1 in sera and found a prevalence of 59% in control subjects from the general population. Peptide mapping of VP1, however, reveals that conformational epitopes present in assembled viral particles provide higher sensitivity and specificity for measuring past MCV exposure. Expression of MCV VP1 and VP2 in

cells generates self-assembling virus-like particles (VLPs) that can be used as antigens for immunoassays. Using this approach, Tolstov and colleagues (44) found an age-related increase in MCV seroprevalence to age 50, at which 80% of healthy North American blood donors showed evidence for past MCV exposure. Using MCV VLPs, Touze et al. (78) reported similar results. Antibody responses to MCV VLPs are not diminished by cross-competition with other polyomavirus antigens, which indicates that they are highly specific for MCV infection. When a reporter gene is incorporated into the VLPs, the pseudovirions can be used to infect susceptible cells, thereby allowing measurement of neutralizing antibodies that may be even more sensitive and specific than the enzyme immunoassay based on VLP (26).

Thus, similar to other human polyomaviruses, MCV appears to be a common infection of childhood, and primary infection may be asymptomatic. The rate of seroprevalence in children younger than 4 years of age is 9%, which increases to 35% by 4–13 years of age (79). In a longitudinal study of healthy adult gay and bisexual men, aged 18 years and up, who were recruited and followed for signs and symptoms related to HIV and AIDS, 79% had antibodies to MCV at entry into the study, and subsequently 31 (5.5%) of the men seroconverted to MCV positivity (80). No apparent signs, symptoms, or common laboratory test results were significantly associated with primary MCV infection among these men.

Prognostic Value of Merkel Cell Polyomavirus Detection

MCV status in MCC may have a clinical prognostic value. In a survey of Finnish MCC patients, Sihto et al. (67) found significantly reduced disease-specific mortality for patients with MCV-positive MCC compared with patients with MCV-negative MCC. A French study (21) revealed similar results for tumors with one or more viral copies per tumor cell but concluded that MCC patients with detectable MCV virus in peripheral blood cells fared worse than MCC patients without virus shedding.

These and other studies are complicated by the ready detection of viral DNA by PCR in nontumor tissues. Although MCV is abundant in most MCC tumors, it is evident that the virus can be found as normal flora at various body sites (5, 20, 81–87). MCV immunohistochemistry, a less sensitive but more robust technique than PCR, has confirmed that MCV-positive tumors are associated with better prognosis in two studies (57, 88) but not in a third (89). Tests for serum antibodies to MCV T antigen show that elevated MCV T antigen antibodies are also a risk for disease progression (90). If MCV-positive tumors are less malignant or more amenable to therapy, these findings may be due in part to cellular immune responses to MCV antigens. Disease progression may be marked by loss of controlling immune surveillance, which can lead to detectable viremia and increased antibodies against viral early antigens (26, 44, 91).

Association of Merkel Cell Polyomavirus with Other Cancers

Common human infection with MCV complicates the establishment of a significant relationship between MCV infection and other cancers. Population registries have repeatedly revealed significant associations between MCC and other cancers as either primary or secondary neoplasms. Chronic lymphocytic leukemia (CLL), salivary gland cancers, non-Hodgkin's lymphomas, and various non-MCC skin cancers, for example, are elevated either prior or subsequent to MCC (68, 92, 93). The relationship between MCC and CLL is particularly well established and suggests the possibility of a common etiology.

Non-MCC skin tumors have been examined for MCV infection by PCR and immunoblotting because of their frequent cooccurrence with MCC (94). In general, MCV is not commonly found in melanomas, basal cell carcinomas, and squamous cell carcinomas (83 95–97),

although one group (98) has reported detection of the viral genome in these tumors more frequently than expected. To resolve this issue, we examined a panel of basal cell and squamous cell carcinomas in patients with previous or subsequent MCC. None of the non-MCC skin tumors were positive for MCV T antigen expression, which suggests that MCV does not directly contribute to these tumors but, instead, that these tumors may share risk factors such as excessive sun exposure (99).

Varying reports exist on the presence of MCV genome detection in CLL tumors as well. MCV has been reported to have a lymphotropism (20, 100), and quantitative PCR has detected MCV in lymphocyte populations from MCC patients (20, 21). Patients with MCV-positive MCC tumors have an elevated risk for CLL compared with patients with MCV-negative MCC tumors (101), but direct examination of CLL tumors does not reveal a pattern of MCV genome positivity (20, 52). In one study, MCV genome and protein expression was identified from a subset of CLL tumors, and importantly, recovered viral genome was defective, which is reminiscent of MCV infection in MCC (102). In contrast, a study (103) of two patients with concurrent MCC and CLL demonstrated that MCV protein is expressed only in the MCC component and not in the CLL component (Figure 8). Comparison between MCV antibody positivity in CLL patients and in a leukemia control group also did not reveal differences in the rates of MCV prevalence (103).

The repeated demonstration that MCC and CLL are linked together in cancer patient surveys remains an important and interesting correlation. Although most CLL tumors are not positive for MCV, the virus may contribute to a significant fraction of CLL cases. Data from initial studies on this topic are contradictory and will require extended studies using the rapidly improving technologies that can detect MCV infection. If the virus does not directly contribute to CLL, then it is likely that other common risk factors or CLL-related immunosuppression plays a role in this correlation.

Merkel Cell Polyomavirus and Merkel Cell Carcinoma: Causality

Determining whether a virus causes cancer is fraught with contention and is highly dependent on the criteria used to determine proof (104, 105). The best-known criteria, Koch's postulates (106), are a nineteenth-century construct that do not take into consideration modern concepts of asymptomatic carriage, species specificity of infection, cell-dependent viral replication, and endogenous retroviruses. Although Koch's postulates are frequently cited by nonepidemiologists, they are not considered useful for most human viral pathogens, and some investigators have attempted to modernize these concepts (107).

In the 1960s, A.B. Hill (108) promoted a list of criteria to establish causality between a factor and human disease that rely mainly on epidemiologic correlations. Hill's criteria have been used successfully for viruses such as Kaposi's sarcoma-associated herpesvirus (109), but they are tractable mainly for pathogens, unlike MCV, in which infection prevalence is low. Clonal integration of MCV within tumors is evidence for MCV infection of the individual preneoplastic cells prior to tumorigenesis; this finding fulfills Hill's temporal-relationship requirement, which is the most important of the epidemiologic criteria (5).

More than 40 published studies from around the world confirm the strong relationship between a subset of MCCs and MCV. Serologic studies suggest that, like Burkitt's lymphoma-associated Epstein-Barr virus infection, MCV is a common childhood infection that only rarely leads to cancer. Other factors and events are required for an MCV infection to evolve into a cancer. Hill's criteria, developed to address the association between cigarette smoking and lung cancer, are useful in designing natural history studies to study these questions, but critics have raised doubts about their universal use in determining whether particular infectious agents cause specific cancers (24). More probabilistic Bayesian

approaches may ultimately be needed to establish whether common viral infections contribute to cancers.

The strongest direct evidence for MCV's role in MCC is experimental. Fully transformed and tumorigenic MCV-positive and MCV-negative MCC lines have been established (5, 110). If the viral T antigen locus is knocked down with short-hairpin, small interfering RNA vectors, MCV-positive cells arrest and initiate nonapoptotic cell death (23). MCV-negative cell lines are unaffected by MCV T antigen knockdown, as expected given that the virus is not present. Thus, the virus directly contributes to signaling events that maintain the tumor phenotype in MCV-positive cells, and it appears to be necessary for the tumor. Although no tumors other than MCC are consistently infected at high copy numbers, only a small fraction of tumor types has been examined. It is possible that additional tumors that have not yet been examined harbor MCV infection.

As with all other human cancer viruses, MCV infection alone is not sufficient for tumors to develop. MCC tumors require specific mutations (both T antigen truncation and genomic integration) and possibly immunosuppression or, at least, loss of specific surveillance for MCV epitopes and additional cellular changes or mutations that allow outgrowth of the tumor. In short, the evidence that MCV contributes to most MCCs is overwhelming, but additional risk factors and changes to the virus must occur before clinically apparent MCC will emerge.

FUTURE PERSPECTIVES

We are entering a phase of MCV research in which causality for MCC is widely accepted, but the mechanistic details of MCC tumorigenesis remain unexplored. As with other human tumor viruses, identifying the cellular targets of MCV will undoubtedly lead to new fundamental insights into cancers other than those caused by MCV. Thus, an uncommon and previously obscure cancer may contribute to profound discoveries applicable to more common noninfectious cancers.

MCC, like other virus-associated cancers, is highly immunologically responsive due to the presence of foreign viral antigens in tumor cells. An unusual quality of MCCs concerns the spontaneous regression of disseminated tumors (e.g., Reference 111), possibly due to improved immune surveillance. The viral epitopes recognized by immune surveillance pathways have not been defined, but this line of investigation holds promise for more effective and targeted immunity-based therapies for this cancer. MCV and other viral cancers are the most promising candidates for therapeutic vaccines, and the lessons learned from these cancers could be applied to noninfectious cancers as well.

A precise understanding of the signaling pathways targeted by MCV also points toward new approaches to treating this cancer. The surprising finding that MCV sT rather than LT initiates in vitro cell transformation suggests the importance of cap-dependent translation dysregulation to MCV-positive MCC. New small-molecule inhibitors of cap-dependent translation and mTOR signaling have potential for specific molecular therapies for this previously poorly responsive cancer. The search for additional cellular mutations that act in concert with MCV to induce MCC may provide novel signaling insights that can be extended to other cancers.

The identification of MCV has already provided a more precise molecular pathology diagnosis for MCC, and rapid scientific progress that promises to improve the diagnosis of and clinical care for MCV is under way. Although critics of the Human Genome Project have recently suggested that few clinical benefits have emerged from this project, this

statement is clearly contradicted by the discovery of MCV, which would not have been possible without the availability of the human genomic sequence.

Acknowledgments

The authors are supported by National Institutes of Health grants CA136363 and CA120726 and by American Cancer Society research professorships.

LITERATURE CITED

1. Toker C. Trabecular carcinoma of the skin. *Arch Dermatol.* 1972; 105:107–10. [PubMed: 5009611]
2. Engels EA, Frisch M, Goedert JJ, Biggar RJ, Miller RW. Merkel cell carcinoma and HIV infection. *Lancet.* 2002; 359:497–98. [PubMed: 11853800]
3. Agelli M, Clegg LX. Epidemiology of primary Merkel cell carcinoma in the United States. *J Am Acad Dermatol.* 2003; 49:832–41. [PubMed: 14576661]
4. Hodgson NC. Merkel cell carcinoma: changing incidence trends. *J Surg Oncol.* 2005; 89:1–4. [PubMed: 15611998]
5. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science.* 2008; 319:1096–100. [PubMed: 18202256]
6. Gross L. Transmission of Ak leukemic agent into newborn mice of the C57 brown/cd inbred line. *Proc Soc Exp Biol Med.* 1954; 86:734–39. [PubMed: 13204339]
7. Sweet BH, Hilleman MR. The vacuolating virus, SV 40. *Proc Soc Exp Biol Med.* 1960; 105:420–27. [PubMed: 13774265]
8. Engels EA. Cancer risk associated with receipt of vaccines contaminated with simian virus 40: epidemiologic research. *Expert Rev Vaccines.* 2005; 4:197–206. [PubMed: 15889993]
9. Poulin DL, DeCaprio JA. Is there a role for SV40 in human cancer? *J Clin Oncol.* 2006; 24:4356–65. [PubMed: 16963733]
10. Lane DP, Crawford LV. T antigen is bound to a host protein in SV40-transformed cells. *Nature.* 1979; 278:261–63. [PubMed: 218111]
11. Linzer DI, Levine AJ. Characterization of a 54 kD cellular SV40 tumor antigen present in SV40-transformed cells and uninfected embryonal carcinoma cells. *Cell.* 1979; 17:43–52. [PubMed: 222475]
12. Lanford RE, Butel JS. Construction and characterization of an SV40 mutant defective in nuclear transport of T antigen. *Cell.* 1984; 37:801–13. [PubMed: 6086146]
13. Waga S, Stillman B. Anatomy of a DNA replication fork revealed by reconstitution of SV40 DNA replication in vitro. *Nature.* 1994; 369:207–12. [PubMed: 7910375]
14. Li JJ, Kelly TJ. Simian virus 40 DNA replication in vitro. *Proc Natl Acad Sci USA.* 1984; 81:6973–77. [PubMed: 6095264]
15. Gardner SD, Field AM, Coleman DV, Hulme B. New human papovavirus (BK) isolated from urine after renal transplantation. *Lancet.* 1971; 1:1253–57. [PubMed: 4104714]
16. Padgett BL, Walker DL, Zur Rhein GM, Eckroade RJ, Dessel BH. Cultivation of papova-like virus from human brain with progressive multifocal leucoencephalopathy. *Lancet.* 1971; 1:1257–60. [PubMed: 4104715]
17. Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, et al. Identification of a third human polyomavirus. *J Virol.* 2007; 81:4130–36. [PubMed: 17287263]
18. Gaynor AM, Nissen MD, Whitley DM, Mackay IM, Lambert SB, et al. Identification of a novel polyomavirus from patients with acute respiratory tract infections. *PLoS Pathog.* 2007; 3:e64. [PubMed: 17480120]
19. zur Hausen H, Gissmann L. Lymphotropic papovaviruses isolated from African green monkey and human cells. *Med Microbiol Immunol.* 1979; 167:137–53. [PubMed: 226854]
20. Shuda M, Arora R, Kwun HJ, Feng H, Sarid R, et al. Human Merkel cell polyomavirus infection. I. MCV T antigen expression in Merkel cell carcinoma, lymphoid tissues and lymphoid tumors. *Int J Cancer.* 2009; 125:1243–49. [PubMed: 19499546]

21. Laude HC, Jonchere B, Maubec E, Carlotti A, Marinho E, et al. Distinct merkel cell polyomavirus molecular features in tumour and non tumour specimens from patients with merkel cell carcinoma. *PLoS Pathog.* 2010; 6:8.
22. Sastre-Garau X, Peter M, Avril MF, Laude H, Couturier J, et al. Merkel cell carcinoma of the skin: pathological and molecular evidence for a causative role of MCV in oncogenesis. *J Pathol.* 2009; 218:48–56. [PubMed: 19291712]
23. Houben R, Shuda M, Weinkam R, Schrama D, Feng H, et al. Merkel cell polyomavirus–infected Merkel cell carcinoma cells require expression of viral T antigens. *J Virol.* 2010; 84:7064–72. [PubMed: 20444890]
24. Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat Rev Cancer.* 2010; 10:878–89. [PubMed: 21102637]
25. Wetzels CT, Hoefnagel JG, Bakkers JM, Dijkman HB, Blokx WA, Melchers WJ. Ultrastructural proof of polyomavirus in Merkel cell carcinoma tumour cells and its absence in small cell carcinoma of the lung. *PLoS ONE.* 2009; 4:e4958. [PubMed: 19305499]
26. Pastrana DV, Tolstov YL, Becker JC, Moore PS, Chang Y, Buck CB. Quantitation of human seroresponsiveness to Merkel cell polyomavirus. *PLoS Pathog.* 2009; 5:e1000578. [PubMed: 19750217]
27. Pastrana DV, Pumphrey KA, Cuburu N, Schowalter RM, Buck CB. Characterization of monoclonal antibodies specific for the Merkel cell polyomavirus capsid. *Virology.* 405:20–25. [PubMed: 20598728]
28. Johne R, Muller H, Rector A, van Ranst M, Stevens H. Rolling-circle amplification of viral DNA genomes using phi29 polymerase. *Trends Microbiol.* 2009; 17:205–11. [PubMed: 19375325]
29. Schowalter RM, Pastrana DV, Pumphrey KA, Moyer AL, Buck CB. Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. *Cell Host Microbe.* 2010; 7:509–15. [PubMed: 20542254]
30. van der Meijden E, Janssens RW, Lauber C, Bouwes Bavinck JN, Gorbalenya AE, Feltkamp MC. Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an immunocompromised patient. *PLoS Pathog.* 2010; 6:e1001024. [PubMed: 20686659]
31. Wyatt AJ, Sachs DL, Shia J, Delgado R, Busam KJ. Virus-associated trichodysplasia spinulosa. *Am J Surg Pathol.* 2005; 29:241–46. [PubMed: 15644782]
32. Scuda N, Hofmann J, Calvignac-Spencer S, Ruprecht K, Liman P, et al. A novel human polyomavirus closely related to the African green monkey–derived lymphotropic polyomavirus (LPV). *J Virol.* 2011; 85:4586–90. [PubMed: 21307194]
33. Kaplan DR, Whitman M, Schaffhausen B, Pallas DC, White M, et al. Common elements in growth factor stimulation and oncogenic transformation: 85 kd phosphoprotein and phosphatidylinositol kinase activity. *Cell.* 1987; 50:1021–29. [PubMed: 2441878]
34. Cantalupo P, Doering A, Sullivan CS, Pal A, Peden KW, et al. Complete nucleotide sequence of polyomavirus SA12. *J Virol.* 2005; 79:13094–104. [PubMed: 16189011]
35. Pipas JM. Common and unique features of T antigens encoded by the polyomavirus group. *J Virol.* 1992; 66:3979–85. [PubMed: 1318392]
36. Wright CM, Seguin SP, Fewell SW, Zhang H, Ishwad C, et al. Inhibition of Simian virus 40 replication by targeting the molecular chaperone function and ATPase activity of T antigen. *Virus Res.* 2009; 141:71–80. [PubMed: 19200446]
37. Kelley WL, Georgopoulos C. The T/t common exon of simian virus 40, JC, and BK polyomavirus T antigens can functionally replace the J-domain of the *Escherichia coli* DnaJ molecular chaperone. *Proc Natl Acad Sci USA.* 1997; 94:3679–84. [PubMed: 9108037]
38. Campbell KS, Mullane KP, Aksoy IA, Stubdal H, Zalvide J, et al. DnaJ/hsp40 chaperone domain of SV40 large T antigen promotes efficient viral DNA replication. *Genes Dev.* 1997; 11:1098–110. [PubMed: 9159391]
39. Pallas DC, Cherington V, Morgan W, DeAnda J, Kaplan D, et al. Cellular proteins that associate with the middle and small T antigens of polyomavirus. *J Virol.* 1988; 62:3934–40. [PubMed: 2845116]

40. Noda T, Satake M, Yamaguchi Y, Ito Y. Cooperation of middle and small T antigens of polyomavirus in transformation of established fibroblast and epithelial-like cell lines. *J Virol.* 1987; 61:2253–63. [PubMed: 3035224]
41. Hahn WC, Counter CM, Lundberg AS, Beijersbergen RL, Brooks MW, Weinberg RA. Creation of human tumour cells with defined genetic elements. *Nature.* 1999; 400:464–68. [PubMed: 10440377]
42. Kwun HJ, Guastafierro A, Shuda M, Meinke G, Bohm A, et al. 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.* 2009; 83:12118–28. [PubMed: 19759150]
43. Feng H, Kwun HJ, Liu X, Gjoerup O, Stolz DB, et al. Cellular and viral factors regulating Merkel cell polyomavirus replication. *PLoS ONE.* 2011 In press.
44. Tolstov YL, Pastrana DV, Feng H, Becker JC, Jenkins FJ, et al. Human Merkel cell polyomavirus infection. II. MCV is a common human infection that can be detected by conformational capsid epitope immunoassays. *Int J Cancer.* 2009; 125:1250–56. [PubMed: 19499548]
45. Calder KB, Smoller BR. New insights into merkel cell carcinoma. *Adv Anat Pathol.* 17:155–61. [PubMed: 20418670]
46. Tang CK, Toker C. Trabecular carcinoma of the skin: an ultrastructural study. *Cancer.* 1978; 42:2311–21. [PubMed: 719609]
47. Sibley RK, Dehner LP, Rosai J. Primary neuroendocrine (Merkel cell?) carcinoma of the skin. I. A clinicopathologic and ultrastructural study of 43 cases. *Am J Surg Pathol.* 1985; 9:95–108. [PubMed: 2579594]
48. Rao P, Balzer BL, Lemos BD, Liegeois NJ, McNiff JM, et al. Protocol for the examination of specimens from patients with merkel cell carcinoma of the skin. *Arch Pathol Lab Med.* 2010; 134:341–44. [PubMed: 20196661]
49. Duncavage EJ, Le BM, Wang D, Pfeifer JD. Merkel cell polyomavirus: a specific marker for Merkel cell carcinoma in histologically similar tumors. *Am J Surg Pathol.* 2009; 33:1771–77. [PubMed: 19809278]
50. Busam KJ, Jungbluth AA, Rekhman N, Coit D, Pulitzer M, et al. Merkel cell polyomavirus expression in Merkel cell carcinomas and its absence in combined tumors and pulmonary neuroendocrine carcinomas. *Am J Surg Pathol.* 2009; 33:1378–85. [PubMed: 19609205]
51. Bhatia K, Goedert JJ, Modali R, Preiss L, Ayers LW. Merkel cell carcinoma subgroups by Merkel cell polyomavirus DNA relative abundance and oncogene expression. *Int J Cancer.* 2010; 126:2240–46. [PubMed: 19551862]
52. Teman CJ, Tripp SR, Perkins SL, Duncavage EJ. Merkel cell polyomavirus (MCPyV) in chronic lymphocytic leukemia/small lymphocytic lymphoma. *Leuk Res.* 2011; 35:689–92. [PubMed: 21414663]
53. McCluggage WG, Kennedy K, Busam KJ. An immunohistochemical study of cervical neuroendocrine carcinomas: neoplasms that are commonly TTF1 positive and which may express CK20 and P63. *Am J Surg Pathol.* 2010; 34:525–32. [PubMed: 20182342]
54. Bhatia K, Goedert JJ, Modali R, Preiss L, Ayers LW. Immunological detection of viral large T antigen identifies a subset of Merkel cell carcinoma tumors with higher viral abundance and better clinical outcome. *Int J Cancer.* 2010; 127:1493–96. [PubMed: 20041469]
55. Shuda M, Kwun HJ, Feng H, Chang Y, Moore PS. Human Merkel cell polyoma small T antigen is an oncoprotein targeting the 4E-BP1 translation regulator. *J Clin Invest.* 2011 In press.
56. Kuwamoto S, Higaki H, Kanai K, Iwasaki T, Sano H, et al. Association of Merkel cell polyomavirus infection with morphologic differences in Merkel cell carcinoma. *Hum Pathol.* 2011; 42:632–40. [PubMed: 21277612]
57. Paulson KG, Lemos BD, Feng B, Jaimes N, Penas PF, et al. Array-CGH reveals recurrent genomic changes in Merkel cell carcinoma including amplification of L-Myc. *J Invest Dermatol.* 2009; 129:1547–55. [PubMed: 19020549]
58. Lewis JS Jr, Duncavage E, Klonowski PW. Oral cavity neuroendocrine carcinoma: a comparison study with cutaneous Merkel cell carcinoma and other mucosal head and neck neuroendocrine carcinomas. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2010; 110:209–17. [PubMed: 20659699]

59. Shuda M, Feng H, Kwun HJ, Rosen ST, Gjoerup O, et al. T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. *Proc Natl Acad Sci USA*. 2008; 105:16272–77. [PubMed: 18812503]
60. Kassem A, Schopflin A, Diaz C, Weyers W, Stickeler E, et al. Frequent detection of Merkel cell polyomavirus in human Merkel cell carcinomas and identification of a unique deletion in the *VPI* gene. *Cancer Res*. 2008; 68:5009–13. [PubMed: 18593898]
61. Paulson KG, Iyer JG, Tegeder AR, Thibodeau R, Schelter J, et al. Transcriptome-wide studies of Merkel cell carcinoma and validation of intratumoral CD8⁺ lymphocyte invasion as an independent predictor of survival. *J Clin Oncol*. 2011; 29:1539–46. [PubMed: 21422430]
62. Houben R, Adam C, Baeurle A, Hesbacher S, Grimm J, et al. 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*. 2011 In press.
63. Liu X, Hein J, Richardson SC, Basse PH, Toptan T, et al. Merkel cell polyomavirus large T antigen disrupts lysosome clustering by translocating human Vam6p from the cytoplasm to the nucleus. *J Biol Chem*. 2011; 286:17079–90. [PubMed: 21454559]
64. Pallas DC, Shahrik LK, Martin BL, Jaspers S, Miller TB, et al. Polyoma small and middle T antigens and SV40 small T antigen form stable complexes with protein phosphatase 2A. *Cell*. 1990; 60:167–76. [PubMed: 2153055]
65. Miller RW, Rabkin CS. Merkel cell carcinoma and melanoma: etiological similarities and differences. *Cancer Epidemiol Biomarkers Prev*. 1999; 8:153–58. [PubMed: 10067813]
66. Heath M, Jaimes N, Lemos B, Mostaghimi A, Wang LC, et al. Clinical characteristics of Merkel cell carcinoma at diagnosis in 195 patients: the AEIOU features. *J Am Acad Dermatol*. 2008; 58:375–81. [PubMed: 18280333]
67. Sihto H, Kukko H, Koljonen V, Sankila R, Bohling T, Joensuu H. Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. *J Natl Cancer Inst*. 2009; 101:938–45. [PubMed: 19535775]
68. Kaae J, Hansen AV, Biggar RJ, Boyd HA, Moore PS, et al. Merkel cell Carcinoma: incidence, mortality, and risk of other cancers. *J Natl Cancer Inst*. 2010; 102:793–801. [PubMed: 20424236]
69. Reichgelt BA, Visser O. Epidemiology and survival of Merkel cell carcinoma in the Netherlands. A population-based study of 808 cases in 1993–2007. *Eur J Cancer*. 2010; 47:579–85. [PubMed: 21144740]
70. Formica M, Basolo B, Funaro L, Mazzucco G, Segoloni GP, Piccoli G. Merkel cell carcinoma in renal transplant recipient. *Nephron*. 1994; 68:399. [PubMed: 7838275]
71. Douds AC, Mellotte GJ, Morgan SH. Fatal Merkel-cell tumour (cutaneous neuroendocrine carcinoma) complicating renal transplantation. *Nephrol Dial Transplant*. 1995; 10:2346–48. [PubMed: 8808240]
72. Catlett JP, Todd WM, Carr ME Jr. Merkel cell tumor in an HIV-positive patient. *Va Med Q*. 1992; 119:256–58. [PubMed: 1390976]
73. Beral V, Bull D, Jaffe H, Evans B, Gill N, et al. Is risk of Kaposi's sarcoma in AIDS patients in Britain increased if sexual partners came from United States or Africa? *Br Med J*. 1991; 302:624–25. [PubMed: 2012875]
74. Viscidi RP, Clayman B. Serological cross reactivity between polyomavirus capsids. *Adv Exp Med Biol*. 2006; 577:73–84. [PubMed: 16626028]
75. Carter JJ, Madeleine MM, Wipf GC, Garcea RL, Pipkin PA, et al. Lack of serologic evidence for prevalent simian virus 40 infection in humans. *J Natl Cancer Inst*. 2003; 95:1522–30. [PubMed: 14559874]
76. Kean JM, Rao S, Wang M, Garcea RL. Seroepidemiology of human polyomaviruses. *PLoS Pathog*. 2009; 5:e1000363. [PubMed: 19325891]
77. Carter JJ, Paulson KG, Wipf GC, Miranda D, Madeleine MM, et al. Association of Merkel cell polyomavirus-specific antibodies with Merkel cell carcinoma. *J Natl Cancer Inst*. 2009; 101:1510–22. [PubMed: 19776382]
78. Touze A, Gaitan J, Arnold F, Cazal R, Fleury MJ, et al. Generation of Merkel cell polyomavirus (MCV)-like particles and their application to detection of MCV antibodies. *J Clin Microbiol*. 2010; 48:1767–70. [PubMed: 20181914]

79. Chen T, Hedman L, Mattila PS, Jartti T, Ruuskanen O, et al. Serological evidence of Merkel cell polyomavirus primary infections in childhood. *J Clin Virol.* 2011; 50:125–29. [PubMed: 21094082]
80. Tolstov YL, Knauer A, Chen JG, Kensler TW, Kingsley LA, et al. Asymptomatic primary Merkel cell polyomavirus infection among adults. *Emerg Infect Dis.* 2011 In press.
81. Foulongne V, Dereure O, Kluger N, Moles JP, Guillot B, Segondy M. Merkel cell polyomavirus DNA detection in lesional and nonlesional skin from patients with Merkel cell carcinoma or other skin diseases. *Br J Dermatol.* 2009; 162:59–63. [PubMed: 19678822]
82. Duncavage EJ, Zehnbauser BA, Pfeifer JD. Prevalence of Merkel cell polyomavirus in Merkel cell carcinoma. *Mod Pathol.* 2009; 22:516–21. [PubMed: 19252474]
83. Becker JC, Houben R, Ugurel S, Trefzer U, Pfohler C, Schrama D. MC polyomavirus is frequently present in Merkel cell carcinoma of European patients. *J Invest Dermatol.* 2008; 129:248–50. [PubMed: 18633441]
84. Goh S, Lindau C, Tiveljung-Lindell A, Allander T. Merkel cell polyomavirus in respiratory tract secretions. *Emerg Infect Dis.* 2009; 15:489–91. [PubMed: 19239773]
85. Katano H, Ito H, Suzuki Y, Nakamura T, Sato Y, et al. Detection of Merkel cell polyomavirus in Merkel cell carcinoma and Kaposi's sarcoma. *J Med Virol.* 2009; 81:1951–58. [PubMed: 19774683]
86. Loyo M, Guerrero-Preston R, Brait M, Hoque MO, Chuang A, et al. Quantitative detection of Merkel cell virus in human tissues and possible mode of transmission. *Int J Cancer.* 2010; 126:2991–96. [PubMed: 19588496]
87. Bergallo M, Costa C, Terlizzi ME, Astegiano S, Curtoni A, et al. Quantitative detection of the new polyomaviruses KI, WU and Merkel cell virus in transbronchial biopsies from lung transplant recipients. *J Clin Pathol.* 2010; 63:722–25. [PubMed: 20595181]
88. Bhatia K, Modali R, Goedert JJ. Merkel cell polyomavirus is not detected in mesotheliomas. *J Clin Virol.* 2010; 47:196–98. [PubMed: 20006539]
89. Schrama D, Peitsch WK, Zapatka M, Kneitz H, Houben R, et al. Merkel cell polyomavirus status is not associated with clinical course of Merkel cell carcinoma. *J Invest Dermatol.* 2011; 131:1631–38. [PubMed: 21562568]
90. Paulson KG, Carter JJ, Johnson LG, Cahill KW, Iyer JG, et al. Antibodies to Merkel cell polyomavirus T antigen oncoproteins reflect tumor burden in merkel cell carcinoma patients. *Cancer Res.* 2010; 70:8388–97. [PubMed: 20959478]
91. Touze A, Le Bidre E, Laude H, Fleury MJ, Cazal R, et al. High levels of antibodies against Merkel cell polyomavirus identify a subset of patients with Merkel cell carcinoma with better clinical outcome. *J Clin Oncol.* 2011; 29:1612–19. [PubMed: 21422439]
92. Howard RA, Dores GM, Curtis RE, Anderson WF, Travis LB. Merkel cell carcinoma and multiple primary cancers. *Cancer Epidemiol Biomarkers Prev.* 2006; 15:1545–49. [PubMed: 16896047]
93. Koljonen V, Kukko H, Tukiainen E, Bohling T, Sankila R, et al. Second cancers following the diagnosis of Merkel cell carcinoma: a nationwide cohort study. *Cancer Epidemiol.* 2010; 34:62–65. [PubMed: 20061203]
94. Bzhalava D, Bray F, Storm H, Dillner J. Risk of second cancers after the diagnosis of Merkel cell carcinoma in Scandinavia. *Br J Cancer.* 2011; 104:178–80. [PubMed: 21081931]
95. Ridd K, Yu S, Bastian BC. The presence of polyomavirus in non-melanoma skin cancer in organ transplant recipients is rare. *J Invest Dermatol.* 2009; 129:250–52. [PubMed: 18650847]
96. Varga E, Kiss M, Szabo K, Kemeny L. Detection of Merkel cell polyomavirus DNA in Merkel cell carcinomas. *Br J Dermatol.* 2009; 161:930–32. [PubMed: 19438857]
97. Andres C, Belloni B, Puchta U, Sander CA, Flaig MJ. Prevalence of MCPyV in Merkel cell carcinoma and non-MCC tumors. *J Cutan Pathol.* 2009; 27:28–34. [PubMed: 19615033]
98. Kassem A, Technau K, Kurz AK, Pantulu D, Loning M, et al. Merkel cell polyomavirus sequences are frequently detected in nonmelanoma skin cancer of immunosuppressed patients. *Int J Cancer.* 2009; 125:356–61. [PubMed: 19384948]
99. Reisinger DM, Shiffer JD, Coggnetta AB Jr, Chang Y, Moore PS. Lack of evidence for basal or squamous cell carcinoma infection with Merkel cell polyomavirus in immunocompetent patients with Merkel cell carcinoma. *J Am Acad Dermatol.* 2010; 63:400–3. [PubMed: 20584559]

100. Toracchio S, Foyle A, Sroller V, Reed JA, Wu J, et al. Lymphotropism of Merkel cell polyomavirus infection, Nova Scotia, Canada. *Emerg Infect Dis*. 2010; 16:1702–9. [PubMed: 21029527]
101. Koljonen V, Kukko H, Pukkala E, Sankila R, Bohling T, et al. Chronic lymphocytic leukaemia patients have a high risk of Merkel-cell polyomavirus DNA-positive Merkel-cell carcinoma. *Br J Cancer*. 2009; 101:1444–47. [PubMed: 19755994]
102. Pantulu ND, Pallasch CP, Kurz AK, Kassem A, Frenzel L, et al. Detection of a novel truncating Merkel cell polyomavirus large T antigen deletion in chronic lymphocytic leukemia cells. *Blood*. 2010; 116:5280–84. [PubMed: 20817850]
103. Tolstov YL, Arora R, Scudiere SC, Busam K, Chaudhary PM, et al. Lack of evidence for direct involvement of Merkel cell polyomavirus (MCV) in chronic lymphocytic leukemia (CLL). *Blood*. 2010; 115:4973–74. [PubMed: 20538816]
104. Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. *Nat Rev Cancer*. 2010; 10:878–89. [PubMed: 21102637]
105. Sarid R, Gao SJ. Viruses and human cancer: from detection to causality. *Cancer Lett*. 2010; 305:218–27. [PubMed: 20971551]
106. Koch, R. 1882 The aetiology of tuberculosis. In: Clark, DH., editor. *Source Book of Medical History*. New York: Dover; 1942. p. 392–406.
107. Fredericks DN, Relman DA. Sequence-based identification of microbial pathogens: a reconsideration of Koch's postulates. *Clin Microbiol Rev*. 1996; 9:18–33. [PubMed: 8665474]
108. Hill AB. Environment and disease: association or causation? *Proc R Soc Med*. 1965; 58:295–300. [PubMed: 14283879]
109. Sarid R, Olsen SJ, Moore PS. Kaposi's sarcoma-associated herpesvirus: epidemiology, virology and molecular biology. *Adv Virus Res*. 1999; 52:139–232. [PubMed: 10384236]
110. Fischer N, Brandner J, Fuchs F, Moll I, Grundhoff A. Detection of Merkel cell polyomavirus (MCPyV) in Merkel cell carcinoma cell lines: Cell morphology and growth phenotype do not reflect presence of the virus. *Int J Cancer*. 2010; 126:2133–42. [PubMed: 19739110]
111. Vesely MJ, Murray DJ, Neligan PC, Novak CB, Gullane PJ, Ghazarian D. Complete spontaneous regression in Merkel cell carcinoma. *J Plast Reconstr Aesthet Surg*. 2007; 61:165–71. [PubMed: 17382612]
112. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, et al. Clustal W and Clustal X version 2.0. *Bioinformatics*. 2007; 23:2947–48. [PubMed: 17846036]
113. Huson DH, Richter DC, Rausch C, Dezulian T, Franz M, Rupp R. Dendroscope: an interactive viewer for large phylogenetic trees. *BMC Bioinformatics*. 2007; 8:460. [PubMed: 18034891]

Figure 1.
Clinical appearance of Merkel cell carcinoma (MCC) lesion. Red, raised, nodular MCC lesion on the arm of a patient. Reproduced courtesy of Dr. Klaus J. Busam, Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York.

Figure 2.

Phylogenetic analysis of large tumor antigens from 12 polyomaviruses. The nine known human polyomaviruses (*red font*) are BK virus (BKV: NC_001538); JC virus (JCV: NC_001699); Washington University polyomavirus (WUV: NC_009539), Karolinska Institute polyomavirus (KIV: NC_009238); Merkel cell polyomavirus (MCV: NC_010277); trichodysplasia spinulosum polyomavirus (TSaPyV: NC_014361); and human polyomaviruses (HPyV) 6 (NC_014406), 7 (NC_014407), and 9 (HQ696595). Also shown are several well-studied nonhuman polyomaviruses (*gray font*): simian vacuolating virus 40 (SV40: NC_001669), murine polyomavirus (MPyV: NC_001515), and African green monkey lymphotropic polyomavirus (LPV: NC_004763). The phylogenetic tree was produced using ClustalX (112, 113). Reproduced courtesy of Dr. Huichen Feng, Cancer Virology Program, Hillman Cancer Center, University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania.

Figure 3.

Genome organization of Merkel cell carcinoma. The wild-type Merkel cell polyomavirus genome is 5,387 bp long and contains a noncoding regulatory region (NCCR), from which the virus bidirectionally encodes for early and late proteins. In addition to the early and late transcriptional promoters, the NCCR also contains the viral origin (*red*), which can be mapped to 71 bp of contiguous sequence recognized by the large tumor antigen (LT). LT, small T antigen (sT), and 57-kT antigen constitute the gene products of the early region. Viral protein 1 (VP1) and VP2 are late proteins required for capsid formation and viral replication.

Figure 4.

Genome, transcript, and protein features of the tumor antigen (T antigen) locus. Merkel cell polyomavirus (MCV) T antigens contain protein motifs similar to those found in other polyomaviruses (*red font*). In its amino-terminal half, preserved even in the presence of tumor-specific truncation mutations, the MCV large T antigen (LT) contains conserved region 1 (CR1)-, DnaJ-, and retinoblastoma (Rb)-binding amino acid sequences. In MCV, ~200 amino acids are inserted immediately following the first exon and before the Rb-binding site. This MCV-unique region (MUR) exhibits no significant homology to other polyomavirus T antigens, which suggests that it may confer unique functions. The C-terminal portion of MCV is occupied largely by motifs required for replication, including the origin-binding domain (OBD) and the zinc finger, leucine zipper, ATPase, and helicase domains. The small T antigen (sT) contains a protein phosphatase 2A (PP2A)-binding motif that is absent in LT and the 57-kT antigen because of the differential splicing of their respective transcripts.

Figure 5. Merkel cell carcinoma (MCC) pathology. (a) Monomorphous clusters of MCC tumor cells in dermis stained with hematoxylin and eosin (20× magnification). (b) Adjacent section immunostained with anti-CK20 antibody, demonstrating distinctive perinuclear localization in a dot-like pattern (20× magnification).

Figure 6.

Expression of Merkel cell polyomavirus (MCV) tumor antigen (T antigen) proteins in Merkel cell carcinoma (MCC). (a) Immunostaining with anti-MCV large T antigen (LT) shows nests of MCC tumor cells infiltrating the dermis (20× magnification). (b) Oil emersion demonstrates the robust expression of MCV LT localized to the nuclear compartment of tumor cells (100× magnification). (c,d) Differential expression of LT and small T antigen (sT) in a case of MCC. Sequential sections of a polymerase chain reaction–confirmed, MCV DNA–positive MCC stained with (c) anti-MCV LT and (d) anti-MCV sT antibodies. Although LT expression was not detected in this lesion, sT expression is clearly seen. Slides are shown at 20× magnification with a hematoxylin counterstain.

Figure 7.

Steps in the molecular evolution of Merkel cell carcinoma (MCC). (1) Merkel cell polyomavirus (MCV) infection is common in the general population and is acquired in early childhood. It is an easily detected component of the normal skin flora. (2) In the setting of decreased immune competence (either iatrogenically induced or age related), reactivation of MCV may occur, as has been observed with other human polyomaviruses. (3) Such a burst of infective virus production can facilitate viral integration in susceptible Merkel cells. (4) Selection pressure is predicted to occur against Merkel cells infected with replication-competent viruses as a result of cell lysis. (5) The expression of MCV tumor antigens (T antigens) in cells with integrated virus harboring T antigen-truncation mutations provides proliferative signals that lead to MCC.

Figure 8.

Merkel cell carcinoma (MCC) metastasis to a lymph node involved with chronic lymphocytic leukemia (CLL). Immunohistochemical study of a lymph node containing both MCC and CLL shows tumor antigen expression only in MCC tumor cells in the typical nuclear pattern, but not in CLL tumor cells. (a) 10× magnification. (b) 100× magnification, oil emersion, hematoxylin counterstain.

Table 1

Human polyomaviruses

Nomenclature	Year identified	Method of identification	Prevalence in human population	Disease associations
BKV	1971	Culture isolation from urine of renal transplant recipient	>90% of adults	Cystitis, polyomavirus-associated nephropathies, ureteral stenosis
JCV	1971	Culture isolation from brain tissue with progressive multifocal leukoencephalopathy	>70% of adults	Progressive multifocal leukoencephalopathy
KIV	2007	Deep sequencing of DNAase-treated respiratory fluids	55–70% of adults	Not defined
WUV	2007	Deep sequencing of DNAase-treated respiratory fluids from children with upper respiratory infections	69–80% of adults	Not defined
MCV	2008	Digital transcriptome subtraction of Merkel cell carcinoma tissue	42–70% of adults (76)	Merkel cell carcinoma
HPyV6	2010	Rolling-circle amplification of skin and hair samples	Not defined	Not defined
HPyV7	2010	Rolling-circle amplification of skin and hair samples	Not defined	Not defined
TSaPyV	2010	Degenerate/consensus PCR of trichodysplasia spinulosum lesion in transplant recipient	Not defined	Transplant-associated trichodysplasia spinulosum
HPyV9	2011	Rolling-circle amplification from serum of renal transplant patient	Not defined	Not defined

Abbreviations: BKV, BK virus; HPyV, human polyomavirus; JCV, JC virus; KIV, Karolinska Institute polyomavirus; MCV, Merkel cell polyomavirus; PCR, polymerase chain reaction; TSaPyV, trichodysplasia spinulosum polyomavirus; WUV, Washington University polyomavirus.