National Toxicology Program
U.S. Department of Health and Human Services

Report on Carcinogens

Monograph on Merkel Cell Polyomavirus

RoC MONOGRAPH 11

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Foreword

The National Toxicology Program (NTP), established in 1978, is an interagency program within the Public Health Service of the U.S. Department of Health and Human Services. Its activities are executed through a partnership of the National Institute for Occupational Safety and Health (part of the Centers for Disease Control and Prevention), the Food and Drug Administration (primarily at the National Center for Toxicological Research), and the National Institute of Environmental Health Sciences (part of the National Institutes of Health), where the program is administratively located. NTP offers a unique venue for the testing, research, and analysis of agents of concern to identify toxic and biological effects, provide information that strengthens the science base, and inform decisions by health regulatory and research agencies to safeguard public health. NTP also works to develop and apply new and improved methods and approaches that advance toxicology and better assess health effects from environmental exposures.

The Report on Carcinogens Monograph series began in 2012. Report on Carcinogens Monographs present the cancer hazard evaluations of environmental agents, substances, mixtures, or exposure circumstances (collectively referred to as “substances”) under review for the Report on Carcinogens. The Report on Carcinogens is a congressionally mandated, science-based, public health document that provides a cumulative list of substances that pose a cancer hazard for people in the United States. Substances are reviewed for the Report on Carcinogens to (1) be a new listing, (2) reclassify the current listing status, or (3) be removed.

NTP evaluates cancer hazards by following a multistep process and using established criteria to review and integrate the scientific evidence from published human, experimental animal, and mechanistic studies. General instructions for the systematic review and evidence integration methods used in these evaluations are provided in the *Handbook for the Preparation of Report on Carcinogens Monographs*. The handbook’s instructions are applied to a specific evaluation via a written protocol. The evaluation’s approach as outlined in the protocol is guided by the nature, extent, and complexity of the published scientific information and tailored to address the key scientific issues and questions for determining whether the substance is a potential cancer hazard and should be listed in the Report on Carcinogens. Draft monographs undergo external peer review before they are finalized and published.

The Report on Carcinogens Monographs are available free of charge on the NTP website and cataloged in PubMed, a free resource developed and maintained by the National Library of Medicine (part of the National Institutes of Health). Data for these evaluations are included in the Health Assessment and Workspace Collaborative. Information about the Report on Carcinogens is also available on the NTP website.

For questions about the monographs, please email NTP or call 984-287-3211.

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This report has been reformatted to meet new NTP publishing requirements; its content has not changed. The proposed substance profile is no longer part of the document because it is published in the 14th Report on Carcinogens.

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Peer Review

Peer review of the Draft RoC Monograph on Merkel Cell Polyomavirus (MCV) was conducted by an ad hoc expert panel at a public meeting held December 17, 2015, in the Rodbell Auditorium at the National Institute of Environmental Health Sciences, David P. Rall Building, Research Triangle Park, NC (see http://ntp.niehs.nih.gov/go/38854) for materials, minutes, and panel recommendations from the peer-review meeting). The selection of panel members and conduct of the peer review were performed in accordance with the Federal Advisory Committee Act and federal policies and regulations. The panel members served as independent scientists, not as representatives of any institution, company, or governmental agency.

The charge to the Peer-Review Panel was as follows:

(1) Comment on the draft cancer evaluation component for MCV, specifically, whether it was technically correct and clearly stated, whether the NTP has objectively presented and assessed the scientific evidence, and whether the scientific evidence is adequate for applying the RoC listing criteria,

(2) Comment on the draft substance profile for MCV, specifically, whether the scientific justification presented in the substance profile supports the NTP's preliminary policy decision on the RoC listing status of the substance (available in the 14th edition of the Report on Carcinogens).

The Panel was asked to vote on the following questions:

(1) Whether the scientific evidence supports the NTP's preliminary conclusion on the level of evidence for carcinogenicity for the specific types of cancer from cancer studies in humans.

(2) Whether the scientific evidence supports the NTP's preliminary listing decision for MCV in the RoC.

This RoC monograph on MCV has been revised based on NTP's review of the Panel's peer-review comments. The Peer-Review Panel Report, which captures the Panel recommendations for listing status of MCV in the RoC and their scientific comments, and the NTP Response to the Peer-Review Report are available on the Peer-Review Meeting webpage for Merkel cell polyomavirus (http://ntp.niehs.nih.gov/go/38854).

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Abstract

Introduction: In the United States, Merkel cell polyomavirus (MCV or MCPyV) infection (as assessed by seroprevalence) ranges from 22% to 88%, with lower rates in children and higher rates in adults. MCV is a stable, nonenveloped DNA virus found in the skin. Transmission of MCV is not fully characterized, but possible transmission through personal contact via saliva or skin has been suggested. MCV establishes a chronic, lifelong, symptomless infection in a large majority of healthy individuals. MCV was discovered in 2008 when nonhuman DNA was detected in human Merkel cell carcinoma cells.

Methods: The National Toxicology Program (NTP) conducted a cancer hazard evaluation of MCV infection for possible listing in the Report on Carcinogens (RoC). The evaluation included the findings from studies reported in the 2008 IARC monograph and from a search for all literature related to MCV. For each cancer site, the evidence from human and mechanistic studies was integrated, considering the following guidelines: Hill's characteristics of causality, multicausality epidemiology issues, and concepts of direct and indirect carcinogenesis proposed by several virus experts. Finally, the RoC's listing criteria were applied to the assessment to reach an overall cancer hazard conclusion.

Results and Discussion: Epidemiological, clinical, and molecular studies demonstrated evidence of a causal association between MCV and Merkel cell carcinoma, a rare cancer most commonly observed in the elderly and in immunosuppressed individuals. This association was seen in studies of populations in different geographical areas. Case-control studies and a nested case-control study found consistent evidence of elevated risk estimates among MCV-infected individuals. Risk estimates were highest among those with high levels of anti-MCV antibodies. Clinical studies found significantly higher MCV antibody levels in MCV-positive Merkel cell carcinoma cases, and case series studies found MCV DNA integrated into tumors. Molecular studies in humans found MCV monoclonally integrated into the cellular genome of tumor cells, providing evidence that virus infection precedes cancer.

Mechanistic studies support the epidemiological evidence. Integration of MCV DNA can lead to the expression of two proteins, including a large T (LT) antigen and a small T antigen, which are required for transformation of the host cell into a cancer cell and for proliferation and survival of the cancer cells. The mutated LT antigen prevents viral replication and allows the virus to evade immune detection. Only the mutated, integrated form of MCV is associated with carcinogenicity, which may explain why only a small percentage of infected individuals develop cancer.

NTP Hazard Conclusion and Significance: The conclusion of the cancer hazard evaluation was that MCV should be listed as *known to be a human carcinogen* in the RoC. The Secretary of Health and Human Services approved the listing of MCV in the 14th RoC. The rationale for the listing was sufficient evidence from studies in humans (human cancer, clinical, and molecular) and supporting mechanistic data. Globally, MCV is estimated to be responsible for approximately 10,000 cancers per year.

Introduction and Methods

This is one of a collection of five monographs that provide cancer hazard evaluations for the following human viruses for potential listing in the Report on Carcinogens (RoC): Epstein-Barr virus, Kaposi sarcoma-associated herpesvirus, human immunodeficiency virus type 1, human T-cell lymphotropic virus type 1, and Merkel cell polyomavirus. Viruses currently listed in the RoC include human papillomaviruses: some genital-mucosal types (HPV), hepatitis B virus (HBV), and hepatitis C virus (HCV). Each virus was selected for review for the RoC based on a large database of scientific information (including authoritative reviews), public health concerns for adverse health outcomes, and evidence that a significant number of people are infected with each virus both in the United States and worldwide.

This section provides background information on the preparation of the monographs as well as a discussion of overarching issues related to evaluating the evidence for cancer from human epidemiological studies and evaluating the causation by viruses.

Monograph Contents

The RoC monograph for each virus reviews the relevant scientific information and assesses its quality, applies the RoC listing criteria to the scientific information, and recommends an RoC listing status. Information reviewed in the monographs, with the exception of information on properties and exposure, comes from publicly available and peer-reviewed sources. All sections of the monographs underwent scientific and quality assurance review by independent reviewers.

The monograph provides the following information relevant to a RoC listing recommendation: Properties and Detection (Section 1), Human Exposure (Section 2), Human Cancer Studies (Section 3), Mechanisms and Other Relevant Data(Section 4), and Overall Cancer Hazard Evaluation and Listing Recommendation (Section 5). Because these viruses are primarily species-specific for humans, we are not conducting an evaluation of the level of evidence for carcinogenicity from studies in experimental animals and are including studies in animals that inform the mechanisms of carcinogenicity in the Mechanistic and Other Relevant Data section of the monographs, which is similar to the approach used by IARC. Also, specific details about the strains of the viruses are given only if needed to provide context, such as in the viral Properties and Detection section. The monographs relied on the information and data provided in previous IARC monographs on these five viruses in addition to newer key studies or reviews published since the IARC monographs; it is a peer-review assessment of available data through August 17, 2015. Additional publications published after that date were added to the monograph based on recommendations from the peer-review panel that reviewed this document on December 17, 2015. Literature search strategies to obtain information relevant to the cancer evaluation are in Appendix A of each virus monograph; search terms were developed in collaboration with a reference librarian.

Evaluating the Evidence from Human Epidemiological Studies

The available studies of specific types of cancer for these human viruses present several challenges with respect to the evaluation of methodological strengths and limitations of the body of evidence. Large prospective cohort studies, particularly those that follow individuals for

whom infection status is documented prior to follow-up or cancer diagnosis, have several potential methodological strengths, including evidence that infection precedes cancer diagnosis, adequate statistical power, and, in some studies, have the ability to analyze dose-response relationships. However, there is the potential for misclassification of exposure in studies with a long follow-up period that measure the virus once and have a long follow-up period as new infections might not be identified. For most types of cancer, only cross-sectional or retrospective cohort studies or hospital- or clinic-based case-control studies are available, all of which lack direct evidence of temporality and may lack power or adequate exposure data, e.g., on viral load. However, molecular evidence from human studies and mechanistic data can be used in the evaluation of temporality, distinguishing latent infections caused by the tumor virus and causality. For some (typically rare) outcomes (e.g., cutaneous T-cell lymphoma and human T-cell lymphotropic virus type 1, or lymphoepithelial carcinoma of the salivary gland and Epstein-Barr virus), only case-comparison studies, in which selection of comparison groups may be biased, unmatched, or inadequately described, or case series are available.

For several rare types of cancer, e.g., adult T-cell leukemia/lymphoma and human T-cell lymphotropic virus type 1, or primary effusion lymphoma and Kaposi sarcoma-associated herpesvirus, the presence of the virus in the tumor cells is used as a diagnostic criterion to define the cancer, and thus, evidence of causality relies on cases defined by this criterion and molecular evidence from human studies rather than on epidemiological population-based studies of the association of the virus with a level of cancer risk.

In addition, methodologically adequate studies should include measurement of cofactors and consider potentially confounding factors; however, relatively few studies have measured a panel of other viruses or taken into account other cofactors. Further, while studies comparing cancer risk in treated vs. untreated populations may provide indirect evidence of the role of human immunodeficiency virus type 1, these studies, in particular calendar-period analyses, may not adequately account for changes in risk attributable to improved survival rates or changes in other risk factors.

Evaluating Causality of Viruses

Approximately 12% of all human cancers have been attributed to viral infections. Although the known oncogenic viruses belong to different virus families, they often share several common traits, such as, viral cancers appear in the context of persistent infections, occur many years to decades after acute infection, and the immune system can play a deleterious or a protective role (Mesri et al. 2014). Many viruses generally increase cancer risk in the context of immunosuppression or chronic inflammation (Mesri et al. 2014). Similar to other carcinogenic agents, only a small percentage of infected or exposed individuals develop cancer, often decades after the initial infection, reflecting the complex nature of oncogenesis. Some cofactors produced by other organisms or agents in conjunction with risk modifiers such as virus-host cell interactions, host genetic factors, immune dysfunction, or chronic inflammation often can contribute to malignant transformation. In addition, severe immunosuppression, as seen with congenital immunodeficiency syndromes, chronic human immunodeficiency virus type 1 infection, or as a result of tissue anti-rejection medication, can severely compromise the immune surveillance capabilities of the patient. There are also other challenges that are somewhat unique to the evaluation of the epidemiological studies of viruses and cancer (discussed below) and thus molecular evidence from human tissues is often considered in the evaluation of causality.

In light of these issues, IARC monographs and several other publications have recommended paths to evaluate causality, which are discussed below and incorporated into the NTP approach for evaluating causality of the viruses. What is important for public health in determination of causation of a health effect, such as risk for cancer, is whether the health effect is eliminated or mitigated by removal of the substance (Rothman and Greenland 2005).

A number of attempts have been made to develop criteria or considerations that address causal associations. However, all of them have limitations, especially when applied to infectious agents (Moore and Chang 2010). The following sections identify factors to consider for evaluating causality, some limitations arising from a strict application of the criteria in the context of virally induced cancers, some alternative approaches, and finally, the NTP's approach for evaluating the role of select viral agents in human cancer.

Hill's Characteristics of Causality

Hill proposed nine characteristics to consider when evaluating causality, primarily for epidemiological studies, although they have been expanded for evaluating mechanistic and other types of data (Table 1). Several considerations—strength of the association, consistency across studies, evidence of an exposure-response gradient, and temporality of exposure (Hill 1965)—are used to help guide the RoC evaluations of the human epidemiological data (see RoC Handbook, NTP (2015)). However, it should be noted that these are not criteria; and, with the exception of temporality, each and every element is not required in order to demonstrate causality (Rothman and Greenland 2005). Hill (1965) avoided discussing the meaning of "causation," noting that the "cause" of an illness could be immediate and direct or remote and indirect. The primary question addressed by Hill was "whether the frequency of the undesirable event B will be influenced by a change in the environmental feature A."

Table 1. Hill's Epidemiological Characteristics for Evaluating Causality

Characteristic	Description
1. Strength of association	A strong association between a virus and a cancer increases the confidence for causality unless confounded by some other exposure. However, a weak association does not give evidence against causality.
2. Consistency	Consistent findings observed among different groups of people, in different places, circumstances, and times.
3. Specificity	A viral exposure is limited only to specific types of cancer; this is considered a weak factor because there are well-established examples in which a virus might cause several types of cancer.
4. Temporality	Exposure to the virus must occur prior to the onset of the cancer, in contrast to a "passenger infection."
5. Biological gradient	The virus is more likely to be found at the tumor site than at non-tumor sites.
6. Plausibility	This characteristic should be applied with caution because it is limited by current medical knowledge (e.g., a currently implausible mechanism may gain acceptance with increased understanding of the underlying biology).
7. Coherence	A virus-cancer association should not seriously conflict with known facts on the cancer's natural history and biology.

Characteristic	Description
8. Experiment	Changing either exposure or continued infection in a randomized clinical trial should change the measure of clinical outcome (e.g., vaccination programs for HPV and HBV).
9. Analogy	Are related viruses clearly established to cause cancers in animals or humans?

Source: Moore and Chang (2014).

Evaluating Mechanistic Data from Human Studies

In their evaluation of the evidence for Epstein-Barr virus, the IARC working group noted that the large majority of people are latently infected with Epstein-Barr virus, thus, epidemiological studies may be limited in determining whether the presence of Epstein-Barr virus in tumor tissue is a cause of the cancer or an effect of the tumor. Therefore, in addition to the Hill characteristics, IARC (1997) considered the following factors in their evaluation of Epstein-Barr virus, which are also applicable to other viruses:

- The proportion of Epstein-Barr virus-positive cases in a given tumor entity.
- The proportion of tumor cells that carry the virus.
- The monoclonality of Epstein-Barr virus in the tumor.
- The expression of Epstein-Barr virus proteins.

zur Hausen (1994; 2001) proposed consideration of the following types of mechanistic or epidemiological evidence for evaluating causality of viruses and cancer:

- The presence and persistence of viral DNA in tumor biopsies and cell lines derived from the same tumor type.
- The growth-promoting activity of specific viral genes or of virus-modified host-cell genes in tissue culture systems or in suitable animal systems.
- The continuous expression of viral oncogenes or the modification of host-cell genes containing viral sequences which maintains the malignant phenotype.
- The epidemiological evidence that the virus infection is a major risk factor.

It is difficult to prove that a virus causes cancer, and such determinations almost always generate considerable controversy and debate (Moore and Chang 2010). Viral cancers employ various mechanisms that involve both direct and indirect modes of interaction (Table 2) (zur Hausen and de Villiers 2014). Understanding and managing viral-induced cancers in humans has been hampered by a lack of suitable animal models, the disparate nature of tumor types, a long latency period between primary infection and cancer development, the different types of oncogenic viruses, and the complex nature of the virus-host cell interactions leading to cancer (Mesri et al. 2014; zur Hausen and de Villiers 2014).

Table 2. Direct and Indirect Modes of Interaction of Viral Infections and Cancers

Type	Description
Direct carcinogenesis	Continued presence and expression of viral oncogenes usually after viral genome integration into host cell DNA. Insertional gene activation or suppression. Continued episomal presence of viral nucleic acid and suppression or activation of cellular genes (e.g., by viral microRNA).
Indirect carcinogenesis	Induction of immunomodulation, activation of latent tumor virus genomes. Induction of oxygen and nitrogen radicals. Amplification of latent tumor virus DNA. Induction of mutations and/or translocations. Prevention of apoptosis.

Source: zur Hausen and de Villiers (2014).

Multicausality Issues

Although thousands of viruses are known to cause infection, only a few have been shown to cause cancer in humans (Moore and Chang 2010). An important consideration regarding causality (not limited to viruses) is "multicausality," that is, the concept that many determinants act together to cause a disease. Rothman and colleagues (2005) defined a sufficient cause as "complete causal mechanism"—not a single factor but a set of minimal factors (i.e., component causes)—that if present in an individual will cause disease. Most causes are neither necessary nor sufficient in the absence of other factors to produce the disease; however, a cause does not have to be either necessary or sufficient for its removal to result in disease prevention (Rothman and Greenland 2005; zur Hausen and de Villiers 2014).

Application of Causality Criteria and Alternative Approaches

Moore and Chang (2010) investigated the difficulties associated with strict application of the Hill characteristics for two of the most recently discovered oncogenic viruses: Kaposi sarcoma-associated herpesvirus and Merkel cell polyomavirus. Kaposi sarcoma-associated herpesvirus was shown to fulfill Hill's characteristics for causality of Kaposi sarcoma; however, the application of the characteristics was problematic in the case of Merkel cell polyomavirus and Merkel cell carcinoma (see the monographs for Kaposi sarcoma-associated herpesvirus and Merkel cell polyomavirus). These two examples illustrate the diversity in the patterns of tumor virus epidemiology. Some of the reasons Hill's characteristics worked for Kaposi sarcoma-associated herpesvirus but not Merkel cell polyomavirus is that all clinical forms of Kaposi sarcoma require infection by Kaposi sarcoma-associated herpesvirus while most studies indicate that not all forms of Merkel cell carcinoma require the presence of Merkel cell polyomavirus. In the case of Merkel cell polyomavirus, additional considerations, as suggested by IARC (1997) and zur Hausen (1994; 2001), provide molecular evidence of the association between Merkel cell polyomavirus and Merkel cell carcinoma, such as mutation and monoclonal integration of the tumor-causing form of the virus into the cellular genome and requirement of tumor cells for the presence of viral oncoproteins for cell survival and proliferation.

While causal criteria can be helpful, there are flaws and practical limitations that restrict their use in cancer biology (Moore and Chang 2010). Therefore, a more probabilistic approach may be more useful for determining whether or not certain viruses cause human cancers. For example, instead of trying to determine if virus A causes cancer B, the probabilistic approach examines if

cancer B is more probable in the presence of virus A. Although a correlation does not imply causation, it can be argued that correlations that are strong, reproducible, and predictive have a similar value as a causative conclusion. In a similar fashion, zur Hausen and de Villiers (2014) also expressed concern over all attempts to summarize criteria for "causality" of infectious agents in cancer development and proposed replacing "causal factor" with "risk factor" and grading them according to their contribution to an individual's cancer risk. This will require a greater understanding of the complexity of factors involved and their mechanistic contribution to individual cancers.

RoC Listing Criteria

Known to Be Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans*, which indicates a causal relationship between exposure to the agent, substance, or mixture, and human cancer.

Reasonably Anticipated to Be Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans*, which indicates that causal interpretation is credible, but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded, OR

there is sufficient evidence of carcinogenicity from studies in experimental animals, which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site, or type of tumor, or age at onset, OR

there is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance, or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either known to be a human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to, dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub-populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals, but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

*This evidence can include traditional cancer epidemiology studies, data from clinical studies, and/or data derived from the study of tissues or cells from humans exposed to the substance in question that can be useful for evaluating whether a relevant cancer mechanism is operating in people.

NTP's Approach

For each virus, the NTP applied the RoC listing criteria (see text box) to the body of literature to reach the listing recommendation. The level of evidence conclusion from studies in humans considers the evidence from epidemiological studies as well as clinical and molecular studies of tissues from exposed (i.e., infected) individuals. In evaluating the mechanistic data and determining the recommendations for its level of evidence conclusion and overall listing recommendation, the NTP considered the principles outlined by Hill (1965), IARC (1997), zur Hausen (1994; 2001; 2014), and Rothman and Greenland (2005) in its assessment of causality for the five viruses reviewed. However, these factors were not used as a strict checklist to either

prove or disprove a causal association but rather as guidance to assess the level of epidemiological or molecular evidence that a virus contributes to a carcinogenic effect.

1. Properties and Detection

This section reviews the biological properties (Section 1.1) and methods for detection (Section 1.2) of the Merkel cell polyomavirus (MCV, MCPyV). The material presented in Sections 1.1 and 1.2 is summarized in Section 1.3.

1.1. Biological Properties

1.1.1. Family and Type

The Merkel cell polyomavirus (MCV) was discovered in 2008 when non-human DNA was detected in human Merkel cell carcinoma cells. The novel sequences had high identity with other known polyomaviruses (IARC 2013; Spurgeon and Lambert 2013). Polyomaviruses were discovered in multiple rodent tumors in the 1950s, thus the term polyoma, meaning multiple tumors (Dalianis and Hirsch 2013; Moens et al. 2015). Polyomaviruses have also been found in birds, fish, cattle, and primates, humans included. MCV shares a high degree of similarity (50% nucleotide identity) with murine polyomavirus and other members of the recently proposed *"Almipolyomavirus*" genus (Carter et al. 2013; IARC 2013; Moens et al. 2015; Moore and Chang 2010; Spurgeon and Lambert 2013). MCV is more distantly related (35% nucleotide identity) to a cluster of highly related primate polyomaviruses that include simian virus 40 (SV40), African Green Monkey lymphotropic polyomavirus, BK polyomavirus (BKV), and JC polyomavirus (JCV). There are 13 polyomaviruses that infect humans, these include MCV, BKV, and JCV (Dalianis and Hirsch 2013; Moens et al. 2015). Serological and PCR-based studies indicate that, like other human polyomaviruses, MCV establishes a chronic lifelong infection in a large majority of healthy individuals (IARC 2013; Moore and Chang 2010). The skin appears to be a primary site of MCV infection.

1.1.2. Virus Structure and Genome

MCV is a non-enveloped virus (40 to 55 nm in diameter) composed of the capsid proteins, virus capsid protein 1 (VP1), 2 (VP2), and the genome (see Figure 1-1) (Dalianis and Hirsch 2013; IARC 2013; Spurgeon and Lambert 2013). The outside layer is composed of only VP1, which will spontaneously self-assemble into virus-like particles with icosahedral symmetry (IARC 2013). The minor capsid protein, VP, associates with pockets on the interior surface of VP1. The surface of the virion has a knobby appearance with indentations where the N- and C-termini of VP1 are located (Dalianis and Hirsch 2013; IARC 2013). These termini form disulfide bridges that give the virus stability, even after heating to 75°C (167°F) for an hour. This stability supports the idea that transmission could occur through environmental exposures, such as contact with sewage, rivers, and surfaces.

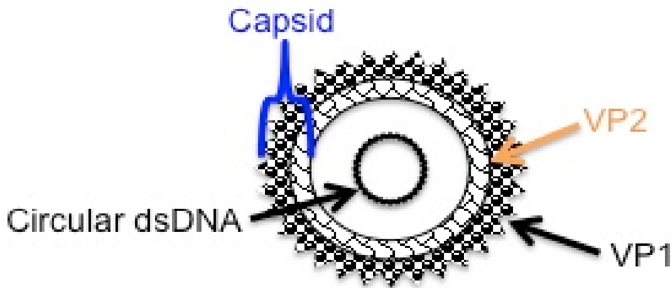

Figure 1-1. Merkel Cell Polyomavirus Particle

Within the capsid is a circular double-stranded (dsDNA) genome of about 5 kb that is wrapped around host cell-derived histones (Dalianis and Hirsch 2013; IARC 2013; Moens et al. 2015; Spurgeon and Lambert 2013). There is little genetic diversity among wild-type MCV, with isolates having genomes with >98.5% nucleotide identity. A single non-coding regulatory region, which contains the origin of replication as well as promoter and enhancer sequences for two flanking protein-coding regions. The two coding regions transcribe in opposite directions and are regulated temporally, with one coding region containing the early regulatory genes (LT, sT, alternate frame of the large T open reading frame [ALTO], 57kT) and the other containing late structural genes (VP1, VP2) which are created by alternative splicing of their respective early or late gene transcripts (Figure 1-2).

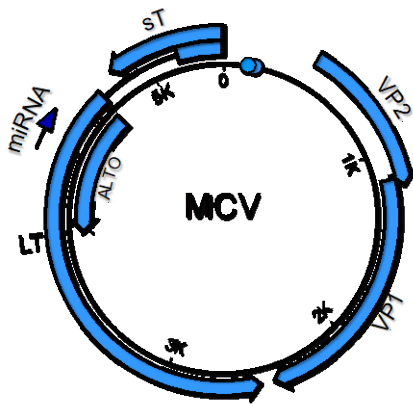

Figure 1-2. Genome Schematic

The early genes, LT and sT, regulate gene expression of viral and host genes, while the function of a multiply spliced LT isoform called 57kT (not shown in the figure) is not well understood (IARC 2013; Moens et al. 2015; Spurgeon and Lambert 2013). Besides the capsid proteins (VP1 and VP2), the late coding strand also expresses a regulatory micro-RNA (miRNA) (IARC 2013; Spurgeon and Lambert 2013). The 3' ends of both transcriptional units are separated by bi-directional polyadenylation sequences, which in the murine polyomaviruses and possibly MCV, cause inefficient termination. This allows for some transcripts to continue into the other transcription unit where the miRNA is located. The miRNA is 22 nucleotides long and appears to negatively regulate LT expression.

1.1.3. Replication

Entry of MCV into the cell requires receptor-mediated endocytosis using a cellular glycan receptor with at least one sialic acid residue binding to VP1 on the surface of the viral capsid and an interaction with heparan sulfate (IARC 2013) (see Figure 1-3). Although it is currently unclear how the non-enveloped MCV virion traverses host cell membranes, other polyomaviruses are thought to traffic to the endoplasmic reticulum, where host-cell chaperone proteins facilitate entry of the polyomavirus into the cytoplasm. The viral genome then enters the nucleus and early genes become expressed, using cellular transcription factors. The early genes promote viral DNA episomal replication. LT binds to the origin of replication, has helicase activity, and also binds host DNA polymerase alpha-primase to initiate viral DNA replication (IARC 2013; Moens et al. 2015; Spurgeon and Lambert 2013). While LT is necessary and sufficient to drive viral DNA replication, sT is not sufficient to drive replication, but may be essential for initial cell transformation and stabilizing LT (Stakaityte et al. 2014). The ability of LT to bind host DNA polymerase alpha-primase is thought to play a major role in determining tissue and host tropism as the cellular receptors needed for endocytosis of the virus are commonly found on many types of cells. The LT early gene can also promote the expression of late genes, allowing for virus formation that can lead to cell lysis. The function of miRNA is believed to be to suppress LT expression and to keep the late and early phase gene expressions temporally separate. The expression of the late genes can become blocked causing the virus to enter a latent phase where a low copy number of viral DNA is maintained episomally and viral genes are not expressed. With little to no viral gene expression, the virus can evade immune detection. The regulation of latent or lytic phases of MCV infection are not fully understood.

MCV DNA has been found integrated into the host DNA of 80% of Merkel cell carcinomas, where it is clonally passed along to daughter cells of both the primary tumor and metastatic tumors (Dalianis and Hirsch 2013; IARC 2013; Moens et al. 2015; Moore and Chang 2010). Integration is thought to occur by non-homologous recombination (Moore and Chang 2010). Because it is clonally expressed in Merkel cell carcinoma, its integration into the host genome is thought to be an early step in carcinogenesis. When integrated into the host genome, the helicase activity of LT would cause uncontrolled replication of the surrounding cellular chromosome, triggering rapid cell death. However, in tumors, integrated MCV genomes contain a truncated LT gene deficient in helicase activity and are unable to replicate, allowing for cell viability.

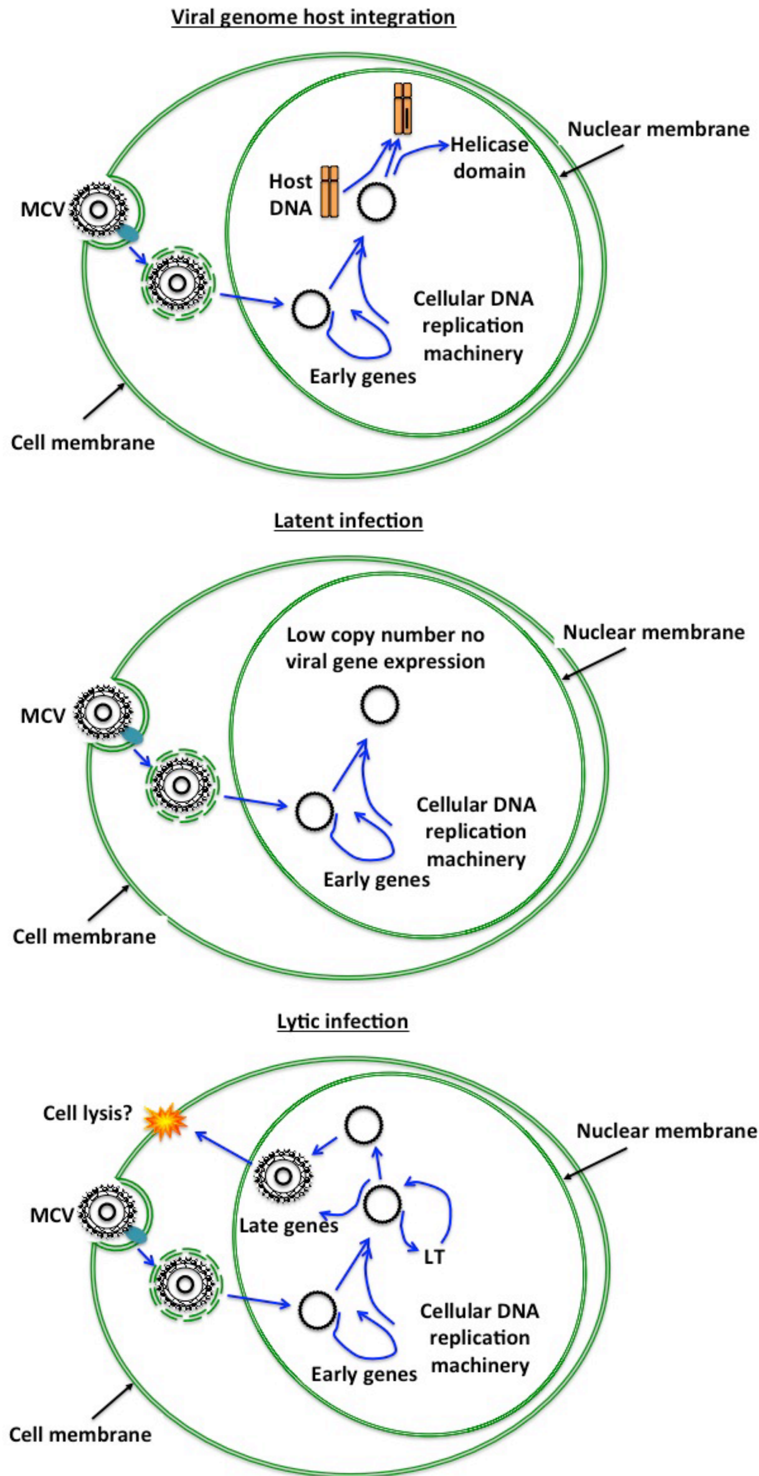

Figure 1-3. MCV Infection and Replication Cycle

1.2. Detection

MCV exposure is commonly found in the general population, starting in newborns and increasing in prevalence as age increases, and at a high prevalence in Merkel cell carcinoma

patients (Dalianis and Hirsch 2013; IARC 2013). MCV infections can be identified by detecting viral DNA and antibodies against MCV. These biomarkers can be examined in the blood, saliva, urine, or specific tissues.

1.2.1. Detection in Fluids

Antibodies

Detection of MCV antibodies in the blood can be achieved by several different immunoassay methods (IARC 2013; Xu et al. 2015). Anti-MCV antibodies are detected by enzyme-linked immunosorbent assays (ELISA), luminex-based multiplex serological assays using VP1 or VP1 plus VP2 virus-like particles (VLP) produced in insect cells, 299 TT cells, glutathione S-transferase (GST)-VP1 recombinant protein (capsomeres), or neutralization assays using MCV pseudovirions produced in human embryonic kidney 293TT cells (Coursaget et al. 2013). High levels of VP1 antibodies are seen in only 7% of people without Merkel cell carcinoma, but they are detected in 65% of Merkel cell carcinoma patients. However, serological tests to detect anti-VP1 antibodies are not equivalent; e.g., assays using VP1 monomers have been shown to underestimate MCV seroprevalence compared with assays using VLPs (Coursaget et al. 2013; Kean et al. 2009). Neutralization assays using MCV pseudovirions have been used to confirm the specificity of the MCV reactivity (Coursaget et al. 2013; Pastrana et al. 2009). Antibody assays have low cross reactivity for other polyomaviruses, like JCV, BKV, or lymphotrophic papovavirus. The level of antibodies correlates with the viral load on the skin and active viral shedding and increases in Merkel cell carcinoma patients.

Detection of antibodies in the blood against early gene products, such as LT or sT, are rare in MCV-infected people who do not have Merkel cell carcinoma (0.9%) compared with Merkel cell carcinoma patients (41%) (Dalianis and Hirsch 2013; IARC 2013). The levels of LT and sT antibodies in patients with Merkel cell carcinoma change with the severity of the cancer and could be used to predict prognosis (IARC 2013).

DNA

MCV DNA can be detected in the blood, saliva, or urine by PCR, nested PCR, real-time PCR, quantitative PCR, and rolling circle amplification (IARC 2013). Common MCV-specific genes used for detection include early genes, LT and sT as well as the late gene VP1. Detection of MCV DNA in bodily fluids indicates an active infection, but will not clearly identify the tissue that is infected (IARC 2013; Xu et al. 2015).

1.2.2. Detection in Cells

Antigens

Antibodies specific for MCV early gene products, sT and LT, have identified MCV by immunohistochemical staining in Merkel cell carcinoma cells and tumor biopsy specimens, producing similar findings as DNA detection (IARC 2013). Viral LT protein expression in Merkel cell carcinoma was demonstrated using monoclonal antibody to a conserved epitope of LT. sT antigen is found in Merkel cell carcinoma more often than LT, and some Merkel cell carcinomas express sT without detectable LT. It appears that Merkel cell carcinoma patients whose tumors score robustly positive for T antigen expression have better survival than MCV-negative Merkel cell carcinoma patients (Moore and Chang 2014; Paulson et al. 2010).

Therefore, antibodies to sT and LT could be used to predict the prognosis of Merkel cell carcinoma patients.

DNA

MCV DNA can be detected in tissues and tumor specimens (Dalianis and Hirsch 2013; IARC 2013; Moens et al. 2015; Moore and Chang 2010). PCR can be used on tissues that have been fixed in formaldehyde and embedded in paraffin, though formaldehyde can lead to DNA fragmentation and may give misleading results in tissues with very low viral loads. Therefore, DNA in fixed tissue might not accurately indicate viral load.

Specific MCV genes (LT and miRNA) have been used for DNA detection in tissue. MCV DNA has been detected in about 80% of Merkel cell carcinoma tumors and most have a truncated LT gene (Dalianis and Hirsch 2013; Moens et al. 2015; Moore and Chang 2010). Since a truncated LT gene that lacks helicase activity is needed for stable host genomic integration and carcinogenesis, the detection of truncated LT gene might be a biomarker specific for carcinogenic risk. The miRNA has been found in about 50% of Merkel cell carcinoma patients and the level of expression correlates with the number of copies of MCV DNA.

1.3. Summary

Merkel cell polyomavirus is a very stable non-enveloped DNA virus found in the skin and integrated into the genome of most Merkel cell carcinomas. Once MCV enters a host cell, its genome is maintained in a form that allows it either to replicate independently or to integrate into the host cell’s genetic material for replication. MCV can exist in either a lytic phase (in which the infected cell is destroyed and viral particles are released) or a latent phase (in which the virus does not replicate). During the latent phase, little viral gene expression occurs, and the virus can evade immune detection. MCV establishes a chronic lifelong infection in a large majority of healthy individuals. The skin appears to be a primary site of MCV infection, and healthy individuals have been shown to chronically shed MCV DNA from the skin surface (Schowalter et al. 2010). MCV has also been reported to infect saliva and mouth, esophagus, and colon (Loyo et al. 2010). MCV is stable at temperatures up to 167°F, so infection can occur from contact with the virus left on surfaces or in water.

2. Human Exposure

This section describes prevalence and transmission (Section 2.1) and non-cancer diseases, prevention, and treatment of MCV (Section 2.2). The material presented in Sections 2.1 and 2.2 is summarized in Section 2.3.

2.1. Prevalence and Transmission

MCV infection is acquired early in life, is near-ubiquitous in adults, is generally asymptomatic, and can result in persistent, life-long infection (Arora et al. 2012a; Chang and Moore 2012; Chen et al. 2011; Chen et al. 2014; Coursaget et al. 2013; IARC 2013; Spurgeon and Lambert 2013).

Age-specific MCV seroprevalence has been reported as 20% in children ages 1 to 5 years, 35% to 50% for those under 10 to 15 years old, and 46% to 87.5% in adults (Chen et al. 2011; IARC 2013; Tolstov et al. 2009; Viscidi et al. 2011). In a study of children with acute lymphoblastic leukemia, neonatal blood spots on filter paper (Guthrie cards) were examined for the presence of polyomaviruses including MCV. All test subjects (N = 50) and controls (N = 100) were found to be MCV negative by real-time PCR assay (Gustafsson et al. 2012). Gustafsson et al. (2012) noted that primers from both the VP1 and LT regions of MCV were used (i.e., coupled LT and VP1 positivity for MCV detection), and all samples were negative for the LT region of MCV; however, 23 of the 150 samples were weakly reactive for the VP1 region of MCV, possibly due to nonspecific amplification or to a very low viral copy number and technical difficulties in amplifying sufficient quantities for sequencing.

U.S. MCV seroprevalence rates have been reported to range from 23% to 88%, indicating that a significant number of people in the United States are exposed to MCV (see Table 2-1) (Carter et al. 2009; Kean et al. 2009; Pastrana et al. 2009; Tolstov et al. 2011; Tolstov et al. 2009; Viscidi et al. 2011). Several test methods are available to determine these levels as described in Section 1.2.1. No analyses of MCV prevalence in blood, serum, or urine specimens from the National Health and Nutrition Examination Survey (NHANES) have been identified.

Table 2-1. U.S. MCV Seroprevalence Rates

Type of Study Group	Total Samples (N)	Prevalence (%)	Detection Method	Reference
Healthy adult blood donors (Denver, CO)	1,501	MCV 350[a] (25%) MCV 339[a] (42%)	VP1 capsomere-based enzyme-linked immunosorbent assay	Kean et al. (2009)
Pediatric plasma samples (Denver, CO)	721	MCV 350 (23%) MCV 339 (34%)		
Age- and sex-matched population-based controls (Seattle, WA)	76	MCPyV w162 (53%)	Multiplex antibody-binding assay and recombinant proteins containing VP1 from MCPyV fused to glutathione S-transferase	Carter et al. (2009)
Serum or plasma from general public control population (Seattle, WA)	451	MCPyV w162 (59%)		
Sera from U.S. commercial donors aged 47 to 75 (Kerrville, TX and Novi, MI)	48	88% MCV	Reporter vector-based neutralization assay to quantitate MCV-specific serum antibody responses in human subjects	Pastrana et al. (2009)
U.S. blood donors in Arizona, Pennsylvania, and New York	166	64% MCV	MCV VLP enzyme-linked immunosorbent assay	Tolstov et al. (2009)
U.S. commercial blood donors (Kerrville, TX and Novi, MI)	100	63% MCV		
Plasma from controls (Tampa, FL)	37	68% MCV	MCV VLP enzyme-linked immunosorbent assay	Viscidi et al. (2011)

MCV or MCPyV = Merkel cell polyomavirus; VLP = virus-like particle; VP1 = viral capsid protein 1.
[a]MCV 350, MCV 339, and MCPyV w162 are different strains of MCV.

Among 5,548 study participants in a large rural Chinese population, overall MCV seroprevalence was 61.0%; seroprevalence was significantly higher in males than in females (64.5% versus 57.7%, $p < 0.001$), and showed a trend to increase with age for both genders (male $p_{trend} < 0.001$, female $p_{trend} < 0.001$) (Zhang et al. 2014). In two study populations in Cameroon, Central Africa, overall seroprevalences of antibodies directed against MCV were 59% (N = 458, 68% of whom were children) and 81% (N = 584, median age = 19 years) (Martel-Jantin et al. 2013). In the study population consisting mostly of children (N = 458), seroprevalence from birth to age 4 months was very similar to seroprevalence in women of childbearing age (approximately 70%). Seroprevalence then decreased with age and reached 0% at age 15 to 16 months, and then increased beginning at age 17 months and reached approximately 60% to 80% at age 4 to 5 years. Martel-Jantin et al. (2013) noted that this seroprevalence pattern in young children is consistent with prevalence of maternal antibodies in very young children, i.e., maternal antibodies progressively disappear, and most children rapidly acquire infection beginning at about age 16 to 18 months.

MCV is found in many different tissues, predominantly on skin surfaces, and healthy individuals have been shown to chronically shed MCV DNA from the skin surface (Pastrana et al. 2012). MCV DNA has been detected in skin samples at up to approximately 28% by polymerase chain reaction (PCR) or nested PCR, up to 40% by rolling circle amplification, and up to 100% by real-time PCR or quantitative PCR (IARC 2013). MCV has also been reported to infect saliva and mouth, esophagus, and colon, as well as the blood and urine; this suggests a systemic distribution (IARC 2013; Loyo et al. 2010). Merkel cell carcinoma has been found to contain up to 0.8 MCV DNA copies per cell (IARC 2013; Shuda et al. 2011). Additionally, MCVs found on one area of skin are genetically identical to the virus found on other areas of skin, further supporting a systemic distribution. It is thought that the viral load is usually higher in the mouth, but that the frequency of detection is higher on the skin where MCV is considered part of normal skin flora.

The mode(s) of transmission of MCV are not fully characterized (IARC 2013). Because MCV has not been detected in fetal autopsy samples, vertical transmission from mother to child does not appear to occur, but the possibility of perinatal transmission at time of delivery has not been excluded. A study of familial aggregation, i.e., the tendency for MCV infection to occur within families, of MCV infection status in Cameroon, Central Africa found statistically significant sib-sib correlation (odds ratio [OR] = 3.2, 95% CI = 1.27 to 9.19, $p = 0.014$), especially between siblings close together in age (<7 years), and a trend for mother-child correlation (OR = 2.71, 95% CI = 0.86 to 8.44, $p = 0.08$), suggesting MCV can be transmitted through close personal contact involving saliva or skin, between young siblings, and between mothers and their children (Martel-Jantin et al. 2013). Further, a cross-sectional study of a large rural Chinese population found that poor personal hygiene (e.g., infrequent bathing) may increase risk of cutaneous transmission of MCV, and that among heterosexual couples, MCV seropositivity of one spouse was significantly related to that of the other partner (adjusted OR = 1.32, 95% CI = 1.07 to 1.62, $p = 0.009$) (Zhang et al. 2014).

MCV DNA detected in the gastrointestinal tract and in urban sewage suggests a possible fecal-oral mode of transmission (Spurgeon and Lambert 2013). MCV has been detected in 85% of environmental surface samples, indicating that transmission from environmental sources to humans is possible (Foulongne et al. 2011; IARC 2013). Because most adults have MCV antibodies, blood transmission is not expected to play a large role in transmission (IARC 2013).

MCV DNA has also been detected in nasopharyngeal aspirates (0.6% to 1.3% in children and 2.1% to 8.5% in adults), tonsils (3.5%), lung tissues (6.7%), bronchoalveolar and bronchoaspirates (17.2%), suggesting possible aerodigestive transmission.

MCV seropositivity has not been found to be associated with other chronic viral infections (e.g., human immunodeficiency virus-1, hepatitis B virus, or hepatitis C virus) (IARC 2013; Tolstov et al. 2011) or with sexual activity (Carter et al. 2009; Tolstov et al. 2011; Zhang et al. 2014). However, concordance for MCV seropositivity between heterosexual couples exists and is likely due to increased non-sexual transmission via respiratory, fecal-oral, or cutaneous routes from frequent close contact or shared family environment (Zhang et al. 2014).

2.2. Diseases, Prevention, Treatment

MCV has not been associated with any other disease or symptoms to date (IARC 2013).

Some cancer treatments target MCV oncoproteins (e.g., MCV-specific treatment based on T antigens to manage MCV-positive Merkel cell carcinomas) (Samimi et al. 2015). At this time, there is no vaccine against MCV (CDC 2011; FDA 2015), and limited vaccine development efforts are ongoing (Gomez et al. 2013; Pastrana et al. 2009; Samimi et al. 2015; Zeng et al. 2012).

2.3. Summary

U.S. seroprevalence study data indicate that a significant number of people living in the United States are exposed to Merkel cell polyomavirus. MCV infection is acquired early in life, is near-ubiquitous in adults, is generally asymptomatic, and can result in persistent, life-long infection. The mode(s) of transmission of MCV are not fully characterized. Studies of MCV infection within families suggest that MCV can be transmitted through close personal contact involving saliva or skin, between young siblings, and between mothers and their children. Vertical transmission from mother to child does not appear to occur, but the possibility of perinatal transmission at time of delivery has not been excluded. Based on detections of MCV DNA in the gastrointestinal tract and in urban sewage, a fecal-oral mode of transmission is possible. Detections of MCV in environmental surface samples indicate that transmission from environmental sources to humans is also possible. Poor personal hygiene (e.g., infrequent bathing) may increase risk of cutaneous transmission of MCV. There is currently no vaccine against MCV, although limited vaccine development efforts are ongoing.

3. Human Cancer Studies

Merkel-cell polyomavirus (MCV) is a recently (2008) discovered polyomavirus that has been studied in relationship to Merkel cell carcinoma. The NTP used the body of knowledge published by IARC (2013) on MCV for studies conducted between 2008 and 2012, combined with new studies that were identified (published between 2012 and 2015) to evaluate the scientific evidence for specific cancer endpoints independently of IARC's conclusions. Where available, IARC data tables of the effect estimates have informed the cancer hazard assessment.

IARC primarily evaluated the relationship between MCV and Merkel cell carcinoma; other cancer endpoints were discussed by IARC, but not reviewed in detail. In this section, only the Merkel cell carcinoma endpoint is evaluated in depth due to sparsely available published literature on other endpoints. MCV detection methods varied across studies, with exposure determined primarily through amplification of viral DNA and other techniques in tissue, and via multiplex-binding assays of serum.

The cancer hazard evaluation of MCV from human cancer studies is divided into three parts: the first briefly summarizes the approach for identifying and selecting the literature specific to MCV (Section 3.1); the second discusses the cancer hazard evaluation for specific cancer endpoints (Sections 3.2 to 3.4); and the last part summarizes the evaluations across endpoints (Section 3.5). The level of evidence from cancer studies in humans also considers studies of tissues from humans in addition to epidemiological studies and is provided in Section 5.

3.1. Selection of the Relevant Literature

A systematic literature search of major databases, citations, and other authoritative sources from 2012 to August 2015 was conducted. Details on the literature search strategy can be found in Appendix A. For the MCV review, all case-control and cohort studies, regardless of cancer endpoint, were identified. These included studies reviewed by IARC (2013) and new epidemiological studies identified in the literature search. The case-control studies may range from broadly defined, non-matched hospital- or population-based case-control designs to formal matched case-control designs. Case-series studies of five patients or more on the relationship between MCV and Merkel cell carcinoma, and published since 2012, were also included in the review. Data from the epidemiological studies included case-series studies and were considered in the overall assessment.

3.2. Cancer Hazard Evaluation: Merkel Cell Carcinoma

This section provides a brief background on Merkel cell carcinoma, summarizes the study findings for each study design, discusses relevant cofactors and integrates the evidence for the association between Merkel cell carcinoma and MCV across study designs. The review consists of six case-series studies, three case-control studies, and one nested case-control study on MCV and Merkel cell carcinoma.

3.2.1. Background Information

Merkel cell carcinoma is a rare and highly aggressive form of skin cancer, with an incidence rate of approximately 4 cases per million (Hodgson 2005). It is most common in whites, males, and

those over 60 (Agelli and Clegg 2003; Schrama et al. 2012). Merkel cell carcinoma has a five-year relative survival rate of 60% to 70% (relative to stage and anatomic site at diagnosis) (IARC 2013; Schrama et al. 2012). There are several risk factors for Merkel cell carcinoma, which include age, with a mean age of onset around 75 years of age, male gender, and race, with Merkel cell carcinoma occurring primarily in Caucasians (Agelli and Clegg 2003; Schrama et al. 2012). Additionally, ultraviolet light is a potential risk factor, with Merkel cell carcinoma most often diagnosed in sun-exposed areas of the skin (Mogha et al. 2010; Spurgeon and Lambert 2013). Merkel cell carcinoma occurs most often in immunocompromised individuals, including transplant recipients (Clarke et al. 2015) and those who are HIV-1 positive (Engels et al. 2002).

3.2.2. Case-series Studies of MCV and Merkel Cell Carcinoma

Since its discovery in 2008, MCV has been identified in up to 80% of Merkel cell carcinoma cases (IARC 2013), with newer studies suggesting over 98% of Merkel cell carcinoma tumors contain MCV (Rodig et al. 2012). Other studies suggest that there are two varieties of Merkel cell carcinoma, one that is MCV infected, and one that is not; however, the existence of a MCV-negative subset is controversial (Moore and Chang 2014). In a meta-analysis of mixed case-series and mechanistic studies, the global prevalence of MCV in Merkel cell carcinoma cases was 79% (Santos-Juanes et al. 2015). In 21 case-series studies of 5 patients or more since 2008, MCV was detected in 716 of 885 Merkel cell carcinoma cases (see Table 2.3 in IARC (2013)). All but six of these case-series studies were reviewed by IARC (2013); the remaining studies are detailed in Table 3-1 below. In the IARC (2013) review, several of the case-series studies reviewed included a control group; however, the IARC Working Group did not consider these true case-control studies, due to convenience sampling or the lack of comparability of exposure measures. In the studies presented here, evidence of MCV infection in Merkel cell carcinoma cases ranged from 42% to 100%; however, only 23% of cytokeratin 20 (CK-20) negative Merkel cell carcinoma cases presented by Miner et al. (2014) were positive for MCV. CK-20 is an epithelial marker positive in approximately 95% of Merkel cell carcinomas. A variety of detection methods were employed in these case-series studies, which may account for the differences in the percentage of cases in which MCV was detected.

Table 3-1. Recent Case-series Studies of MCV and Merkel Cell Carcinoma Published Since the IARC (2013) Review

Author (Year)	n/N Exposed Cases	Percent Exposed to MCV	MCV Tissue Detection Method	Location/Comments
Ly et al. (2012)	17/27	63.0	IHC staining with CM2B4 anti-large T antigen	Canada
Ota et al. (2012)	9/9	100.0	Viral load calculated using digital PCR (small-T DNA primers); anti-CK20, chromogranin A, and synaptophysin IHC; anti-CM2B4 IHC and conventional PCR (6 nested primer sets)	Japan
Chun et al. (2013)	6/7	85.7	PCR and quantitative PCR, and IHC	Korea

Author (Year)	n/N Exposed Cases	Percent Exposed to MCV	MCV Tissue Detection Method	Location/Comments
Hourdequin et al. (2013)	11/11	100.0	Quantitative PCR assays for the detection of 4 MCV genomic targets	United States
Leitz et al. (2014)	19/32	59.4	Qualitative PCR for LT sequences	Germany (not all cases tested with all three methods)
	16/29	55.2	Quantitative PCR for LT sequences in DNA	
	13/31	41.9	IHC staining with CM2B4 anti-large T antigen	
Miner et al. (2014)	3/13	23.0	Quantitative PCR for LT and sT antigens	United States (CK-20-negative MCC)

CK-20 = cytokeratin 20, CM2B4 = antibody to MCV, LT = large T-antigen, IHC = immunohistochemistry, MCC = Merkel cell carcinoma, MCV = Merkel cell polyomavirus, PCR = polymerase chain reaction, sT = small T antigen.

3.3. Case-control Studies

Three case-control studies were identified that investigated the association between MCV and Merkel cell carcinoma. These three studies were reviewed previously by IARC (2013), and are detailed in Table 3-2. In the first study, Carter et al. (2009) investigated the association between MCV antibodies (antibodies to MCVw162 VP1) and Merkel cell carcinoma in 41 (27 male and 14 female) consecutively identified Merkel cell carcinoma cases and 76 (51 male and 25 female) hospital controls frequency matched on age and sex. They found that 36 of 41 cases carried MCV antibodies, compared with 40 of 76 controls (OR 6.6, 95% CI = 2.3 to 18.8, adjusted for age and sex).

A second study by Paulson et al. (2010) included 139 Merkel cell carcinoma cases (including 79 males and 60 females) and 530 controls. Controls were identified through random-digit dialing and were frequency matched by age (within 5 years) and sex to cases. Significant associations were seen between MCV capsid, (OR = 5.5, 95% CI = 2.9 to 11.2), as well as two markers of early gene expression, large T oncoproteins (31 of 139 exposed cases; OR = 16.9, 95% CI = 7.8 to 36.7) and small T oncoproteins (51 of 139 exposed cases; OR = 63.2, 95% CI = 24.4 to 164.0) and Merkel cell carcinoma. Imprecise estimates, particularly for small T oncoproteins, were a result of few seropositive controls within the study.

In a third study (Viscidi et al. 2011), 33 Merkel cell carcinoma patients (25 males and 8 females) and 37 healthy controls were recruited from a family medicine clinic. Of 33 cases, 30 were seropositive for MCV capsid IgG, detected via VLP-based ELISA, while 25 of 36 controls were seropositive ([OR = 4.4, 95% CI = 1.10 to 17.53], p = 0.02; OR and CI were calculated by IARC (2013). After adjusting for age, this association was attenuated (p = 0.32); however, the adjusted effect estimate and confidence intervals were not reported.

Table 3-2. Case-control and Nested Case-control Studies of MCV and Merkel Cell Carcinoma

Author (Year) Country	Serum Detection Method	Exposure Group (MCV + cases; controls)	OR (95% CI)[a]	Covariates	Comments
Case-control studies					
Carter et al. (2009)[b] United States	Multiplex antibody-binding assay to detect MCV VP1 antibody	MCV VPI + (36/41; 40/76)	6.6 (2.3–18.8)	Age, sex	Controls selected from a previous case-control study, matched on age and sex
Paulson et al. (2010)[b] United States	Multiplex antibody-binding assay to detect antibodies to MCV VP1; MCV sT; MCV LT	MCV VPI (139/530) MCV sT (50/139; 5/530) MCV LT (31/139; 9/530)	5.5 (2.9–11.2) 63.2 (24.4–164.0) 16.9 (7.8–36.7)	Age, sex	Controls selected by RDD and frequency matched on age and sex; 66 individuals not matched and not included in analysis
Viscidi et al. (2011) Italy	VLP-based ELISA	VLP + (30/33; 25/36)	[4.4 (0.9–26.7)]	Age	Controls selected from cancer-free individuals attending screenings at a clinic
Nested case-control study					
Faust et al. (2014) Sweden and Norway	Antibodies to MCV measured by neutralization assay and by IgG antibodies to MCV pseudovirions	All participants Any (22/13) High (1/22; 14/79) Neutralizing (10/22; 12/75) Females Any (12/13; 33/47) High (9/13; 10/47) Neutralizing (8/13; 8/47) Males Any (7/9; 24/29) High (2/9; 4/29) Neutralizing (2/9; 4/29)	All participants 2.6 (0.7–15.0) 4.4 (1.3–17.4) 5.3 (1.3–32.3) Females 6.0 (0.8–277) 7.0 (1.6–42.8) 14.3 (1.7–677) Males 1.0 (0.1–12.6) 1.3 (0.1–19.9) 1.3 (0.1–19.9)	Enrollment method, age, sex, county, and length of follow up	Cases and controls from two large biobank cohorts. Matched by enrollment method, age, sex, county, and length of follow up

CI = confidence interval; ELISA = enzyme-linked immunosorbent assay; IgG = immunoglobulin G; LT = large T antigen; MCC = Merkel cell carcinoma; MCV = Merkel cell polyomavirus; OR = odds ratio; PCR = polymerase chain reaction; RDD = random digit dialing; sT = small T antigen; VLP = virus-like particle; VP1 = viral capsid protein 1.
[a]ORs in brackets were calculated by NTP.
[b]Study populations for Carter et al. (2009) and Paulson et al. (2010) overlap to an unknown extent.

3.3.1. Nested Case-control Study

A prospective nested case-control study (Faust et al. 2014) utilized two large biobank cohorts containing samples from over 856,000 individuals in Sweden and Norway. Cases were identified through cancer registries and linked to samples in the biobanks. A total of 22 cases with samples in the biobank were identified. Four healthy controls (alive and cancer free at the time the case was diagnosed) were matched to each case on enrollment method, age, sex, county, number of samples, and length of follow-up. The risk for future Merkel cell carcinoma was associated with both baseline presence of neutralizing MCV antibodies (10 of 22 cases exposed; OR = 5.3, 95% CI = 1.3 to 32.3) and with the presence of high levels of MCV antibodies (11 of 22 cases exposed; OR = 4.4, 95% CI = 1.3 to 17.4), along with an elevated, but non-significant risk for any level of MCV antibodies (19 of 22 cases exposed; OR = 2.6, 95% CI = 0.7 to 15.0). When stratified by gender, the risk for Merkel cell carcinoma in females was significantly associated with both the baseline presence of neutralizing MCV antibodies (8 of 13 cases exposed; OR = 14.3, 95% CI = 1.7 to 677, and the presence of high levels of MCV antibodies (9 of 13 cases exposed; OR = 7.0, 95% CI = 1.6 to 42.8). There was also an elevated, but statistically non-significant association between Merkel cell carcinoma and any level of MCV antibodies (OR = 6.0, 95% CI = 0.8 to 277). No association between Merkel cell carcinoma and MCV was seen in males, which may be related to the small sample size of males in this study. This study was described in Table 3-2.

3.3.2. Cofactors

There is limited and conflicting evidence as to whether co-infection with HIV-1 increases the risk of MCV infection (Tolstov et al. 2011; Wieland and Kreuter 2011). However, prior to or shortly after the discovery of MCV, several studies reported increased risks of Merkel cell carcinoma among HIV-1-positive populations (Engels et al. 2002; Izikson et al. 2011; Lanoy et al. 2009), organ transplant recipients, and other immunocompromised patients (Clarke et al. 2015; Heath et al. 2008; Lanoy and Engels 2010); see also accompanying monograph on HIV-1. To date, no studies have been identified that have measured MCV viral load in healthy tissues from Merkel cell carcinoma cases, however, and no other cofactors of the relationship between MCV and Merkel cell carcinoma have been identified to date. Additionally, no studies have been identified to date that have measured MCV among HIV-1-positive or immunocompromised Merkel cell carcinoma cases.

Ultraviolet (UV) radiation has been identified as a risk factor for Merkel cell carcinoma. UV radiation is both mutagenic and immunosuppressive and evidence that exposure to UV light is an important cofactor in Merkel cell carcinoma development includes the following: Merkel cell carcinoma incidence is higher at equatorial latitudes, more than 80% of primary tumors occur on sun-exposed skin, and Caucasians have the highest risk (Agelli et al. 2010; Amber et al. 2013; Becker et al. 2009a). An in vivo study using human volunteers showed that after UV exposure, there was induction of the sT transcript that was attributed to MCV activation and a luciferase-based in vitro study confirmed that the sT promoter was UV-inducible (Mogha et al. 2010).

These and other potential cofactors in the Merkel cell carcinoma/MCV relationship have not been evaluated in epidemiological studies.

3.3.3. Integration of the Evidence across Studies

MCV is present in over 80% of Merkel cell carcinoma cases. As MCV is a newly identified virus, only a handful of epidemiological studies have been conducted; however, there is credible evidence of an association between Merkel cell carcinoma and MCV. The three case-control and one nested case-control studies found statistically significant associations, with ORs ranging from 4.4 to 63.2. Nevertheless, it is noteworthy that the study populations of Carter et al. (2009) and Paulson et al. (2010) overlap to an unknown extent; although there are at least 98 unique cases in Paulson et al. (2010). Additionally, there is some evidence of an increased risk at higher levels of MCV exposure, evidenced by Faust et al. (2014), where ORs in populations with high levels of antibodies were higher than those for any MCV exposure. Faust et al. (2014) also demonstrated a temporal relationship between Merkel cell carcinoma and MCV. This study is also suggestive of an effect modification by gender, with a stronger association in females than in males; however, fewer male cases were reported in the study. The study by Paulson et al. (2010) demonstrated a strong association between MCV T antigen (both LT and sT) antibodies and virus detection in Merkel cell carcinomas. Further, antibodies to T antigens (but not to MCV capsid protein) varied greatly over time in infected patients and reflected tumor burden. Due to the paucity of studies and lack of known risk factors for MCV infection in relation to Merkel cell carcinoma, confounding cannot be ruled out. Moreover, the lack of a gold standard detection method for MCV is a limitation in each of the reviewed studies, which might lead to exposure misclassification.

3.4. Cancer Hazard Evaluation: Other Cancer Endpoints

A small number of case-control studies have investigated the association between MCV and other cancer endpoints, including acute lymphoblastic leukemia (Gustafsson et al. 2012), skin squamous-cell carcinoma (Rollison et al. 2012), bladder cancer (Polesel et al. 2012; Robles et al. 2013), esophageal cancer (Sitas et al. 2012), chronic lymphocytic leukemia (Robles et al. 2015; Robles et al. 2012). To date, there is insufficient evidence to fully evaluate these cancer endpoints; however, two cancer endpoints (chronic lymphocytic leukemia and lung carcinoma) are shown in Table 3-3 and discussed below. For findings on other endpoints, see IARC (2013) Table 2.2.

Four case series and reports have been identified investigating the prevalence of MCV in chronic lymphocytic leukemia cases and included a total of 345 chronic lymphocytic leukemia cases. Between 4% and 74% of cases were positive for MCV, depending on the MCV detection method (Cimino et al. 2013; Imajoh et al. 2012; Peretti et al. 2014; Tolstov et al. 2010). In addition to differences in methods for measuring exposure to MCV (e.g., viral load, MCV DNA) this wide range in prevalence may be due to differences in tissues or aims of the study. For example, in the Peretti et al. (2014) study, 243/293 chronic lymphocytic leukemia cases were MCV positive when MCV was tested in the hair bulb, and 0/293 were positive in skin lesions.

Two case-control studies and one nested case-control study on the association between chronic lymphocytic leukemia and MCV were identified. Robles et al. (2015; 2012) conducted two case-control studies and found mixed results; a non-significant positive association between MCV and chronic lymphocytic leukemia (OR = 1.49, 95% CI = 0.80 to 2.75) in the first study, and non-significant negative association in the later study (OR = 0.79, 95% CI = 0.54 to 1.16). In a nested

case-control study, 42/66 chronic lymphocytic leukemia cases were seropositive for MCV prior to diagnosis (OR = 0.80, 95% CI = 0.47 to 1.37) (Teras et al. 2015).

Nine case-series studies have been identified that looked at the prevalence of MCV in lung carcinomas. In five studies of small-cell lung carcinoma patients, 11% (14/129) were MCV positive (Andres et al. 2009; Helmbold et al. 2009; Joh et al. 2010b; Karimi et al. 2014; Wetzels et al. 2009). In five studies of non-small-cell lung carcinoma patients, 13% (71/529) were MCV positive. A case-series study on extrapulmonary small-cell lung carcinoma (Hourdequin et al. 2013) found 19% (3/16) of cases to be MCV positive.

Table 3-3. Case-control Studies of MCV Lymphomas or Leukemia

Author (Year)	Detection Method	Cancer Endpoint	MCV+ Cases/ Total Cases (%)	MCV+ Controls/ Total Controls (%)	OR (95% CI)	Covariates
Chronic lymphocytic leukemia						
Robles et al. (2015)	Bead-based multiplex serology	CLL	All cases 233/289 (80.5%)	260/310 (83.8%)	All cases 0.79 (0.54–1.16)	–
			1st Tertile: 52/123 (42.6%)		Reference group	
			2nd Tertile: 41/109 (37.7%)		0.92 (0.63–1.34)	
			3rd Tertile: 11/57 (19.7%)		0.46 (0.30–0.70)	
Robles et al. (2012)	MCV VP1 VLP enzyme immunoassay	All lymphomas	400/468 (85%)	448/552 (81.2%)	1.34 (0.95–1.88)	Age, sex, study center
		CLL	94/108 (87%)	NR	1.49 (0.80–2.75)	
Teras et al. (2015)[a]	MCV serology	CLL/SLL	All cases 42/66 (63.75%)	383/557 (68.8%)	All cases 0.80 (0.47–1.37)	Age, sex, race, birth, blood draw date
			High titer 19/42 (45.2%)[b]	Low titer 23/42 (54.8%)	1.0 (ref, low titer) 0.83 (0.44–1.59)[b]	

CLL = chronic lymphocytic leukemia; MCC = Merkel cell carcinoma; MCV = Merkel cell polyomavirus; OR = odds ratio; PCR = polymerase chain reaction; SLL = small lymphocytic lymphoma; VLP = virus-like particle; VP1 = viral capsid protein 1.

[a]Nested case control study.

[b]Among seropositive participants only, median fluorescence intensity value for MCV antigen was used as the cutpoint.

3.5. Synthesis across Cancer Endpoints

A summary of the evidence for MCV infection and the different cancer endpoints from epidemiological studies is provided in Table 3-4. The level of evidence from cancer studies in humans also considers studies of tissues from humans in addition to epidemiological studies and is provided in Section 5.

Table 3-4. Summary of MCV Cancer Endpoints and Strength of the Epidemiological Evidence

Cancer Endpoint	Strength of Evidence
Merkel cell carcinoma	Consistent evidence seen in several epidemiological studies, including one prospective study. All studies found a positive association between MCC and MCV. MCV found in >80% of MCC tumors.
Chronic lymphocytic leukemia	Inconsistent evidence of an association in case series and epidemiological studies.
Lung carcinoma	Inconsistent evidence of an association seen in case series. No adequate epidemiological studies have been conducted.

MCC = Merkel cell carcinoma, MCV = Merkel cell polyomavirus.

4. Mechanisms and Other Relevant Data

To date, Merkel cell carcinoma is the only neoplasm associated with MCV (see Section 3); however, an etiologic role has been suggested for some cases of small-cell lung cancer (Helmbold et al. 2009), non-small cell lung cancer (Gheit et al. 2012; Hashida et al. 2013; Joh et al. 2010a; Lasithiotaki et al. 2013), and some hematologic malignancies (Teman et al. 2011). MCV was identified as a causal factor in Merkel cell carcinoma after it was found clonally integrated into the cellular DNA of approximately 80% of Merkel cell carcinoma tumors examined (Becker et al. 2009b; Feng et al. 2008; Kassem et al. 2008; Martel-Jantin et al. 2014; Rodig et al. 2012). In contrast, MCV DNA is maintained as a circular episome in the host cell during productive infection (Pastrana et al. 2009). This section reviews the following topics: the general characteristics of MCV and risk factors for Merkel cell carcinoma (Section 4.1), MCV and cancer hallmarks (Section 4.2), the mode of action and evidence for MCV's role in Merkel cell carcinoma (Section 4.3), and provides a brief synthesis of the mechanistic data (Section 4.4).

4.1. Characteristics and Risk Factors

The biological properties and other characteristics of MCV were described in Section 1. MCV is part of the normal human skin flora as it is chronically shed from human skin (Schowalter et al. 2010). It follows that asymptomatic MCV infection is common (Amber et al. 2013; Chen et al. 2011; IARC 2013; Tolstov et al. 2009). Merkel cell carcinoma is a rare but aggressive primary neuroendocrine carcinoma that is thought to arise in Merkel cells (a type of mechanoreceptor cell located in the stratum basale of the skin) (Stakaityte et al. 2014). These tumors occur most frequently (>90%) in sun-exposed areas, particularly around the head and neck, but can occur almost anywhere on the body.

Oncogenic viruses, including MCV, generally cause cancer by dysregulation of cell growth and proliferation; however, additional factors (e.g., immunosuppression, chronic inflammation, environmental agents) increase the risk for malignant transformation (Mesri et al. 2014). The infectious nature of oncogenic viruses distinguishes them somewhat from other cancer-causing agents (Ahuja et al. 2014). Although only a small percentage of individuals infected with an oncogenic virus develop cancer (this is typical for exposure to any carcinogenic agent), chronic infection provides the virus with a prolonged opportunity to mount mutagenic and epigenetic events that increases the risk of cell transformation and malignancy. Several critical alterations in a cell's physiology (i.e., cancer hallmarks) have been identified that are required for malignant transformation (Hanahan and Weinberg 2000; 2011). The following sections discuss cofactors and cancer hallmarks that have been associated with MCV-induced Merkel cell carcinoma.

In addition to MCV, additional risk factors for Merkel cell carcinoma include immunosuppression, UV exposure, and advanced age (Agelli et al. 2010; Amber et al. 2013; Becker et al. 2009a; Dalianis and Hirsch 2013; Spurgeon et al. 2015; Spurgeon and Lambert 2013; Teman et al. 2011). Chronically immunosuppressed individuals (e.g., chronic lymphocytic leukemia, autoimmune disease, organ transplant, and HIV/AIDS patients) are more than 15 times more likely to develop Merkel cell carcinoma than their age- and sex-matched controls (Becker et al. 2009a). Merkel cell carcinoma in immunosuppressed individuals also has a higher mortality rate than in non-immunosuppressed individuals and occurs at a significantly younger age (about 50% <50 years compared with a mean age of 70 for all Merkel cell carcinoma cases).

Further, partial regression of metastatic Merkel cell carcinoma has been reported following discontinuation of immunosuppressive therapy. Severe immunosuppression appears to increase the risk of Merkel cell carcinoma more than that of malignant melanoma (ratio of melanoma to Merkel cell carcinoma in the general population of 65:1 compared to 6:1 in the post-transplant population) (Agelli et al. 2010).

UV radiation is both mutagenic and immunosuppressive and may contribute to Merkel cell carcinoma development (see Section 3.3.2). In addition, C to T and CC to TT transition mutations have been identified in the *p53* and H-*ras* genes of Merkel cell carcinoma and are considered diagnostic of UV-induced DNA damage (Agelli et al. 2010; Popp et al. 2002; Van Gele et al. 2000).

4.2. MCV and Cancer Hallmarks

All mammalian cells carry similar molecular machinery that regulates proliferation, differentiation, and cell death (Hanahan and Weinberg 2000; 2011). Transformation of a normal cell into a cancer cell is a multistep process that involves genetic and epigenetic changes that disrupts the cell's molecular machinery and promotes malignant growth. These changes result in several critical alterations that are recognized as hallmarks of cancer and include: (1) sustained growth factor signaling, (2) evading growth suppressors, (3) resisting cell death, (4) enabling replicative immortality, (5) inducing angiogenesis, (6) activating invasion and metastasis, (7) evading immune destruction, and (8) reprogramming of energy metabolism. Genomic instability and inflammation underlie these changes and foster their acquisition and development. Several of these cancer hallmarks have been identified in the pathogenesis of MCV-positive Merkel cell carcinoma and are briefly reviewed here.

4.2.1. Growth Factor Signaling

Merkel cell carcinoma is a rare but aggressive skin tumor that grows rapidly and, if untreated, may double in size within a week (Becker et al. 2009a; Houben et al. 2009). Normal cells require mitogenic growth signals in order to move from a quiescent state into an active proliferative state (Hanahan and Weinberg 2000). These signals are transmitted into the cell via transmembrane receptors that bind distinctive classes of signaling molecules. In contrast, tumor cells are able to generate their own growth signals and are not as dependent on exogenous growth stimulation. Many oncogenes in cancer cells act by mimicking normal growth factor signals. Further, growth factor receptors are overexpressed or modified in many cancers, resulting in an enhanced response to circulating levels of exogenous growth factor signals or constitutive expression. Relevant growth factor signaling changes reported for Merkel cell carcinoma include a novel single heterozygous base change in exon 10 of the platelet-derived growth factor (PDGF) receptor and heterozygous loss of chromosome 10 or the long arm of chromosome 10 where the tumor suppressor phosphatase and tensin homologue (PTEN) is encoded (Fernandez-Figueras et al. 2007; Houben et al. 2009; Swick et al. 2008; Van Gele et al. 2001; Van Gele et al. 1998). However, it was not clear if the base change in PDGF represented a true mutation or a single nucleotide polymorphism (Houben et al. 2009; Swick et al. 2008). No mutations were observed that affected the mitogen activated protein kinase (MAPK) pathway (Houben et al. 2006).

4.2.2. Evading Growth Suppressors

Normal tissues maintain cellular quiescence and homeostasis through multiple antiproliferative signals or growth suppressors (Hanahan and Weinberg 2000; 2011). In addition to inducing and sustaining growth-stimulatory signals, cancer cells must evade powerful antigrowth signals, many of which depend on the actions of tumor suppressor genes. In particular, the retinoblastoma and p53 tumor suppressor pathways are interconnected and operate as central control nodes to regulate cell proliferation, senescence, and apoptosis (Hanahan and Weinberg 2000; 2011; Houben et al. 2009; Yamasaki 2003). Retinoblastoma transduces growth-inhibitory signals originating primarily from outside the cell while p53 receives input from stress sensors, e.g., DNA damage, within the cell (Hanahan and Weinberg 2011; Houben et al. 2009). When in a hypophosphorylated state, retinoblastoma protein blocks cell proliferation by sequestering and altering the function of E2F transcription factors that control the expression of genes essential for progression from G1 to S phase. Phosphorylation of retinoblastoma by cyclin/cyclin-dependent kinase complexes causes dissociation of the retinoblastoma-E2F complex and cell-cycle entry (Sihto et al. 2011). The retinoblastoma and/or p53 pathways are dysregulated in virtually all human tumors (Yamasaki 2003).

Several studies have shown that the retinoblastoma pathway is critical to Merkel cell carcinoma pathogenesis while *p53* mutations are rare (Bhatia et al. 2010; Borchert et al. 2014; Cimino et al. 2014; Harms et al. 2013; Higaki-Mori et al. 2012; Houben et al. 2012; Kuwamoto 2011; Lassacher et al. 2008; Sahi et al. 2014; Sihto et al. 2011). Merkel cell carcinoma retinoblastoma expression has a strong positive association with MCV DNA and MCV large T-antigen (LT) expression, suggesting that retinoblastoma inhibition is important for MCV-induced tumorigenesis (Sihto et al. 2011). Although LT expression was not associated with expression of phosphorylated retinoblastoma, LT binds to retinoblastoma, thus reducing retinoblastoma-E2F complex formation and inhibiting its cell-cycle regulation function. The oncogenic role of LT is discussed further in Section 4.3.2.

Cimino et al. (2014) reported that the retinoblastoma pathway was dysregulated in both MCV-negative and MCV-positive Merkel cell carcinoma cases and proposed two separate pathways of Merkel cell carcinoma oncogenesis. In MCV-positive cases, the retinoblastoma protein is functionally inactivated as described above. In MCV-negative cases, Merkel cell carcinoma tumors had truncating, nonsense mutations in the retinoblastoma gene. Mutations in the *p53* gene were found primarily in LT- or retinoblastoma-negative tumors suggesting possible involvement of p53 in MCV-negative tumors (Sihto et al. 2011). Additionally, hypermethylation of the *p14ARF* promoter DNA has been reported in about 40% of Merkel cell carcinoma samples (Lassacher et al. 2008). Silencing of *p14ARF* could cause inactivation of the p53 pathway. Asioli et al. (2007) also reported that 25 of 47 cases of Merkel cell carcinoma were positive for p63 expression (a member of the p53 family) and that these cases demonstrated a more aggressive clinical course. In contrast, Higaki-Mori et al. (2012) showed no significant correlation of MCV infection and survival with p63 expression. However, the p63 gene is frequently amplified or overexpressed in human cancers (Asioli et al. 2007).

4.2.3. Apoptosis

Apoptosis is controlled by both intrinsic and extrinsic signaling pathways and involves counterbalancing pro- and antiapoptotic members of the Bcl-2 family of regulatory proteins

(Adams and Cory 2007; Hanahan and Weinberg 2011). Bcl-2, and related proteins inhibit apoptosis by binding to and suppressing proapoptotic triggering proteins (Bax and Bak) that are embedded in the mitochondrial outer membrane. Overexpression of anti-apoptotic proteins or loss of pro-apoptotic signals results in loss of tissue homeostasis and supports oncogenesis by allowing cancer cells to evade programmed cell death. Bcl-2 overexpression is a common finding in many tumors and has been observed in approximately 67% to 85% of Merkel cell carcinoma tumors examined (Feinmesser et al. 1999; Houben et al. 2009; Kennedy et al. 1996; Sahi et al. 2012). However, Bcl-2 protein expression was not correlated with the MCV status of the tumors (Sahi et al. 2012). The presence of MCV also was associated with deregulated expression of the *Bcl-2* gene in several cases of non-small-cell lung cancer (Lasithiotaki et al. 2013). *Bcl-2* expression was downregulated in MCV-positive lung tumors compared with MCV-negative tumors ($p = 0.05$) or healthy tissue ($p = 0.047$), and the *Bax/Bcl-2* ratio was 0.97 for the lung cancer group compared to 8.06 for the controls. A *Bax/Bcl-2* ratio of <1 is associated with a lower apoptotic index (Brambilla et al. 1996). In addition, *Bcl-2* inhibition was associated with Merkel cell carcinoma tumor shrinkage in an in vivo SCID mouse/human Merkel cell carcinoma xenograft model (Schlagbauer-Wadl et al. 2000).

The antiapoptotic protein survivin was also overexpressed in Merkel cell carcinoma tissue and was found to have a critical role in the survival of MCV-positive Merkel cell carcinoma cells (Arora et al. 2012b; Dresang et al. 2013; Kim and McNiff 2008; Sahi et al. 2012). A sevenfold increase in mRNA encoding the survivin oncoprotein was reported for MCV-positive compared with MCV-negative Merkel cell carcinoma tumors (Arora et al. 2012b). Xie et al. (2014) reported that decreased transcript and protein detection of the survivin gene in MCV-negative Merkel cell carcinoma cells was due to overexpression of microRNA (miRNA) miR-203. miR-203 functions as a tumor suppressor that is downregulated in certain cancers, and its expression was significantly lower in MCV-positive tumors compared with MCV-negative tumors. Nuclear staining for survivin was also associated with an aggressive clinical course and poor prognosis (Kim and McNiff 2008).

4.2.4. Angiogenesis

New blood vessel growth, or angiogenesis, is essential to sustain neoplastic development. To accomplish this growth, an “angiogenic switch” is almost always activated and remains on, causing normally quiescent vasculature to sprout new vessels to supply the growing tumor (Hanahan and Weinberg 2011). Increased expression of vascular endothelial growth factor (VEGF) via activation of hypoxia-inducible factor (HIF-1) is commonly observed in cancer. Although HIF pathway activation was not demonstrated with Merkel cell carcinoma, there was a significant association between metastatic tumor spread and elevated expression of VEGF (Fernandez-Figueras et al. 2007). Another study reported that VEGF-A, VEGF-C, and VEGF-receptor 2 were expressed in 91%, 75%, and 88%, respectively, of the 32 Merkel cell carcinomas examined (Brunner et al. 2008).

4.2.5. Immune Evasion

The initial event in MCV-induced Merkel cell carcinoma is most likely a loss of immune surveillance for the virus as evidenced by increased risk in immunosuppressed populations (Amber et al. 2013; Hughes and Gao 2013; Moore and Chang 2010). MCV-related cases of Merkel cell carcinoma display vigorous antibody responses to MCV structural proteins;

however, Merkel cell carcinoma tumors do not express detectable amounts of MCV VP1 capsid protein (Pastrana et al. 2009). These data suggest that the strong humoral responses in Merkel cell carcinoma patients are primed by an unusually robust MCV infection rather than Merkel cell carcinoma tumor viral antigen expression and that loss of cellular immune control may allow more extensive viral spread before tumor development (Moore and Chang 2010; Pastrana et al. 2009). Other mechanisms of immune evasions include downregulation of genes associated with the innate immune response (e.g., CCL20, CXCL-9, IL-2, IL-8, TANK) (Moens et al. 2015; Stakaityte et al. 2014), downregulation of Toll-like receptor 9 (TLR9) (Griffiths et al. 2013), and disruption of inflammatory signaling via inhibition of the NF- κB essential modulator (NEMO) adaptor protein (Shahzad et al. 2013). TLR9 is a key receptor in the host innate immune response that senses viral or bacterial dsDNA. Mouchet et al. (2014) compared transcriptional profiles in MCV-positive Merkel cell carcinoma cells and normal Merkel cells and reported that most of the downregulated genes were related to immune interactions.

A common feature of oncogenic viruses is that they persist in the host as a latent or pseudo-latent infection that generally does not replicate to form infectious virus particles (also known as lytic replication) in tumor cells (Moore and Chang 2010). Latent infection serves as an immune evasion strategy that allows the virus to hide from the immune system by turning off unnecessary viral proteins that might be detected by cell-mediated immunity. When latent viruses switch to lytic infection, virus replication generates pathogen-associated molecular patterns that trigger DNA damage responses and innate immune signaling. These cellular responses result in death of the infected cell and release of infectious virions. Integration of small DNA tumor viruses, such as MCV, into the nascent tumor cell eliminates their ability to replicate as virions (a state of pseudo-latency). Monoclonal integration of viral DNA within individual tumors provides the primary evidence that MCV causes most cases of Merkel cell carcinoma (Moore and Chang 2010; Pastrana et al. 2009). These concepts are discussed further in the following sections.

4.3. Mode of Action and Evidence for Cancer Causation

As discussed in the Overview and Introduction Section, it is difficult to apply stringent criteria, such as Hill's considerations, for determining that a human tumor virus is oncogenic (Moore and Chang 2010; zur Hausen 2001). Moore and Chang (2010) concluded that the MCV association with Merkel cell carcinoma could not be established strictly by using Hill's epidemiological considerations for several reasons. First, MCV infection is ubiquitous while Merkel cell carcinoma is very rare and measurement of the total MCV burden does not reflect the tumor-causing form of the virus (i.e., the virus must be mutated and integrated into the host genome). Secondly, many studies report two clinical forms of Merkel cell carcinoma—one type that is MCV infected (predominant form) and one type that is not. These studies also suggest the MCV-positive cases have a better prognosis than MCV-negative cases. In addition to the usual criteria used by epidemiologists to determine causality, other factors, including molecular evidence, should be considered as proposed by IARC (1997) and zur Hausen (1994; 2001). The major lines of evidence supporting the role of MCV in Merkel cell carcinoma include the following: (1) the increased incidence of Merkel cell carcinoma in immunodeficient individuals indicates an infectious etiology, (2) the infectious agent was identified as MCV, (3) the MCV genome was monoclonally integrated in most Merkel cell carcinoma samples, (4) the integration event invariably was associated with truncating mutations in the large T antigen-coding sequence that lead to loss of the helicase domain but retention of the retinoblastoma binding domain and small

T antigen coding, (5) expression of T antigens only in tumor cells in MCV-infected tumors, and (6) knockdown of T antigens leading to growth arrest and cell death in MCV-positive Merkel cell carcinoma samples (Gjoerup 2012). As a result, the available data provide strong support that MCV is an etiologic factor in most cases of Merkel cell carcinoma and are reviewed below.

4.3.1. Presence and Persistence of MCV in Merkel Cell Carcinoma

MCV has been detected in approximately 70% to 97% of Merkel cell carcinoma cases (Amber et al. 2013; Feng et al. 2008; IARC 2013; Rodig et al. 2012; Sihto et al. 2009; Spurgeon and Lambert 2013; Stakaityte et al. 2014). The data suggest that Merkel cell carcinoma can develop through both a virus-mediated pathway in which MCV promotes tumorigenesis and possibly, in a minority of cases, a nonvirus-mediated pathway (Amber et al. 2013; Martel-Jantin et al. 2012). However, some have suggested that the presence of MCV in Merkel cell carcinoma is more common than reported and that improved detection methods might reveal that all Merkel cell carcinoma specimens contain MCV DNA (Rodig et al. 2012). Martel-Jantin et al. (2012) also reported that MCV detection was much higher in fresh-frozen biopsies than in formalin-fixed paraffin-embedded biopsies. Carter et al. (2009) reported that while 77% (24/31) of Merkel cell carcinoma samples were positive for MCV, 92% (22/24) of these patients were positive for antibodies to MCV. These results raise the possibility that MCV is involved in Merkel cell carcinoma initiation but may not be required to maintain the cancer phenotype and may explain why some Merkel cell carcinomas are negative for MCV DNA. Virus-negative carcinomas might indicate advanced tumor stages with a secondary loss of virus genomes (Niller et al. 2011). It is well documented that viral genomes, either inserted into the host cellular DNA or co-replicating with it in episomal form, can be subsequently lost from neoplastic cells (i.e., a "hit and run" mechanism). The number of copies of the MCV genome found integrated in Merkel cell carcinoma ranges widely from less than one to several thousand copies (Bhatia et al. 2010; DeCaprio and Garcea 2013; Laude et al. 2010; Rodig et al. 2012; Shuda et al. 2009).

4.3.2. Viral Oncogenes and Maintenance of the Malignant Phenotype

The MCV genome consists of about 5,400 base pairs that are divided into early and late coding regions separated by a noncoding regulatory region (see Section 1.1.2) (Chang and Moore 2012; Spurgeon and Lambert 2013; Stakaityte et al. 2014). The early coding region expresses overlapping nonstructural transcripts from a single T antigen locus that are differentially spliced to form small T (sT) and large T (LT) antigens. The late region encodes MCV structural proteins and a miRNA (MCV-miR-M1-5p) that is encoded antisense to the LT coding region. The miRNA is thought to downregulate expression of the early genes and may have a role in cellular transformation (Lee et al. 2011; Seo et al. 2009). Viral miRNA was detected in half of MCV-positive tumors but was not detected in MCV-negative tumors (Lee et al. 2011).

The sT and LT antigens are critical for viral replication, manipulation of the host cell cycle, and cellular transformation (Amber et al. 2013; Angermeyer et al. 2013; Stakaityte et al. 2014). About 75% of Merkel cell carcinoma tumor samples are positive for LT, while about 92% are positive for sT (Stakaityte et al. 2014). In normal infections in permissive cells, MCV completes its replication cycle in the cell nucleus to form virions without inducing tumorigenesis. The T antigens are transcribed immediately upon entry into the nucleus of the host cell and induce the cell to enter S-phase. However, mutagenic events can cause MCV integration into host cell DNA and truncation of LT (see In vitro studies Section below). In these circumstances, expression of

sT and mutated LT dysregulate cell proliferation and prevent apoptosis primarily through interactions with retinoblastoma and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) (Houben et al. 2012; Hughes and Gao 2013; Shuda et al. 2011). Expression of truncated LT also inhibits key responses to UV radiation-induced DNA damage (i.e., DNA repair and cell-cycle defects) and suggests that progressive MCV-mediated genomic instability contributes to Merkel cell carcinoma (Demetriou et al. 2012). This may explain why most cases of Merkel cell carcinoma occur on chronically sun-exposed skin. Mutational analyses have yet to identify other signature mutations in Merkel cell carcinoma (Erstad and Cusack 2014). However, Van Gele et al. (1998) reported that Merkel cell carcinoma cases showed a characteristic pattern of chromosomal gains and losses that were similar to that seen in small-cell lung carcinoma. The roles of LT and sT in cellular transformation and oncogenesis are discussed in the following sections.

In vivo Studies

Recent studies show that MCV T antigens have oncogenic activity in vivo in transgenic mice (Spurgeon et al. 2015; Verhaegen et al. 2014). Spurgeon et al. (2015) developed a mouse model that used keratin 14-mediated Cre recombinase-induced expression of MCV truncated LT and wild-type sT antigens in the skin (*K14Cre-MCPyV168* mice). Expression of Merkel cell carcinoma tumor-derived MCV T antigens promoted hyperplasia, hyperkeratosis, acanthosis, and papilloma formation in the stratified epithelium of the skin with additional abnormalities occurring in footpads, whisker pads, and eyes. Evidence for neoplastic progression included increased cellular proliferation, unscheduled DNA synthesis, increased E2F-responsive genes, disrupted differentiation, and activation of DNA damage response. Similarly, Verhaegen et al. (2014) reported that expression of MCV sT antigen alone was sufficient for rapid neoplastic transformation in vivo in a panel of transgenic mouse models. sT antigen-expressing embryos exhibited hyperplasia, impaired differentiation, increased proliferation, apoptosis, and activation of a DNA damage response in epithelia. Mutation of the sT antigen-binding domain resulted in loss of transforming activity, thus, identifying this domain as critical for in vivo transformation. Mogha et al. (2010) demonstrated that simulated solar radiation caused a dose-dependent increase of sT antigen transcripts in human volunteers infected with variants of MCV in episomal form. These data might explain the association between Merkel cell carcinoma and UV exposure.

In vitro Studies

All LT sequences recovered from primary Merkel cell carcinoma tumors or tumor-derived cell lines harbor signature mutations (Borchert et al. 2014; Schmitt et al. 2012; Shuda et al. 2008; Stakaityte et al. 2014). These mutations cause premature truncation of the entire C-terminal domain, which leads to the loss of domains associated with viral replication (i.e., origin binding domain and the ATPase/helicase region). Deletions of C-terminal LT sequences appear to be a highly specific surrogate marker for MCV-induced malignancy (Schmitt et al. 2012). Although the sites of mutations are randomly distributed from different tumors, the retinoblastoma-binding motif is preserved (Borchert et al. 2014; Shuda et al. 2008). Integration of MCV genomes with full-length LT capable of initiating host DNA replication would result in unlicensed replication, replication fork collision, DNA breakage, and cytopathic cell death (Shuda et al. 2008; Stakaityte et al. 2014). Full-length MCV LT also showed a decreased potential to support cellular proliferation, focus formation, and anchorage-independent cell growth via activation of host DNA damage responses and upregulation of p53 downstream target genes (Li et al. 2013).

Infected cells containing a wild-type episomal MCV genome can be transformed into a tumor cell containing multiple copies of an integrated mutant viral genome via two distinct models (DeCaprio and Garcea 2013). LT truncation and amplification of viral genome copy number may occur before or after random integration into the host genome (Figure 4-1). If wild-type MCV is integrated into the host genome, then it must be followed by an LT mutation to disable viral replication. The integrated mutant genome could subsequently undergo copy number amplification. In cases where the LT mutation occurs first, the mutant genome could undergo rolling-circle amplification prior to integration (DeCaprio and Garcea 2013; Stakaityte et al. 2014). Therefore, at least two mutation events are required prior to tumorigenesis and this may explain why Merkel cell carcinoma is rare. In either case, there is a strong selection pressure within the Merkel cell carcinoma tumors to eliminate viral replication capabilities and retain only replication-deficient copies of MCV.

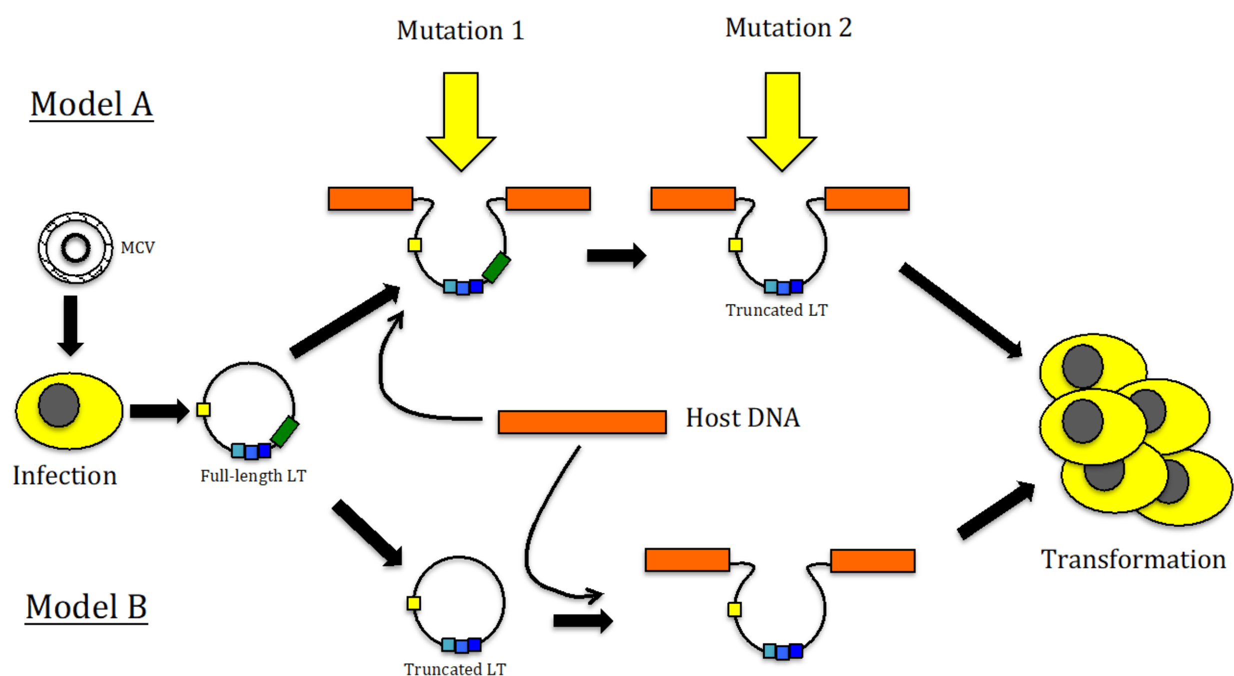

Figure 4-1. Models of MCV-induced Cell Transformation

Source: Adapted from Stakaityte et al. (2014).
Immunosuppression and loss of immunosurveillance leads to virus proliferation. At least two mutations are needed for the virus to transform cells. In model A, the first mutation leads to integration of the full-length viral genome into the host DNA, while the second mutation leads to LT truncation. In model B, LT is truncated prior to integration.

Cheng et al. (2013) also demonstrated that truncated LT was more efficient than wild-type LT at inducing cellular proliferation. Knockdown of T antigen expression in MCV-positive Merkel cell carcinoma cell lines induced cell-cycle arrest and apoptosis in vitro and regression of established xenograft tumors in vivo (Houben et al. 2012; Houben et al. 2010). These effects were largely due to the interaction of LT with retinoblastoma. LT also was required for MCV-positive Merkel cell carcinoma cell growth and survival (Angermeyer et al. 2013; Shuda et al. 2011). Borchert et al. (2014) reported that truncated LT antigens exhibit a very high binding affinity for retinoblastoma and that both wild-type and truncated LT antigens could transform baby rat

kidney epithelial cells. However, truncated LT antigen did not bind to p53 or reduce p53-dependent transcription. Since the constructs used in this study were likely able to express both LT and sT antigens, sT might have contributed to the transformation events. Liu et al. (2011) also identified human Vam6p (hVam6p) cytoplasmic protein as a novel target for MCV LT. MCV LT translocates hVam6p to the nucleus, sequestering it from its normal function in lysosomal processing. Although this study suggested that hVam6p sequestration was more likely to play a role in MCV replication than in tumorigenesis, the data were insufficient to rule out possible contributions to cell growth and proliferation.

sT expression was sufficient to induce cell transformation, loss of contact inhibition, and anchorage-independent growth in rodent fibroblast, and serum independent growth in human fibroblasts (Angermeyer et al. 2013; Shuda et al. 2011). Silencing of sT expression by sT-specific short hairpin RNAs lead to variable degrees of growth retardation; however, these effects were not sT specific because MCV-negative cell lines were similarly affected. MCV sT-induced cell transformation may be mediated by reducing the turnover of hyperphosphorylated 4E-BP1 (a downstream component of the PI3K-Akt-mTOR signaling pathway) (Shuda et al. 2011; Stakaityte et al. 2014). The PI3K-Akt-mTOR signaling cascade is an important pathway in cell-cycle regulation that is overactive in many cancers and is often targeted by oncogenic viruses. Hyperphosphorylation prevents 4E-BP1 from sequestering the eukaryotic cap-dependent translation initiation factor 4E (eIF4E), allowing free eIF4E to form the cap assembly on mRNA and initiate translation. eIF4E is part of a multisubunit eIF4F complex (composed of eIF4E, eIF4A, and eIF4G). Overexpression of eIF4F can induce cell transformation in rodent and human cells in vitro (Avdulov et al. 2004; Lazaris-Karatzas et al. 1990; Stakaityte et al. 2014). An alternative pathway for regulation of cap-dependent translation during mitosis is through cyclin-dependent kinase 1 (CDK1) hyperphosphorylation of 4E-BP1 (Shuda et al. 2015). sT-induced cell transformation was reversed by expression of a constitutively active mutant 4E-BP1 protein that could not be inactivated by MCV sT (Stakaityte et al. 2014). sT also contributes to LT expression by blocking proteasomal degradation of LT by the cellular SCF^{Fbw7} E3 ligase (Kwun et al. 2015; Kwun et al. 2013). sT inhibits E3 ligase through its LT stabilization domain (LSD) and consequently stabilizes other cellular Fbw7 targets such as the cell-cycle regulators c-myc and cyclin E.

These data suggest a synergistic role for both sT and mutated LT antigens during Merkel cell carcinoma tumorigenesis (Borchert et al. 2014; Houben et al. 2012; Shuda et al. 2011). sT may be essential for initial cell transformation and stabilizing LT, while LT is necessary for subsequent survival and proliferation of transformed cells (Stakaityte et al. 2014). Some of the primary molecular targets and biological effects associated with MCV LT and sT antigens are illustrated in Figure 4-2.

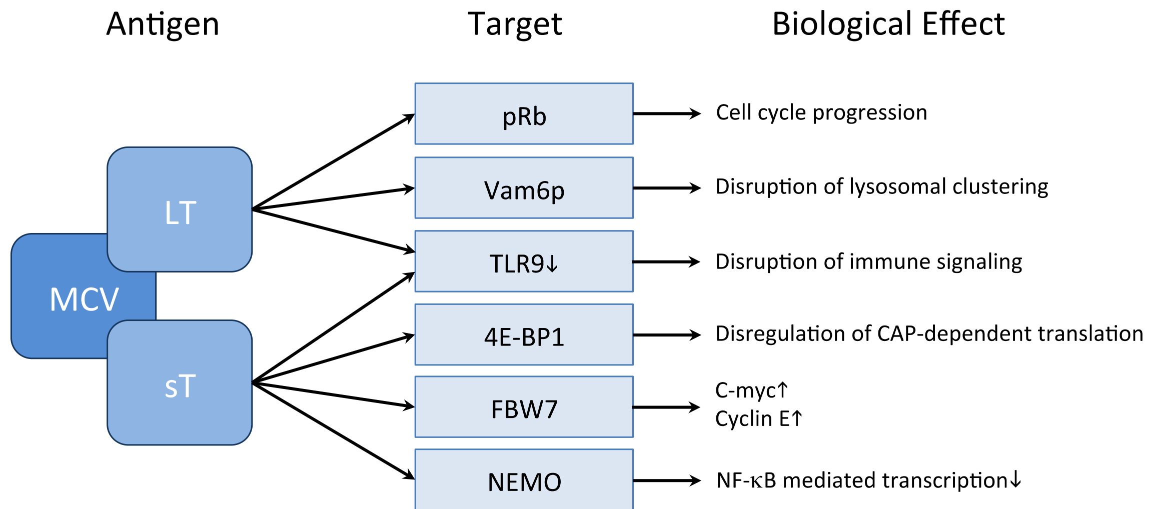

Figure 4-2. Molecular Targets and Biological Effects of MCV LT and sT Antigens

Source: Adapted from White et al. (2014).
4E-BP1 = eukaryotic translation initiation factor 4E-binding protein 1; FBW7 = F-box/WD repeat-containing protein 7; NEMO = NF-κB essential modulator; pRB = retinoblastoma protein; Vam6p = vacuolar protein-sorting gene product; TLR9 = Toll-like receptor 9.

4.3.3. MCV as a Major Risk Factor for Merkel Cell Carcinoma

Although MCV infection is common, Merkel cell carcinoma is rare (Chang and Moore 2012). Early clinical findings identified immunosuppression as a major risk factor for Merkel cell carcinoma and pointed toward an infectious etiology. Epidemiological studies (see Section 3) support an association of Merkel cell carcinoma cases with MCV infection. MCV antibody levels are significantly higher in MCV-positive Merkel cell carcinoma cases compared with healthy controls that are MCV seropositive which suggests that development of Merkel cell carcinoma is preceded by an unusually robust MCV infection (Agelli et al. 2010; Pastrana et al. 2009). MCV also is monoclonally integrated into the host genome in Merkel cell carcinoma primary tumors and metastases providing strong evidence that viral infection precedes clonal expansion of the neoplastic cell (Feng et al. 2008; Laude et al. 2010). Viral genome integration is a typical feature of virus-mediated oncogenesis and refutes the possibility that MCV is merely a coincidental passenger infection in Merkel cell carcinoma (Chang and Moore 2012; Kuwamoto 2011). These data, combined with several studies showing that expression of MCV T antigens are required to sustain tumor growth (see Section 4.2.2), provide strong evidence that MCV is a major risk factor for Merkel cell carcinoma. The key events identified for MCV-induced Merkel cell carcinoma are shown in Figure 4-3.

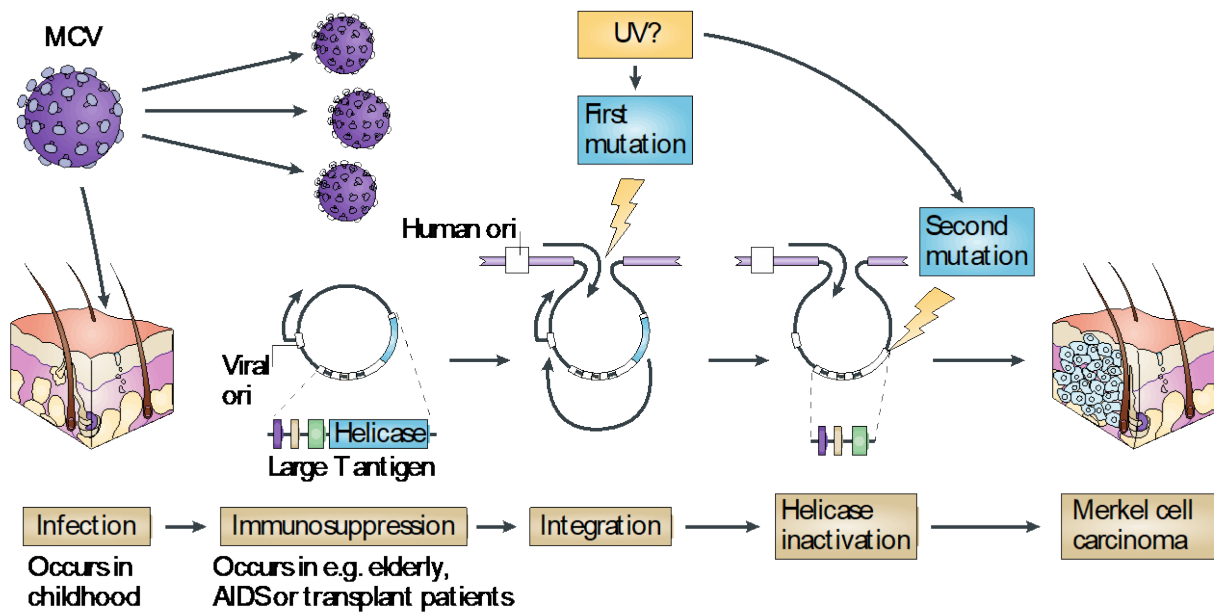

Figure 4-3. Key Events Leading from MCV Infection to Merkel Cell Carcinoma

Source: Moore and Chang (2010) (used by permission: Nature Publishing Group, License No. 3642570512432).
Although MCV is a common infection, loss of immune surveillance through aging, AIDS, or transplantation and subsequent treatment with immunosuppressive drugs may lead to resurgent MCV replication in skin cells (Pastrana et al. 2009). If a rare integration mutation into the host cell genome occurs (Feng et al. 2008), the MCV T antigen can activate independent DNA replication from the integrated viral origin that will cause DNA strand breaks in the proto-tumor cell (Shuda et al. 2008). A second mutation that truncates the T antigen, eliminating its viral replication functions but sparing its RB1 tumor suppressor targeting domains, is required for the survival of the nascent Merkel tumor cell. Exposure to sunlight (possibly UV irradiation) and other environmental mutagens may enhance the sequential mutation events that turn this asymptomatic viral infection into a cancer virus.

4.4. Synthesis

Human viral oncogenesis is a complex process that involves interactions among many viral, host, and environmental factors. Although MCV infection is common, very few people develop Merkel cell carcinoma. Therefore, as is the case with most oncogenic viruses, several cofactors are associated with a higher risk of developing Merkel cell carcinoma (e.g., immune suppression, chronic UV exposure, and advanced age). The key events associated with MCV-induced Merkel cell carcinoma cases include immunosuppression and immune evasion, monoclonal integration of the MCV genome and expression of T antigens in tumor cells, mutations causing truncation of the LT antigen, and dysregulation of cell-cycle control and apoptosis. The major lines of evidence linking MCV to Merkel cell carcinoma include the following:

- Immunosuppression is an important cofactor based on an increased risk of developing Merkel cell carcinoma in AIDS patients, organ transplant patients, and the elderly, which is consistent with an infectious etiology;
- MCV has been identified as an infectious agent in 80% or more of Merkel cell carcinoma cases;
- The MCV genome is monoclonally integrated in most Merkel cell carcinoma samples;

- A signature feature of MCV-positive Merkel cell carcinoma tumors is the presence of mutations that truncate the LT protein at its carboxy-terminus leading to loss of viral replication while preserving transforming activity;
- Molecular targets for truncated LT antigen include retinoblastoma and TLR9 that promote cell-cycle progression and disrupt immune signaling;
- Molecular targets for sT include 4E-BP1, NEMO, TLR9, and FBW7 that dysregulate CAP-dependent translation, downregulate NF-κB transcription, disrupt immune signaling, and upregulate c-myc and cyclin E;
- MCV T antigens transform cells in vitro and in vivo, are expressed only in tumor cells of MCV-infected tumors, and are required to maintain tumor growth and survival.

5. Overall Cancer Hazard Evaluation and Listing Recommendation

Merkel cell polyomavirus (MCV) is known to be a human carcinogen based on sufficient evidence from studies in humans. This conclusion is based on epidemiological studies showing that it causes Merkel cell carcinoma (MCC) in humans, together with supporting evidence from mechanistic studies demonstrating the biological plausibility of its carcinogenicity in humans (Table 5-1).

Data are inadequate to evaluate the association between MCV and either chronic lymphocytic leukemia or lung carcinoma, both of which have inconsistent evidence from epidemiological studies and no available evidence from mechanistic studies.

The following table provides the level of evidence recommendation for the carcinogenicity of MCV for Merkel cell carcinoma from studies in humans, including the key data from both epidemiological and molecular studies in humans.

Table 5-1. Summary of the Evidence for MCV and Merkel Cell Carcinoma from Human Studies

Types of Studies	Merkel Cell Carcinoma
Epidemiological	
Studies with positive associations or dose-response	21 Case series (716 MCV/855 MCC cases) 3/3 Case-control studies; moderate to highly statistically significant OR; 1 nested case-control study—statistically significant; increase in risk in females but only modest nonsignificant risk in males
Molecular (human tissue)	
Clonality	Monoclonal
% MCV-infected tumors	>80% of MCC
MCV protein expression	Large T (LT), small T (sT) antigens
Level of evidence	Sufficient

LT = large T antigen; MCV = Merkel cell polyomavirus; MCC = Merkel cell carcinoma; OR = odds ratio; sT = small T-antigen.

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Glossary

Case series: A collection of subjects (usually, patients) with common characteristics used to describe some clinical, pathophysiological, or operational aspect of a disease, treatment, exposure, or diagnostic procedure. A case series does not include a comparison group and is often based on prevalent cases and on a sample of convenience. Common selection biases and confounding severely limit their power to make causal inferences.

Case-comparison study (case-control study, case referent study): The observational epidemiological study of persons with the disease (or another outcome variable) of interest and a suitable control group of persons without the disease (comparison group, reference group). The potential relationship of a suspected risk factor or an attribute to the disease is examined by comparing the diseased and non-diseased subjects with regard to how frequently the factor or attribute is present (or, if quantitative, the levels of the attribute) in each of the groups (diseased and non-diseased).

Cellular immunity: immunity independent of antibody but dependent on the recognition of antigen by T cells and their subsequent destruction of cells bearing the antigen or on the secretion by T cells of lymphokines that enhance the ability of phagocytes to eliminate the antigen.

Convenience sample: Samples selected by easily employed but basically non-probabilistic (and probably biased) methods. “Man-in-the-street” surveys and a survey of blood pressure among volunteers who drop in at an examination booth in a public place are in this category.

Diagnostic criteria: The specific combination of signs, symptoms, and test results that a clinician uses to identify a person as representing a case of a particular disease or condition.

Familial aggregation: A tendency of some diseases to cluster in families, which may be the result of genetic and epigenetic mechanisms, shared environmental exposures (e.g., diet), or both.

Humoral response: An immune response in which antibodies produced by B cells cause the destruction of extracellular microorganisms and prevent the spread of intracellular infections.

Immunoassay: A laboratory technique that uses the binding between an antigen and its homologous antibody to identify and quantify the specific antigen or antibody in a sample.

Innate immune response: The fast-acting, non-specific immunological actions of an organism that recognize an infection and attempt to clear it from the organism. The innate immune system can be thought of an organism's front line of defense against pathogens.

Latent phase: A phase of the virus life cycle during which the virus is not replicating.

Lytic phase: A phase of the virus life cycle during which the virus replicates within the host cell, releasing a new generation of viruses when the infected cell lyses.

microRNA: small, non-coding RNA molecules approximately 22 nucleotides in length that act post translationally in a regulatory role to target messenger RNAs for cleavage or translational expression.

Polymerase chain reaction: A laboratory technique used to produce large amounts of specific DNA fragments. Polymerase chain reaction is used for genetic testing and to diagnose disease.

Titer: A laboratory measurement of the concentration of a substance in a solution (e.g., an antibody titer measures the presence and amount of antibodies in the blood).

Vertical transmission: The transmission of infection from one generation to the next (e.g., from mother to infant prenatally, during delivery, or in the postnatal period via breast milk.

Abbreviations

4EBP-1: 4E-binding protein 1

AIDS: Acquired Immune Deficiency Syndrome

ALTO: alternate frame of the large T open reading frame

BKV: BK polyomavirus

CDC: Centers for Disease Control and Prevention

CI: confidence interval

CK-20: cytokeratin 20

CLL: chronic lymphocytic leukemia

CM2B4: antibody to MCV

DNA: deoxyribonucleic acid

dsDNA: double stranded deoxyribonucleic acid

ELISA: enzyme-linked immunoassay

FBW7: F-box/WD repeat-containing protein 7

FDA: Food and Drug Administration

HIF-1: hypoxia-inducible factor

HIV: human immunodeficiency virus

IARC: International Agency for Research on Cancer

IgG: immunoglobulin G

IHC: immunohistochemistry

JCV: JC polyomavirus

LT: large T antigen

MCC: Merkel cell carcinoma

MCPyV: Merkel cell polyomavirus

MCV: Merkel cell polyomavirus

N: number

NEMO: NF-κB essential modulator

MAPK: mitogen activated protein kinase

miRNA: microRNA

NHANES: National Health and Nutrition Examination Survey

NTP: National Toxicology Program

OR: odds ratio

PCR: polymerase chain reaction
PDGF: platelet-derived growth factor
pRB: retinoblastoma protein
PTEN: phosphatase and tensin homologue
RDD: random digit dialing
SLL small lymphocytic lymphoma
sT: small T-antigen
SV40: simian virus 40
TLR9: Toll-like receptor 9
USA: United States of America
UV: ultraviolet radiation
Vam6p: vacuolar protein-sorting gene product
VEGF: vascular endothelial growth factor
VL: virus-like particle
VLP: virus-like particle
VP1: viral capsid protein 1
VP2: viral capsid protein 2

Appendix A. Literature Search Strategy

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The objective of the literature search approach is to identify published literature that is relevant for evaluating the potential carcinogenicity of the Merkel cell polyomavirus (MCV). As discussed in the Viruses Concept Document (https://ntp.niehs.nih.gov/ntp/roc/concept_docs/2014/virusesconcept_508.pdf), the monograph relies on the IARC monograph and studies published since the monograph (new studies). The literature search strategy was used to identify new human cancer studies and recent reviews of mechanistic data.

A.1. General Approach

Database searching encompasses selecting databases and search terms and conducting the searches. Searches of several citation databases are generally conducted using search terms for the individual viruses of interest, combined with search terms for cancer and/or specific topics, including epidemiological and mechanistic studies. A critical step in the process involves consultation with an information specialist to develop relevant search terms. These terms are used to search bibliographic databases. IARC used literature found by searching PubMed for MCV through 2012. Because the body of literature for this virus was small, PubMed, Web of Science and Scopus were searched for any information about MCV without date limitations up to August 2015. Table A-1highlights the general concepts searched with selected example terms. To review all the terms used, please refer to the full search strings below.

Table A-1. Major Topics Searched

Topics	Example Terms
Merkel cell polyomavirus	Merkel cell polyomavirus, Merkel cell virus, Merkel cell carcinoma

The literature for MCV was searched without using narrowing terms within the bibliographic databases. The results were then processed in EndNote to remove duplicates before being transferred to DistillerSR for screening.

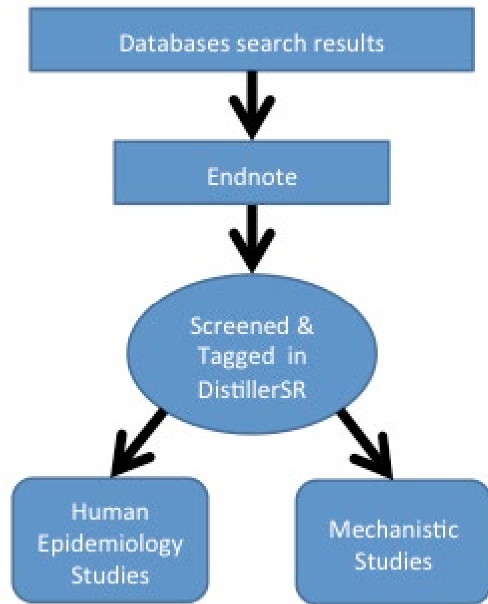

Figure A-1. Literature Processing Flow

The bibliographic database search results (1869) were processed in Endnote then imported into DistillerSR for first and second tier screening. Relevant studies found through the citations of review articles and other secondary searched were also included. Tagging in DistillerSR categorized the useful articles into Human Epidemiologic literature (119) or Mechanistic literature (199).

A.2. Search Strings for MCV Searches

PubMed, Scopus and WOS

"Merkel cell polyomavirus" OR "Merkel cell virus" OR "Merkel cell carcinoma" AND (polyomavirus OR virus)

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