New Virus Associated With Merkel Cell Carcinoma Development

Dana E. Rollison, PhD; Anna R. Giuliano, PhD; and Jürgen C. Becker, MD

Abstract
Merkel cell carcinoma (MCC) is a rare, aggressive neuroendocrine malignancy of the skin with an annual incidence in the United States of 0.34 and 0.17 per 100,000 men and women, respectively. MCC incidence rates are also higher among severely immunosuppressed populations, including people who have undergone organ transplantation, have lymphoma, and are HIV-infected. Given the increased risk for MCC observed with immunosuppression and the established associations between viral infections and other cancers that occur more often in immunosuppressed populations, MCC was a prime cancer candidate for a viral cause. Therefore, Feng et al. used digital transcriptome subtraction techniques to search for and identify a novel virus in MCC tumor tissues. Their investigation discovered a genome encompassing 5387 base pairs of a new polyomavirus, subsequently named the Merkel cell polyomavirus (MCV or MCPyV). Polyomaviruses are small, double-stranded DNA viruses that infect multiple species, such as the simian virus 40 (SV40), which infects the rhesus macaques. Until 2007, the only polyomaviruses known to naturally infect humans were JC virus (JCV) and BK virus (BKV), both discovered in 1971. The potential link between polyomavirus infections and cancer garnered initial attention with the observation that SV40 could induce tumor formation in experimentally infected hamsters. This finding represented a potential concern for human health given that SV40 was an accidental contaminant of polio vaccines derived from rhesus macaques kidney cell cultures in 1955 to 1961. However, several descriptive, serologic, and tumor-based studies have since shown that SV40 infection is not associated with cancer in humans.

Until 2007, the only polyomaviruses known to naturally infect humans were JC virus (JCV) and BK virus (BKV), both discovered in 1971. Initial infections with JCV and BKV occur in late childhood to early adolescence and are thought to be asymptomatic, with adult seroprevalence estimates ranging
from 44% to 77% and 55% to 85%, respectively. Little is known about the natural history of these infections, including their routes of transmission, although evidence exists for both respiratory and fecal–oral transmission. These viruses establish latency in the kidney and can reactivate under conditions of severe immunosuppression to cause disease, including JCV-associated progressive multifocal leukoencephalopathy in patients with AIDS and BKV-associated nephropathy in those who have undergone renal transplantation.

At the molecular level, all polyomaviruses encode for the large T antigen, a nonstructural protein that can bind to and inactivate tumor suppressor proteins p53 and pRB and can interfere with other cell signaling pathways. However, the role of these polyomavirus infections in the origin of human cancer has not been established in several tumor and observational studies. In 2007, 2 additional human polyomaviruses were isolated from respiratory secretions, KI and WU polyomaviruses, neither of which has been found in human tumor tissues, although only one study has been published. MCC was identified in MCC tissues in 2008 and, in contrast to the inconsistency in results across studies of JCV and BKV infections in relation to cancer, accumulating evidence suggests that MCV may play a causal role in MCC.

**MVC in MCC: Evidence for Causality**

In their initial report of MCV, Feng et al. showed not only the presence of MCV DNA in 80% of 10 MCC tumor tissues tested but also monoclonal integration of the virus into the host genome. Twenty-six studies on 24 unique patient populations subsequently reported the presence of MCV DNA in 43% to 100% of tumors tested using polymerase chain reaction (PCR) techniques (Table 1). These studies included patients from multiple continents (North America, Asia, Europe, and Australia) and sample sizes ranging from 5 to 114 patients with MCC (Table 1). Many of these studies also included comparison groups with normal tissues or other types of tumor tissues (e.g., normal skin tissue, basal and squamous cell carcinomas of the skin), consistently finding these to yield lower proportions of MCV DNA-positive samples compared with MCC tumor tissues.

Variability in estimates of MCV DNA prevalence in MCC tumor tissues across studies may be partly because of the small sample sizes of some studies, sample selection, differences in assay sensitivity as a function of the primers and conditions used for PCR and tissue preparation (i.e., formalin-fixed paraffin-embedded vs. fresh-frozen), or differences in the underlying population prevalence of MCV infection or cofactors that interact with MCV infection to cause MCC. However, despite these variations in prevalence estimates, MCV is clearly present in most MCC tumor tissues across studies.

Viral load, or number of MCV copies per cell equivalent, has also been investigated in 10 of the aforementioned tumor studies using quantitative PCR (qPCR; Table 1). Among the 9 studies that provided the median MCV copy number, the medians ranged from 0.06 to 10 copies per cell equivalent, with 6 of the 9 studies reporting medians above 1.0 MCV copy per cell equivalent. These data are consistent with the model of an MCV-infected Merkel cell undergoing clonal expansion, resulting in an MCC tumor with at least 1 copy of MCV per cell equivalent. However, in all 9 studies that used qPCR, the range of viral copies included values far below 1 MCV copy per cell equivalent. Viral copy number could be low in these tumors because of the presence of nontumor cells, such as adjacent normal cutaneous tissues and infiltrating lymphocytes. Therefore, although these studies consistently show the presence of MCV DNA at high copy numbers in a subset of tumors, clearly a subset of MCV-positive MCC tumors with fewer than 1 viral copy per cell equivalent exists for which the etiologic relevance of MCV remains unclear.

In addition to the presence and quantity of viral DNA in tumor tissue, oncoprotein expression has also been investigated in MCC tumor tissues (Table 1). MCV large T-antigen expression was shown with immunohistochemistry or reverse-transcriptase PCR in 44% to 100% of MCC tumor tissues investigated in 4 studies, with sample sizes ranging from 8 to 36 tissues. Two of the immunohistochemistry studies showed localization of MCV T-antigen expression to the nuclei of MCC tumor cells. Importantly, 3 studies investigating the correlation between the presence of MCV DNA and T-antigen expression in tumors
Table 1  MCV DNA Detection, T-Antigen Expression, and Seropositivity in MCC

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>No. Tumors</th>
<th>T-Antigen Expression</th>
<th>MCV Seropositivity</th>
<th>Correlation Between Markers of MCV Infection Within Patients With MCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feng et al., 2008</td>
<td>United States</td>
<td>10</td>
<td>80%</td>
<td>0.0001–10</td>
<td></td>
</tr>
<tr>
<td>Garneski et al., 2009</td>
<td>North America and Australia</td>
<td>37</td>
<td>43%</td>
<td>(0.0005–1.2)</td>
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<tr>
<td>Kassem et al., 2008</td>
<td>Germany</td>
<td>30</td>
<td>77%</td>
<td></td>
<td></td>
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<tr>
<td>Becker et al., 2009</td>
<td>Germany</td>
<td>75</td>
<td>85%</td>
<td></td>
<td></td>
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<tr>
<td>Fouloungne et al., 2008</td>
<td>France</td>
<td>9</td>
<td>89%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bhatia et al., 2010</td>
<td>United States</td>
<td>34</td>
<td>74%</td>
<td>0.06 (0.0005–1.2)</td>
<td></td>
</tr>
<tr>
<td>Varga et al., 2009</td>
<td>Hungary</td>
<td>8 (6 pts)</td>
<td>83%</td>
<td></td>
<td></td>
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<tr>
<td>Touze et al., 2009</td>
<td>France</td>
<td>32</td>
<td>66%</td>
<td></td>
<td></td>
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<tr>
<td>Sastre-Garau et al., 2009</td>
<td>France</td>
<td>10</td>
<td>100%</td>
<td>3.15 (0.6–62.2)</td>
<td></td>
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<tr>
<td>Duncavage et al., 2009</td>
<td>United States</td>
<td>41</td>
<td>76%</td>
<td></td>
<td></td>
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<tr>
<td>Wetzels et al., 2009</td>
<td>Netherlands</td>
<td>5</td>
<td>40%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helmbold et al., 2009</td>
<td>Germany</td>
<td>98 (91 pts)</td>
<td>92%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Houben et al., 2009</td>
<td>Germany</td>
<td>50</td>
<td>86%</td>
<td>1.0 (0.001 to &gt; 100)</td>
<td></td>
</tr>
<tr>
<td>Sihto et al., 2009</td>
<td>Finland</td>
<td>114</td>
<td>80%</td>
<td>1.6 (0.0003–4224)</td>
<td></td>
</tr>
<tr>
<td>Katano et al., 2009</td>
<td>Japan</td>
<td>13 (11 pts)</td>
<td>55%</td>
<td>0.119 (0.038–0.43)</td>
<td></td>
</tr>
<tr>
<td>Shuda et al., 2009</td>
<td>Spain</td>
<td>10</td>
<td>3.3 (&lt; 0.001–14.3)</td>
<td>21/36 (58%)</td>
<td>5/6 (83%) tumors with &gt; 1 MCV copy per cell vs. 0/3 (0%) of tumors with &lt; 1 MCV copy per cell were TAg+ by IHC</td>
</tr>
<tr>
<td>Andres et al., 2009</td>
<td>Germany</td>
<td>33</td>
<td>64%</td>
<td></td>
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<tr>
<td>Nakajima et al., 2009</td>
<td>Japan</td>
<td>14</td>
<td>79%</td>
<td></td>
<td></td>
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<tr>
<td>Loyo et al., 2010</td>
<td>United States</td>
<td>7</td>
<td>86%</td>
<td>10 (0.05–173)</td>
<td></td>
</tr>
<tr>
<td>Busam et al., 2009</td>
<td>United States</td>
<td>17</td>
<td>88%</td>
<td>27/36 (75%)</td>
<td>10/13 (77%) MCV DNA+ tumors vs. 0/2 (0%) MCV DNA tumors were TAg+ by IHC</td>
</tr>
<tr>
<td>Wieland et al., 2009</td>
<td>Germany</td>
<td>34</td>
<td>88%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Koljonen et al., 2009</td>
<td>Finland</td>
<td>5</td>
<td>100%</td>
<td>5.49 (0.88–444.4)</td>
<td></td>
</tr>
<tr>
<td>Carter et al., 2009</td>
<td>United States</td>
<td>31</td>
<td>77%</td>
<td>36/41 (88%)</td>
<td>22/24 (92%) MCV DNA+ cases vs. 7/7 (100%) MCV DNA cases were MCV seropositive</td>
</tr>
<tr>
<td>Tolstov et al., 2009</td>
<td>United States</td>
<td>27</td>
<td>78%</td>
<td>24/27 (89%)</td>
<td>21/21 (100%) MCV DNA+ cases vs. 3/6 (50%) MCV DNA cases were MCV IgG seropositive (P = .007)</td>
</tr>
</tbody>
</table>
consistently reported that a higher proportion of MCV DNA-positive tumors (77%–80%) showed T-antigen expression compared with MCV DNA-negative tumors (0%–50%).

In addition to measures of the virus in the tumor tissue, serologic markers of MCV infection have been developed, including circulating IgG antibodies to the MCV capsid protein (VP1), and preliminary studies have indicated that seroreactivity to VP1 is significantly greater among patients with MCC than in healthy controls. When assay-specific binary cutpoints were used to define the proportion of patients who were MCV-seropositive, similar estimates of seroprevalence were reported: 88% and 89% of 41 and 27 patients with MCC, respectively. However, in the patients for whom both tumor tissue and serum were available, these 2 studies reported different results when seroreactivity was compared between patients with and without MCV DNA in their tumors (Table 1). In the study by Tolstov et al., 100% of 21 patients with MCV DNA-positive tumors were MCV-seropositive compared with 50% of 6 patients with MCV DNA-negative tumors, a difference that was statistically significant (P = .007). Of 31 patients for whom tissue samples were available, Carter et al. 36 observed no difference in MCV seroprevalence among 24 patients who were MCV DNA-positive (92%) and 7 who were MCV DNA-negative (100%).

**MCV in Relation to MCC Clinical Characteristics and Survival**

Studies have begun to characterize the clinical characteristics of MCV-positive versus MCV-negative MCC. With respect to demographics, 2 studies observed no association between presence of MCV DNA in MCC tumor tissues and age at diagnosis, whereas a third study reported that MCV T-antigen expression in the tumor was associated with younger age at diagnosis (P = .07). One of two studies observed a difference in MCV according to sex, with a higher MCV DNA prevalence in men (P = .109). No differences between the sexes were observed in T-antigen expression.

Among 2 studies, presence of MCV DNA in MCC tumor tissue was not associated with MCC morphology (intermediate vs. trabecular or small cell). In 2 of 3 studies, no association was seen between MCV DNA in tumor tissues and presence

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**Table 1** MCV DNA Detection, T-Antigen Expression, and Seropositivity in MCC (cont.)

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>No. Tumors</th>
<th>Positive</th>
<th>Viral Copies</th>
<th>T-Antigen Expression</th>
<th>MCV Seropositivity</th>
<th>Correlation Between Markers of MCV Infection</th>
<th>Patients With MCC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Woo et al., 2010</td>
<td>Korea</td>
<td>7</td>
<td>100%</td>
<td>0.073 (0.013–11.943)</td>
<td>8/18 (44%)</td>
<td>8/10 (80%) MCV DNA+ tumors vs. 0/8 (0%) MCV DNA tumors were TAg+ according to IHC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nakamura et al., 2010</td>
<td>Japan</td>
<td>19</td>
<td>58%</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations: IHC, immunohistochemistry; MCC, Merkel cell carcinoma; MCV, Merkel cell polyomavirus; PCR, polymerase chain reaction; TAg, T-antigen.

1Unless otherwise indicated, one tumor was obtained from each patient (pt).

1Recent positivity indicates the proportion of tumors that amplified at least one of the PCR primers used. When multiple tumors were included from individuals, % positive indicates the proportion of patients who had at least one MCV DNA-positive tumor.

1Number of MCV copies per cell equivalent expressed as the median (range) among MCV DNA-positive tumors.

1MCV T-antigen (TAg) expression was determined through IHC in all studies except for that by Sastre-Garau et al., in which it was determined by reverse transcriptase PCR.

1Percentage indicates proportion of tumors that were MCV-positive (proportion of patients with at least one MCV-positive tumor was not stated).

1This article presented viral load data for the 10 MCC tumors from the United States, 8 of which had already tested positive for PCR in the study by Feng et al., 10 of 10 had a viral load of 0 copies per cell based on quantitative PCR (qPCR), with 9 of 10 having viral loads that ranged from < 0.0001 to 14.3, as indicated in the table. IHC for TAg was conducted on a separate set of 36 tumors obtained from Spain (indicated in the TAg expression column) in addition to 9 of the 10 tumors tested by quantitative PCR; comparisons between DNA and TAg expression were based on 9 of the 10 tumors from the United States.

1Mean viral copy number (median was not stated).

1All MCCs occurred in patients with chronic lymphocytic leukemia.

1MVC DNA was determined from the 27 tumors in 2 previous publications (Feng et al. and Shuda et al.); correlations with seropositivity were reported by Tolstov et al. 31
of metastases at diagnosis, whereas the third study showed fewer regional metastases at diagnosis among patients with MCV DNA-positive MCC (P = .043). In 250 of 3 studies,4,50,58 MCV DNA-positive tumors tended to occur on the limbs more often than did MCV DNA-negative tumors, with the latter occurring more often on the head, neck, or trunk. The number of MCV viral copies per cell equivalent has been observed to be positively associated with expression of retinoblastoma gene product (pRb), and higher levels of CD44 expression, absent CK7 expression, and lower p53 expression were observed among patients with MCC who expressed both MCV T-antigen and pRb.44 In a separate study, presence of MCV DNA in tumor tissue was not associated with hypermethylation of several tumor suppressor genes (RASSF1A, TP73, FHIT, CDKN2A).40

Presence of MCV DNA in tumor tissue or T-antigen expression has been associated with a 61% to 65% improvement in overall survival in 2 patient populations44,50,59 after adjustment for other prognostic factors. Two separate studies reported no observed differences in survival by MCV status, although no data were provided.33,39

**Future Directions**

Two fundamental questions remain regarding the role of MCV in MCC. The first question is whether MCV infection causes MCC. The tumor studies published to date have consistently shown the existence of a subset of MCV DNA-negative cases, indicating that MCV is not a necessary cause of MCC, unlike human papillomavirus (HPV) and cervical cancer. However, HPV also plays a role in other cancers, such as oropharyngeal and penile cancers, and only a subset of those cancers are HPV-positive.60,61

To more clearly establish a causal role for MCV in a subset of MCC, future tumor studies with larger sample sizes comprising tumor tissues obtained from multiple countries should measure the presence of MCV DNA and its clonal integration, viral load, and T-antigen expression, and its localization to the nucleus. Laser capture microdissection could be used to ensure that only tumor cells are included in the sample, thus improving the interpretation of the number of viral copies per cell equivalent. Importantly, sequencing of the PCR products should be conducted to determine the presence of a truncating mutation in the T-antigen coding region, which may be necessary for MCV-associated carcinogenesis. The use of validated laboratory assays is essential for comparing results across studies, and testing the same panel of samples across multiple laboratories could assist in assay validation.35,63

In addition to tumor case series, additional study designs are needed to estimate the magnitude of the association between MCV infection and MCC. A case-control study based on circulating MCV antibodies targeted to VP1 and, importantly, MCV T-antigen would not only provide this estimate but also facilitate the identification of cofactors that may interact synergistically with MCV infection to induce MCC, such as UV radiation. Given the rarity of MCC, prospective studies are difficult to conduct, although a nested case-control study including samples from all the large established cohorts worldwide may be able to establish the temporal relationship between baseline seroreactivity and subsequent development of MCC.

The second fundamental question is whether patients with MCV-positive MCC have different clinical characteristics and a survival advantage compared with those who have MCV-negative MCC. To address the question of whether MCV is associated with improved MCC survival, a multicentered study enrolling hundreds of MCC patients is needed.59 Careful attention should be paid to the definition of MCV-positive MCC; tumors with viral loads of fewer than 1 copy per cell equivalent with no T-antigen expression should be considered separately from those with 1 or more viral copy per cell, presence of the truncating T-antigen mutation, and expression of T-antigen. Large sample sizes are needed to adequately control for other prognostic factors, including age at diagnosis, stage at diagnosis, and treatment.

Finally, other questions outside of MCC that deserve further investigation revolve around the natural history of MCV infection (e.g., how it is transmitted, what tissues serve as the reservoir of infection, the existence of a latent state and reactivation if infection) and the possible role of MCV infection in other types of cancer. For example, MCV DNA has been detected in 13% to 38% of basal cell carcinomas (BCCs) and 13% to 25% of squamous cell carcinomas (SCCs) among immunocompetent individuals, and in 72% of BCCs and 52% of SCCs among immunocompromised individuals, although other studies did not observe MCV in any BCC.52,53
References


