

Clinical Implications

- Heat treatment induces antiviral factors APOBEC3A and APOBEC3G in genital warts.
- APOBEC3A and APOBEC3G induce mutations in the human papilloma virus E2 gene.
- Hyperthermia may induce regression of genital warts via APOBEC-dependent antiviral and immunological mechanisms.

against the virus. Hyperthermia, in contrast to cryotherapy, would be an elegant way to expose the virus in a meaningful way to immune reactivity and even prevent recurrences, an effect similar to that of imiquimod when used to treat genital warts.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

- Ahmed Z, Kawamura T, Shimada S, Piguet V. The role of human dendritic cells in HIV-1 infection. *J Invest Dermatol* 2015;135:1225–33.
- Huo W, Gao XH, Sun XP, Qi RQ, Hong Y, McHepange UO, et al. Local hyperthermia at 44 degrees C for the treatment of plantar warts: a randomized, patient-blinded, placebo-controlled trial. *J Infect Dis* 2010;201:1169–72.
- Roberts SA, Lawrence MS, Klimczak LJ, Grimm SA, Fargo D, Stojanov P, et al. An APOBEC cytidine deaminase mutagenesis

pattern is widespread in human cancers. *Nat Genet* 2013;45:970–6.

Sheehy AM, Gaddis NC, Choi JD, Malim MH. Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* 2002;418:646–50.

Turelli P, Mangeat B, Jost S, Vianin S, Trono D. Inhibition of hepatitis B virus replication by APOBEC3G. *Science* 2004;303:1829.

Vartanian JP, Guetard D, Henry M, Wain-Hobson S. Evidence for editing of human papillomavirus DNA by APOBEC3 in benign and precancerous lesions. *Science* 2008;320:230–3.

Yang Y, Wang H, Zhang X, Huo W, Qi R, Gao Y, et al. Heat increases the editing efficiency of human papillomavirus E2 gene by inducing upregulation of APOBEC3A and 3G. *J Invest Dermatol* 2017;137:810–8.

Zhu LL, Gao XH, Qi R, Hong Y, Li X, Wang X, et al. Local hyperthermia could induce antiviral activity by endogenous interferon-dependent pathway in condyloma acuminata. *Antiviral Res* 2010;88:187–92.

In North America, the majority of Merkel cell carcinoma (MCC) tumors have Merkel cell polyomavirus (MCPyV) DNA integrated into their genomes. Considering the poor survival rates seen in patients with MCC, it is essential to understand disease risk factors and prognostic markers to guide prevention and treatment strategies. Presently, Moshiri et al. (2017) report the largest retrospective case series to date addressing the impact of MCPyV status on MCC prognosis. As there is no gold standard assay for detecting virus, they employed a consensus of the three methods for virus detection. Analyzing MCC samples from 282 patients, the authors showed improved progression-free survival and disease-specific survival in the 81% of patients with MCPyV-positive tumors. However, the effect of virus status on prognosis was not independent of tumor stage on multivariate analysis.

Currently, the clinical implications of MCC viral status are poorly understood. One reason for this is that there is no validated clinical test for MCPyV detection. The present study used a multimodal viral detection method to show that four in five MCC tumors in their patient population tested positive for MCPyV. More significantly, they showed that detecting MCPyV in tumor samples is imperfect with both immunohistochemistry (IHC) and quantitative PCR (qPCR) approaches. Compared with the consensus virus detection of two antibodies and one set of qPCR primers, staining for MCPyV large T antigen with CM2B4 antibody showed the highest sensitivity (88.2%) and specificity (94.3%). This approach to MCPyV detection can be implemented easily in most pathology labs, and it has the advantage of using a commercially available monoclonal antibody. Their PCR assay was less robust, with a sensitivity of 82.5% and specificity of 81.1%.

Many qPCR approaches have been used to detect MCPyV DNA in patient samples. Published PCR MCPyV detection assays vary in the virus-specific primer sequences used, the number of virus primer sets used, the reference human sequence that is amplified, and the thresholds used to consider a test positive. The present study used only one optimized primer

See related article on pg 819

Seeking Standards for the Detection of Merkel Cell Polyomavirus and its Clinical Significance

Mary Eid¹, Jannett Nguyen² and Isaac Brownell²

Merkel cell carcinoma is a rare skin cancer associated with Merkel cell polyomavirus in most cases. Prior studies associating Merkel cell carcinoma viral status with prognosis have inconsistent findings. Moshiri et al. used multimodal virus detection to determine that the 81% of patients with virus-positive Merkel cell carcinoma tumors had earlier stage disease and better outcomes relative to virus-negative cases.

Journal of Investigative Dermatology (2017) 137, 797–799. doi:10.1016/j.jid.2016.12.024

¹Laboratory of Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; and ²Dermatology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA

Correspondence: Isaac Brownell, Dermatology Branch, 10 Center Drive, 12N240C, Bethesda, Maryland 20892-1908, USA. E-mail: isaac.brownell@nih.gov

© 2017 The Authors. Published by Elsevier, Inc. on behalf of the Society for Investigative Dermatology.



Clinical Implications

- Merkel cell polyomavirus-positive Merkel cell carcinomas are less aggressive.
- Virus status is not an independent prognostic marker for Merkel cell carcinoma.
- Antibody staining works, but a gold-standard assay for Merkel cell polyomavirus is still needed.

set to detect virus DNA, and like most published assays, the threshold to call a sample positive was set at a level well below 1 viral copy per cell (≥ 0.01). Despite this, the authors detected many ($n = 40$) false-negative PCR tests in their study (samples negative by PCR, but positive with both antibodies by IHC). Lowering the PCR test threshold for viral positivity to include more of these tumors would decrease specificity, as detection of background MCPyV was already noted in 10 MCC tumors and 3 of 16 non-MCC skin tumors negative by IHC. These findings are indicative of seemingly inherent difficulties in using PCR to amplify integrated MCPyV DNA from patient samples.

Despite the fact that multiple virome copies can be clonally integrated into a

given MCC tumor genome, it is not uncommon for qPCR to detect only a fraction of virus copies per cell in MCPyV-positive tumor samples. Stromal cells, blood, and adjacent tissues are not expected to harbor MCPyV DNA, but they contribute a minor fraction of the total DNA extracted from clinical tumor samples. An interpretation that only a fraction of tumor cells contain viral DNA is at odds with the concept that clonal viral integration is an early event in MCC carcinogenesis (Feng et al., 2008) and that IHC staining for viral T antigen is typically present in close to all tumor cells in MCPyV-positive tumors (Figure 1). It seems more likely that MCPyV detection by PCR is compromised in some MCC tumor samples rather than virus being absent in up to 99% of tumor cells.

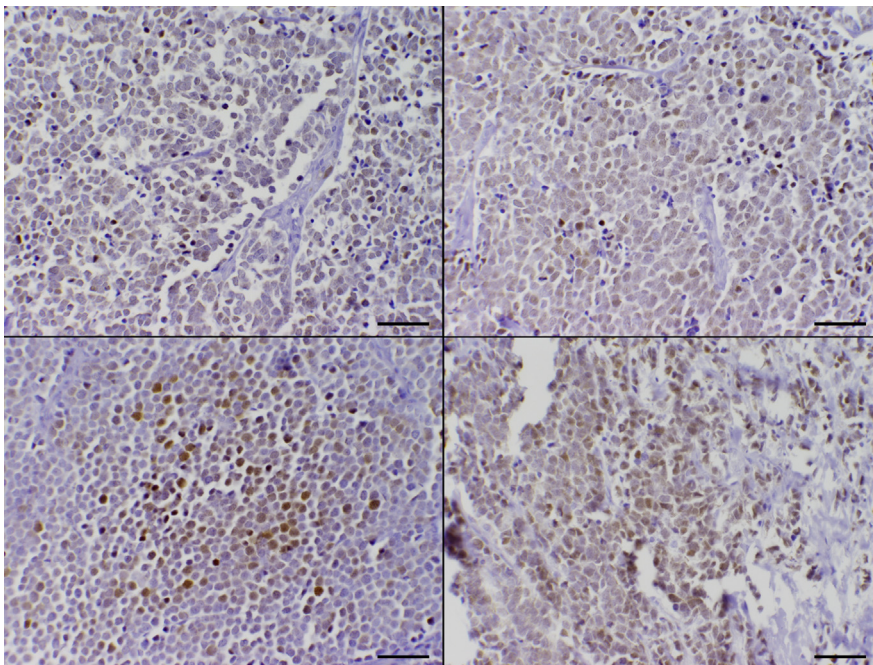


Figure 1. Merkel cell carcinoma with immunostaining for Merkel cell polyomavirus large T antigen using the CM2B4 antibody (brown) and hematoxylin counterstain. Four virus-positive tumors demonstrating that although staining intensity can vary from cell to cell, almost all tumor cells stain for virus. Staining is notably absent in stromal fibroblasts and endothelial cells. Scale bar = 50 μ m.

Regardless of the cause, the low threshold needed to detect MCPyV DNA reliably in virus-positive tumors decreases assay specificity and impedes the use of PCR as a clinical test.

The challenges with PCR detection of MCPyV could explain why so many divergent qPCR assays have been developed and why some laboratories use multiple primer sets. At least four prior studies (Busam et al., 2009; Chun et al., 2013; Leroux-Kozal et al., 2015; Rodig et al., 2012) have used PCR approaches in addition to IHC to detect MCPyV in MCC patient samples. All four studies detected virus in more samples by PCR than by IHC, suggesting that PCR detection is more sensitive. In contrast, the current study detected virus in more tumors using IHC than with their PCR assay. Considering their diversity, it is possible that the most sensitive and specific PCR assay for MCPyV detection has yet to be compared with IHC assays.

The present study found better progression-free survival and MCC-specific survival in patients with virus-positive tumors. Prior studies on the prognostic implications of MCPyV status have generally shown either a trend toward or significantly better survival associated with virus-positive tumors (Bhatia et al., 2010; Brummer et al., 2016). Some studies showed better prognosis with virus-positive tumors on univariate analysis, but viral status was not an independent prognostic factor on multivariate analysis (Nardi et al., 2012). Similarly, the present study also demonstrated that virus status is not an independent prognostic factor when tumor stage is considered. Taken together, these results suggest that MCPyV-positive tumors are less aggressive and that the less favorable outcomes associated with virus-negative tumors are, in part, due to their higher propensity to present with regional or distant metastases.

The better prognosis associated with MCPyV-positive MCC tumors is analogous to the finding that human papillomavirus-positive head and neck squamous cell carcinoma has a more favorable prognosis over human papillomavirus-negative head and neck squamous cell carcinoma (Mallen-St Clair et al., 2016; Okami, 2016). Because of better treatment responses

and more favorable prognosis in human papillomavirus-positive head and neck squamous cell carcinoma, clinical trials for treatment deintensification are underway in an effort to reduce treatment toxicity without jeopardizing efficacy (Okami, 2016). More favorable outcomes have been seen with human papillomavirus-positive vulvar squamous cell carcinomas (Sznurkowski et al., 2016). When considering other virus-associated tumors, one might assume that all virus-negative tumors are associated with worse outcomes. However, this is not always the case. For example, a multivariate analysis by Peng et al. (2016) showed that patients with Epstein-Barr virus-positive nasopharyngeal carcinoma have a worse prognosis than patients with Epstein-Barr virus-negative nasopharyngeal carcinoma, although overall stage reflected the most significant prognostic factor for nasopharyngeal carcinoma. It is useful to understand the significance of MCPyV on the aggressiveness of MCC tumors, as viral status can help guide treatment intensity and monitoring in patients.

Differences in the biological behaviors of virus-positive and virus-negative MCC tumors are not surprising considering recent exome sequencing studies (Goh et al., 2016; Harms et al., 2015) showing high somatic mutational burdens and ultraviolet signature mutations in MCPyV-negative tumors that are absent in MCPyV-positive tumors. As therapies for MCC are developed, it will be important to determine whether MCPyV status predicts response rates. Interestingly, both recent trials of immune checkpoint inhibitors failed to find a significant difference in response rates between virus-positive and virus-negative MCC tumors (Kaufman et al., 2016; Nghiem et al., 2016).

Optimizing MCPyV detection assays and reconciling the discrepancies

between viral detection methods will require further investigation. Based on the present study, it is clear that no single assay performed as well as the multimodal approach, and development of a gold standard clinical test to detect MCPyV is still needed. Whereas the current study focused on the aggressive nature of MCPyV-negative tumors, it also found that advanced stage, male sex, and immunosuppression were independently associated with poor prognosis in MCC. These factors should be taken into account when determining appropriate disease management and follow-up. Considering MCC's rare nature, relatively large studies such as the current one by Moshiri et al. (2017) are important in developing clinical tools for and advancing our understanding of this potentially deadly malignancy.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

- Bhatia K, Goedert JJ, Modali R, Preiss L, Ayers LW. Immunological detection of viral large T antigen identifies a subset of Merkel cell carcinoma tumors with higher viral abundance and better clinical outcome. *Int J Cancer* 2010;127:1493–6.
- Brummer GC, Bowen AR, Bowen GM. Merkel cell carcinoma: current issues regarding diagnosis, management, and emerging treatment strategies. *Am J Clin Dermatol* 2016;17:49–62.
- Busam KJ, Jungbluth AA, Rektman N, Coit D, Pulitzer M, Bini J, et al. Merkel cell polyomavirus expression in Merkel cell carcinomas and its absence in combined tumors and pulmonary neuroendocrine carcinomas. *Am J Surg Pathol* 2009;33:1378–85.
- Chun SM, Yun SJ, Lee SC, Won YH, Lee JB. Merkel cell polyomavirus is frequently detected in Korean patients with Merkel cell carcinoma. *Ann Dermatol* 2013;25:203–7.
- Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 2008;319:1096–100.
- Goh C, Walradt T, Markarov V, Blom A, Riaz N, Doumani R, et al. Mutational landscape of MCPyV-positive and MCPyV-negative Merkel cell carcinomas with implications for immunotherapy. *Oncotarget* 2016;7:3403–15.
- Harms PW, Vats P, Verhaegen ME, Robinson DR, Wu YM, Dhanasekaran SM, et al. The distinctive mutational spectra of polyomavirus-negative Merkel cell carcinoma. *Cancer Res* 2015;75:3720–7.
- Kaufman HL, Russell J, Hamid O, Bhatia S, Terheyden P, D'Angelo SP, et al. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. *Lancet Oncol* 2016;17:1374–85.
- Leroux-Kozal V, Lévêque N, Brodard V, Lesage C, Duzé O, Makeieff M, et al. Merkel cell carcinoma: histopathologic and prognostic features according to the immunohistochemical expression of Merkel cell polyomavirus large T antigen correlated with viral load. *Hum Pathol* 2015;46:443–53.
- Mallen-St Clair J, Alani M, Wang MB, Srivatsan ES. Human papillomavirus in oropharyngeal cancer: the changing face of a disease. *Biochim Biophys Acta* 2016;1866:141–50.
- Moshiri A, Doumani R, Yelistratova L, Blom A, Lachance K, Shinohara MM, et al. Polyomavirus-negative Merkel cell carcinoma: a more aggressive subtype based on analysis of 282 cases using multimodal tumor virus detection. *J Invest Dermatol* 2017;137:819–27.
- Nardi V, Song Y, Santamaria-Barria JA, Cosper AK, Lam Q, Faber AC, et al. Activation of PI3K signaling in Merkel cell carcinoma. *Clin Cancer Res* 2012;18:1227–36.
- Nghiem PT, Bhatia S, Lipson EJ, Kudchadkar RR, Miller NJ, Annamalai L, et al. PD-1 blockade with pembrolizumab in advanced Merkel cell carcinoma. *N Engl J Med* 2016;374:2542–52.
- Okami K. Clinical features and treatment strategy for HPV-related oropharyngeal cancer. *Int J Clin Oncol* 2016;21:827–35.
- Peng H, Chen L, Zhang Y, Guo R, Li WF, Mao YP, et al. Survival analysis of patients with advanced-stage nasopharyngeal carcinoma according to the Epstein-Barr virus status. *Oncotarget* 2016;7:24208–16.
- Rodig SJ, Cheng J, Wardzala J, DoRosario A, Scanlon JJ, Laga AC, et al. Improved detection suggests all Merkel cell carcinomas harbor Merkel polyomavirus. *J Clin Invest* 2012;122:4645–53.
- Sznurkowski JJ, Żawrocki A, Biernat W. The overexpression of p16 is not a surrogate marker for high-risk human papilloma virus genotypes and predicts clinical outcomes for vulvar cancer. *BMC Cancer* 2016;16:465.