OncoImmunology 1:8, 1420–1421; November 2012; © 2012 Landes Bioscience

Tumor-infiltrating lymphocytes and outcome in Merkel cell carcinoma, a virus-associated cancer

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Keywords: cytotoxic T cells, Merkel cell carcinoma, Merkel cell polyomavirus, survival, tumor-infiltrating lymphocytes

Abbreviations: CTLA-4, cytotoxic T-lymphocyte-associated protein 4; MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus; PD-1, programmed death-1; VP, viral capsid protein

An intense immune infiltrate, enriched in T cells, is associated with the presence of Merkel cell polyomavirus (MCPyV) DNA in Merkel cell carcinoma (MCC), a rare skin cancer. High tumor-infiltrating T-cell counts are associated with favorable survival regardless of the tumor MCPyV status. Hence, boosting host immune functions might constitute a new approach for the treatment of MCC.

Merkel cell carcinoma (MCC) is a rare and aggressive endocrine skin cancer that is most often found in elderly Caucasian individuals. Treatment of the primary tumor consists in surgery combined or not with radiation therapy. MCC frequently gives rise to distant metastases, the 5 year disease-specific survival being approximately 65%.[1] Immunodeficient patients, including individuals affected by HIV or chronic lymphocytic leukemia as well as transplantation recipients or subjects undergoing immunosuppressive therapy are at a greater risk for MCC compared with the general population. Exposure to sunlight has also been linked to an increased risk for MCC.[1] Rarely, MCC regresses spontaneously, suggesting that activation of the host immune system, at least in a few instances, is sufficient for eradicating the tumor. A majority of MCC lesions were recently found to harbor the DNA of a novel virus, called Merkel cell polyomavirus (MCPyV), de facto suggesting a viral origin for a subset of MCCs.[2]

A growing body of evidence indicates that MCPyV is an ubiquitous virus in humans and that it plays a central role in MCC tumorigenesis. MCPyV DNA is clonally integrated into the genome of MCC cells, yet contains mutations that prevent viral replication, possibly reducing the lethality of MCPyV infection to host cells.[2,3] The circulating levels of anti-MCPyV IgG antibodies recognizing the MCPyV viral capsid proteins VP1 and VP2 are higher in MCC patients as compared with the unaffected individuals, and MCC tissues contain more viral DNA than tissues from healthy individuals or patients with other types of cancer.[3] The nuclei of MCC cells express MCPyV T antigens, which can bind the retinoblastoma protein and hence inhibit its function in the regulation of the cell cycle.[3,4] Of note, viral T antigen expression is required for the survival of some MCPyV$^+$ MCC cell lines in vitro.[5]

Patients with MCPyV$^+$ MCC may have a more favorable outcome than patients whose cancer is MCPyV$^-$,[4] although this observation is controversial. MCPyV$^+$ and MCPyV$^-$ MCCs appear to differ also at the molecular level. Indeed, MCPyV$^+$ cancers contain fewer genetic aberrations than their MCPyV$^-$ counterparts.[6] Unlike MCPyV$^-$ MCCs, MCPyV$^+$ MCCs usually express the retinoblastoma protein, whereas gene mutations affecting p53 and phosphoinositide-3-kinase (PI3K) are found almost exclusively in MCPyV$^-$ MCCs.[4,7] Besides such cancer-intrinsic aberrations, the host immune response might also impact on MCC dissemination and patient survival. In one study, the circulating levels of anti-T antigen antibodies have been shown to mirror the course of the disease, decreasing in patients that did not relapse after surgery, and rising in parallel with disease recurrence, sometimes even anticipating the detection of metastases.[8]

We investigated the association of several types of tumor-infiltrating leukocytes with the clinical and histopathological features of a Finnish population of patients affected by primary MCC using immunohistochemistry.[9] We found that the presence of the MCPyV DNA in MCC is associated with increased numbers of intratumoral T (CD3$^+$) cells, natural killer (CD16$^+$) cells and macrophages (CD68$^+$), as compared with MCPyV$^-$ MCCs. A high CD3$^+$ cell count was associated with favorable survival in both the MCPyV$^+$ (85 tumors) and MCPyV$^-$ (31 tumors) subsets of MCC. Intratumoral CD3$^+$ cell count was an independent prognostic factor for favorable overall and MCC-specific survival, as assessed by Cox multivariate analyses that also included MCPyV status, presence of locoregional nodal metastases and gender as cofactors.

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Submitted: 05/29/12; Accepted: 06/13/12
http://dx.doi.org/10.4161/onci.21120

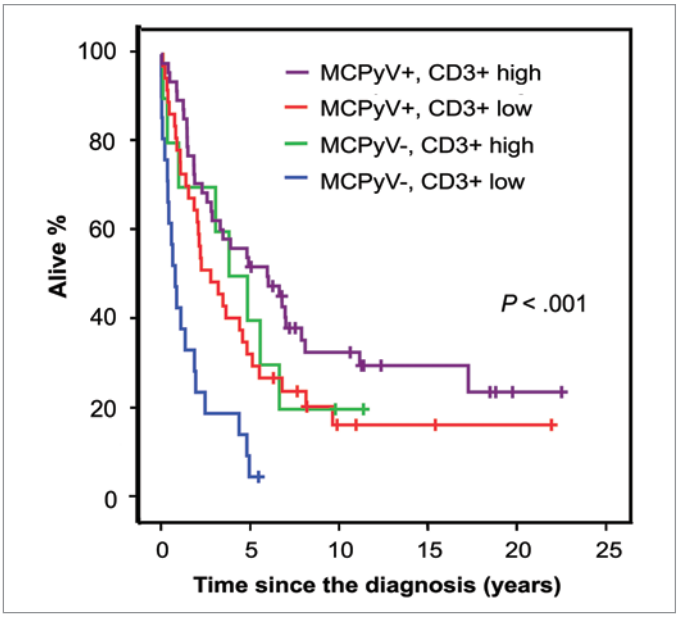

Figure 1. Kaplan-Meyer analysis of overall survival in Merkel cell carcinoma patients stratified according to the presence of MCPyV DNA in the tumor and the median count of intratumoral $CD3^+$ cells. Adapted from Ref. 9 with the permission of the American Association for Cancer Research.

Conversely, intratumoral macrophage or natural killer cell counts were not significantly associated with outcome. In a more detailed analysis of T-cell subsets, high cytotoxic T ($CD8^+$) and regulatory T ($FOXP3^+$) cell counts were each associated with favorable survival, whereas helper T ($CD4^+$) cell counts were not. Patients with a low $CD8^+/CD4^+$ or $FOXP3^+/CD4^+$ cell ratio had a poor outcome. When tumors were stratified based on the presence of the MCPyV DNA and $CD3^+$ cell counts, patients whose cancer contained MCPyV DNA and a high number of $CD3^+$ lymphocytes had the best outcome (**Fig. 1**). A similar association was detected between the presence of the MCPyV DNA and intratumoral $CD8^+$ cell counts.

These data suggest that intratumoral T lymphocytes influence the clinical outcome in MCC patients and that the host immune function may be important not only for MCC tumorigenesis but also for progression and metastasis. Although the presence of the MCPyV DNA is associated with an accumulation of T cells in the tumor, tumor-infiltrating T lymphocytes also influence the outcome of patients with $MCPyV^-$ MCC.

While the mechanisms by which MCC evades immunosurveillance require further study, it is tempting to speculate that some MCC patients might benefit from systemic therapies that boost the immune function. In the absence of randomized clinical trials that would specifically address this question, the benefits of conventional chemotherapy for advanced MCC patients remain controversial. Hence, investigations on the therapeutic potential of immunostimulatory antibodies such as anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and anti-programmed death-1 (PD-1) in this oncological setting is of interest. Inhibition of the PD-1 pathway in $CD8^+$ cells in mice chronically infected with the lymphocytic choriomeningitis virus restored the ability of $CD8^+$ cells to proliferate and secrete cytokines, decreased the viral load and lead to viral eradication.[10] T cells may gradually lose their function during chronic infections, and this might also take place during the development of MCPyV-associated MCC. The identification of MCPyV as a central factor in the pathogenesis of MCC and the recognition of the importance of host immune functions for the progression of MCC may pave the way to novel therapeutic options to manage this rare but highly malignant human cancer.

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