Received: 20 September 2021 | Accepted: 2 November 2021

DOI: 10.1111/1346-8138.16232

THE JOURNAL OF DERMATOLOGY

REVIEW

Immune activity in Merkel cell carcinoma

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Abstract

Merkel cell carcinoma (MCC) is widely known as a highly malignant skin cancer. The pathogenesis of MCC, however, remains mysterious due to the extremely small number of cases and its prevalence in the elderly. Despite its high-grade malignancy, spontaneous regression occurs with some frequency. The immune activity of the tumor underlies this peculiar behavior. In recent years, immune checkpoint blockade therapies, including the anti-programmed death ligand 1 antibody, have provided successful results. These therapies, however, are ineffective in approximately half the patients with advanced MCC and few treatments are available for those patients. In this review, we summarize the increasing body of evidence relating to the immune activity of MCC and immunological biomarkers. The interesting and sometimes peculiar behavior of MCC, such as their spontaneous regression, is largely due to their high immunosensitivity. Understanding the tumor immunokinetics of MCC should provide critical insight for understanding cancer immunotherapy. Here, we introduce a new classification for MCC according to its immune activity. Combined application of programmed death ligand 1 (a prognostic factor and predictor of the efficacy of immune checkpoint inhibitors in various cancers) with glucose-6-phosphate dehydrogenase (a new promising biomarker for MCC) may enable classification of MCC based on its immune status. Whether the new classification can be used to predict the efficacy of immune checkpoint blockade therapies remains to be evaluated in future studies, but the classification may facilitate future treatment selection.

KEYWORDS
biomarker, glucose-6-phosphate dehydrogenase, immune checkpoint inhibitor, Merkel cell carcinoma, programmed death ligand 1

1 | INTRODUCTION

Merkel cell carcinoma (MCC) is a rare and highly malignant skin cancer. In general, MCC has a local recurrence rate of 33–36%, regional lymph node metastasis of 41–55%, and a distant metastasis of 18–35%. It is considered to be highly malignant with high rates of metastasis and recurrence, and the 5-year survival rate is 0–18% in advanced stages.[1] The frequency of MCC is currently approximately 3/1 000 000 persons, but the number of cases is increasing.

Immune checkpoint inhibitors (ICI), including anti-programmed death ligand 1 (PD-L1) antibody or anti-programmed death 1 (PD-1) antibody are administrated systemically for advanced cases and have provided successful results. Approximately half of patients with advanced-stage MCC, however, are non-responders or have low sensitivity to immunotherapy. The discovery of new biomarkers to distinguish between responders and non-responders, and between high-risk and low-risk groups, is therefore critical toward developing new therapies and methods to increase the sensitivity to therapy.

Here, we focus on the immune activity of MCC and describe the peculiar behavior of MCC based on its immune response and immunological biomarkers. We further introduce a new classification enabled by combined application of PD-L1 and glucose-6-phosphate dehydrogenase (G6PD), a promising biomarker that we identified,[2] and present new treatment strategies based on this classification.

2 | PECULIAR BEHAVIOR OF MCC BASED ON ITS HIGH IMMUNOGENICITY

2.1 | Complete spontaneous regression of MCC

Many reports indicate that MCC is a highly immunogenic tumor, which may be reflected in the high rate of complete spontaneous regression (CSR) of MCC. Since the report by O'Rourke and Bell in 1986,[3] a number of cases of CSR have been reported, and it occurs with some frequency in the clinic. The rate of CSR is reported to be 1.7–3.0%,[4] a much higher rate than that of other solid carcinomas. CSR is observed even in some cases of metastatic MCC, and cases with CSR have a better prognosis than those with spontaneous regression of the primary tumor in malignant melanoma.[5,6] Dense lymphocytic infiltration is observed in MCC that begin to shrink after biopsy and appear to be in the process of spontaneous regression, supporting the involvement of tumor immunity in regression. CSR is also sometimes observed in people with weakened immune systems, such as the very old, who are ineligible for treatment, including surgery, or patients with chronic lymphocytic leukemia.[7] These cases suggest that CSR is not caused solely by the patient's active immunity, but rather that there are factors on the tumor side that cause CSR. Inoue *et al.*[8] reported that the TUNEL index, indicating apoptosis, and the number of tumor-infiltrating lymphocytes (TIL) were increased in four cases with CSR compared to three cases of non-regression. This finding suggests that apoptosis and local T-cell-mediated immune response are involved in CSR of MCC. CSR can occur at various times, such as following biopsy, secondary infection,[9] or abandoning treatment.[10] The mechanism underlying the peculiar behavior of these malignant tumors that are considered to be aggressive has yet to be elucidated.

2.2 | MCC with unknown primary origin

As many as 14% of MCC are discovered as lymph node lesions with no obvious skin lesions.[11] These types of MCC have a better prognosis than MCC with a primary origin[12] and are thought to be lymph node metastases that remain after spontaneous regression of the primary tumor. Is it really possible for the primary lesion to disappear without being noticed and without leaving any scars or vestiges? Pan *et al.*[13] described a possible primary MCC arising from a lymph node. According to their report, primary nodal MCC had a significantly lower association with Merkel cell polyomavirus (MCPyV, 31% vs 76%). This debate was settled when a large number of ultraviolet (UV) signature mutations were identified in lymph node lesions of MCC from an unknown primary tumor.[14] Similar to MCC, breast cancer is a malignant tumor in which lymph node metastasis with an unknown primary origin is reported with some frequency. These cancers are called "occult breast cancers" and have a better prognosis than non-occult breast cancers.[15] MCC and breast cancer have a paradoxically better prognosis when the tumor PD-L1 expression is high,[16] suggesting a relationship between cancer of an unknown primary tumor and tumor immunity.

2.3 | MCC in immunosuppressed patients

The high incidence of MCC in immunosuppressed patients with a significantly worse prognosis[17] is also evidence of its high immunogenicity. Approximately 10% of MCC patients are immunosuppressed in some way.[11] Disease-specific survival in immunosuppressed patients is approximately half that in non-immunosuppressed patients. Early-onset MCC is reported in organ transplant recipients and HIV/AIDS patients.[18,19] It is reported that MCC in immunosuppressed patients are mostly $MCPyV^-$.[20] On the other hand, MCPyV is also detected more frequently in immunosuppressed patients with non-melanoma skin cancers, such as basal cell carcinoma and Bowen's disease, than in immunocompetent patients with non-melanoma skin cancers.[21] Although this may indicate that reactivation of MCPyV plays an important role in the development of MCC in immunosuppressed patients and combined MCC (MCC coexisting with other skin cancers such as Bowen's disease or squamous cell carcinoma), combined MCC are mostly $MCPyV^-$,[22] as are MCC in immunosuppressed patients.

3 | IMMUNOLOGICAL BIOMARKERS OF MCC

3.1 | Tumor-infiltrating lymphocytes

Infiltrating lymphocytes and prognosis in MCC have been studied.[23–25] Paulson *et al.*[26] reported a relationship between $CD8^+$ lymphocytes in MCC and a better prognosis. Later, Sihto *et al.*[27] analyzed a group of 116 patients and showed a significant correlation between lower numbers of $CD8^+$ lymphocytes and lower survival of $MCPyV^+$ MCC. They also found that cases with a higher than median number of $CD3^+$ cells in the tumor had a better survival rate than those with a lower than median number of $CD3^+$ cells. Feldmeyer *et al.*[28] reported that the number of $CD8^+$ and $CD3^+$ lymphocytes in the tumor periphery correlates with overall survival in a group of 62 primary MCC cases. In high-density TIL, tertiary lymphoid structures (TLS), defined as $CD20^+$ B-cell follicles juxtaposed with $CD3^+$ T cells, are occasionally observed. TLS are reported in several cancers as prognostic and immunotherapy efficacy markers,[29,30] and in MCC, a significant correlation with recurrence-free survival was reported in 21 $MCPyV^+$ primary MCC in 2014.[31] The number of lymphocytes infiltrating the tumor and tumor periphery reflects the immune activity at that time,

but it is a variable indicator and can differ widely depending on the situation. In fact, the number of TIL changes significantly before and after medical procedures, including biopsies. The immunogenicity of the tumor is thought to play a major role in lymphocyte infiltration into the tumor. Walsh *et al.*[32] analyzed 22 cases and reported that high-density TIL are associated with MCPyV positivity.

3.2 | Merkel cell polyomavirus

Merkel cell polyomavirus was discovered in 2008, and is deeply involved in the development of MCC.[33] Infection with this virus itself is not rare, occurring in approximately half of all healthy individuals. In addition to infection, integration into MCC tumor genomes and a truncating mutation that renders the MCPyV large T-antigen replication incompetent are required for MCC development.[34] MCPyV-derived oncoproteins are highly immunogenic and induce a variety of immune responses. Many studies report a 70–80% positivity rate, but these reports are mostly from North America or Europe. The MCPyV positivity rate exhibits large geographical differences. In Australia, the MCC incidence is approximately twice as high as that in North America and Europe, but the MCPyV positivity rate is only approximately 20%.[35,36] The incidence of MCC is lower in Asia than in the West, and the MCPyV positivity rate was 69% in our Japanese cohort of 71 patients.[37] This geographical difference is related not only to the amount of sun exposure and skin color, but also to variations in the MCPyV genotype itself.[38] The presence or absence of MCPyV infection is thought to be a biomarker for estimating a patient's prognosis.[39] The relationship between MCPyV and immunity has been a focus of attention. The presence of MCPyV infection is associated with high PD-L1 expression in tumor cells[40] and large amounts of TIL, including $CD3^+$, $CD8^+$, $CD16^+$, $FoxP3^+$, and $CD68^+$ cells.[27] A recent study reported that the MCPyV status can inform estimation of the tumor mutation burden (TMB) of MCC.[41]

3.3 | Tumor mutation burden

Merkel cell carcinoma is one of the malignant tumors that exhibits a high TMB.[42] Knepper *et al.*[41] reported that MCC can be clearly divided into high and low TMB groups. They examined 317 MCC samples and reported that the low TMB group (55%) had a median TMB of 1.2 mutations/MB, while the high TMB group (37%) had a median TMB of 53.8 mutations/MB, with a range of 20.4–217.5 mutations/MB. In the high TMB group, MCC had an even higher TMB than malignant melanoma, which is considered to have a high TMB among various cancer types. Interestingly, this TMB classification correlated perfectly with the presence of MCPyV: none of the 117 samples in the high TMB group were $MCPyV^+$, and all $MCPyV^+$ cases had low TMB. On the other hand, 94% (110 samples) of the high TMB group showed a UV signature suggesting carcinogenesis due to accumulated UV damage. The TMB is the amount of genetic mutations in the genome of cancer cells. A high TMB indicates the production of more mutant proteins (neoantigens), which are recognized as foreign by the immune system, with a higher expected effect of immunotherapy. The response rate of ICI for MCC does not correlate with the TMB, however, and high response rates are observed even when the TMB is low ($MCPyV^+$).[43,44] These findings suggest that the MCPyV antigen compensates for the antigenicity in the low TMB group. Therefore, in the presence or absence of MCPyV, TMB values should not be considered predictors of ICI efficacy. $MCPyV^+$ MCC expresses polyomavirus antigens that serve as epitopes for antigen recognition.[45,46] On the other hand, $MCPyV^-$ MCC have a high TMB, and many of them have UV-specific mutation signatures.[41] The high antigenicity due to their induction by viruses or UV supports the high efficacy of immunotherapy in MCC.

3.4 | Programmed death ligand 1

Whether PD-L1 correlates with the prognosis remains inconclusive. While it has been reported that PD-L1 expression does not correlate with prognosis,[47] others have reported that PD-L1 expression correlates with better clinical outcomes in MCC, in contrast to other solid carcinomas such as malignant melanoma.[40] PD-L1 is an immunoinhibitory molecule that suppresses T-cell activation. Overexpression of PD-L1 in cancers usually leads to tumor progression, but in MCC, high expression of PD-L1 is a good sign. As mentioned above, this paradoxical correlation between PD-L1 and prognosis is only observed for MCC and breast cancer.[16] Tumor PD-L1 expression is used to predict the ICI response in other cancer types, but it is not used as a companion diagnostic in MCC. Although PD-L1 expression in MCC provides clues as to whether the tumor is immunologically "hot" or "cold", its clinical use as a prognostic or efficacy marker is difficult due to its high heterogeneity within individual patients.[48] We reported that PD-L1 levels are uniform if multiple primary lesions that appeared at the same time are resected at the same time and immunostained under the same conditions. In that case, surgical invasion results in increased PD-L1 expression.[49] It is clearly established that PD-L1 reflects a patient's immune status. In our other study of 71 patients and 90 samples, we found no correlation between tumor PD-L1 expression and prognosis.[37] On the other hand, in an analysis of metastatic lesions only, there was a strong correlation between high PD-L1 expression in metastases and a good prognosis, but a biomarker that can be used only after the appearance of metastases may have limited usefulness for clinical application. In other words, PD-L1 expression in MCC is a consequence of the active anti-tumor immune response that is currently underway, and may not be predictive of what the immune status will be in the future and after the use of ICI.

3.5 | Glucose-6-phosphate dehydrogenase

As discussed above, known immunological biomarkers for MCC are difficult to use in clinical practice. We therefore propose a new promising biomarker for MCC based on the tumor immune activity. In our study, G6PD is listed as one of the factors that inversely correlates

with tumor immune activity on the basis of comprehensive RNA sequencing using next-generation sequencing.[2] G6PD expression positively correlates with lymph node or distant metastasis during follow-up and negatively correlates with PD-L1 expression, and had the smallest *p*-value in both analyses.[2] G6PD expression measured by immunostaining is less variable than that of PD-L1 within the same patient and correlates with patient outcome. On the other hand, serum G6PD activity sensitively reflects the current therapeutic response and tumor stage. These findings suggest that G6PD is a useful biomarker for predicting prognosis and determining treatment response. G6PD is a key enzyme in the pentose phosphate pathway that provides nicotinamide adenine dinucleotide phosphate (NADPH) to cells, protecting them from oxidative damage. G6PD is highly expressed in various cancers, reflecting poor prognosis.[50,51] G6PD is involved in angiogenesis and apoptosis and considered to be a potential target for cancer therapy.[52,53] In addition, the lack of G6PD will cause autophagy, which will enhance the effect of chemotherapy.[54] and may activate anti-tumor immune response.[55,56] Tumor suppressor p53 plays a role in regulating G6PD, and tumor-associated p53 mutants, which are found in many cancers, are unable to suppress G6PD.[57] p53 is also the most common mutation in MCC, whether MCPyV$^+$ or MCPyV$^-$.[41] In contrast to PD-L1, which reflects the strength of the immune response on the patient side, G6PD may represent the responsiveness of the tumor itself to immunity.

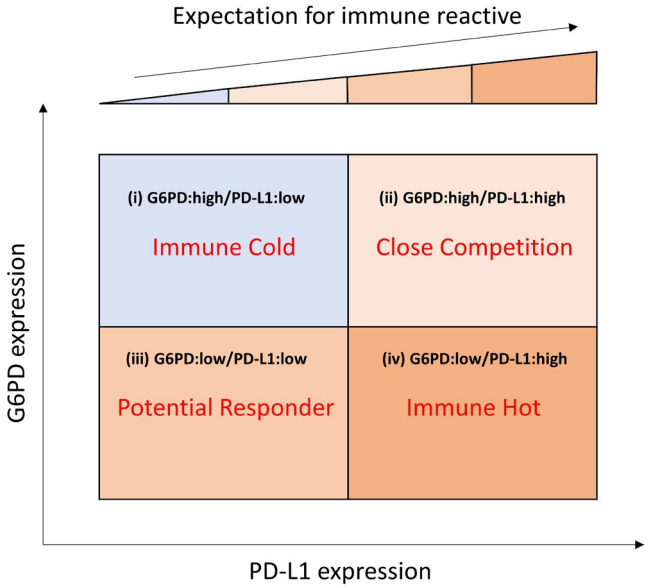

FIGURE 1 Merkel cell carcinoma (MCC) classification based on immune activity. Glucose-6-phosphate dehydrogenase (G6PD) expression reflects potential immune resistance, and programmed death ligand 1 (PD-L1) expression indicates the strength of the immune response at that time. Based on the immunostaining results of these two biomarkers, MCC can be classified into four statuses. The stronger the orange color, the higher the expectation for immune response

4 | TREATMENT STRATEGY OF MCC CONSIDERING THE TUMOR IMMUNITY

4.1 | Classification of MCC based on the immune activity

By combining immunochemistry to evaluate G6PD expression and PD-L1 expression as indicators of the current tumor and patient immune status, we propose the following classification and treatment strategy (Figure 1). In cases with G6PD:high/PD-L1:low (~40% of our cases), the tumor grows aggressively and immune sensitivity is low ("immune cold"). In cases with G6PD:high/PD-L1:high (10% of our cases), tumor immunity is fighting against an aggressive tumor in close competition. In cases with G6PD:low/PD-L1:low (20% of our cases), the tumor has potentially high immune activity but the immune system is quiescent or suppressed, and removal of immunosuppression and additional stimulation is required. Lastly, in cases with G6PD:low/PD-L1:high (30% of our cases), the tumor has high immune activity ("immune hot") and a better prognosis is expected. Further validation studies are required before this classification can actually be applied in clinical practice, but it may facilitate the selection of treatment for MCC.

4.2 | Is a wide excision necessary?

Various guidelines, including those of the National Comprehensive Cancer Network[58] or the European Association of Dermato-Oncology/European Organization for Research and Treatment of Cancer,[59] recommend resection of MCC with 1- to 2-cm margins. In a study of 45 stage-I MCC treated with Mohs micrographic surgery, the mean margin required to obtain a negative pathological margin was 16.7 mm, and positive margins at 2 cm and 3 cm were 25% and 12%, respectively.[60] Even a 3-cm margin is not sufficient for reliable resection. On the other hand, the condition of the resection margin is not associated with local recurrence in patients that undergo postoperative radiation therapy.[61,62] In some cases, it may be an option to limit the resection margin to simply a suturable margin on the assumption that postoperative radiation therapy will be performed. For tumors that are too large to be removed or the location prohibits removal, subtotal resection and radiation is an option. As mentioned above, although this may be a very rare case, there is a report that surgical invasion activated tumor immunity.[48]

4.3 | Lymph node dissection and radiation therapy to lymph nodes

Aggressive treatments to regional lymph nodes, including lymph node dissection or radiation therapy to lymph nodes, should only be selected for cases with obvious lymph node metastasis. Considering the possibility of future immunotherapy, prophylactic dissection and prophylactic irradiation are not wise choices because they inhibit immune cell recruitment. Wright *et al.*[63] analyzed 1473 stage-III

patients and found that lymph node dissection is associated with better overall survival than radical radiation therapy, although it should be noted that the dissection group included those who received postoperative radiation. In contrast, Bhatia *et al.*[64] analyzed 2065 patients with stage-III MCC and reported no significant difference in overall survival between the lymph node dissection group and the radiotherapy alone group or the combined surgery and radiotherapy group. The order of priority between lymph node dissection and radiation therapy is still not clearly defined. Depending on the immune activity of the tumor, ICI should also be considered prior to invasive treatment of the regional lymph nodes.

4.4 | Immune checkpoint inhibitors

Malignant tumors, including MCC, express PD-L1 protein and protect themselves by binding to PD-1 protein expressed on cytotoxic T cells, thus slowing the immune response. Avelumab, a PD-L1 inhibitor, and pembrolizumab, a PD-1 inhibitor, have been approved by the US Food and Drug Administration for MCC. The overall response rates for advanced MCC in patients receiving no previous systemic therapy are 62% for avelumab[44,65] and 56% for pembrolizumab.[66] While these results are groundbreaking, nearly half of patients still do not benefit from these drugs. It is necessary to determine more appropriate adaptation of ICI treatment.

In the future, ICI treatment is expected to be expanded to postoperative adjuvant therapy and preoperative neoadjuvant therapy. Adjuvant therapy is already being used in malignant melanoma and other malignant tumors, where ICI is administrated for a defined period of time after radical resection to reduce the possibility of recurrence and metastasis. For MCC, the ADAM (Adjuvant Avelumab in Merkel) trial (NCT 03271372), a phase III trial of adjuvant therapy using avelumab, is ongoing in the USA. In the aforementioned CheckMate 358 study, nivolumab was administrated to 39 patients with resectable MCC, and except for three patients who could not be operated on due to disease progression (n = 1) and adverse events (n = 2), 36 patients underwent surgery, and 17 patients (47.2%) achieved complete pathological remission.[67] Classification based on immune activity by G6PD and PD-L1 may help determine the indications for adjuvant or neoadjuvant therapy.

5 | CONCLUSIONS AND PERSPECTIVES

In this review, we summarize some phenomena based on the high immunogenicity of MCC and previously reported immunological biomarkers, and propose a classification and treatment strategy based on the immune activity of MCC. The immunokinetics of MCC are not entirely clear, and no definitive immunological biomarkers have been identified. G6PD, which may be useful for the classification of MCC on the basis of its immune activity, has not yet been confirmed to be useful for predicting the effect of ICI. Further cohort studies in MCC patients who have been treated with ICI are required. Understanding the tumor immunity dynamics of MCC will facilitate the development of immunotherapies for other carcinomas as well. MCC with its various characteristics derived from immune responses is a valuable representative model for tumor immunology research.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

None declared.

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How to cite this article: Nakamura M, Morita A. Immune activity in Merkel cell carcinoma. *J Dermatol*. 2022;49:68–74. https://doi.org/10.1111/1346-8138.16232