Letters to the Editor

Comment on 'Cytokeratin 20-negative Merkel cell carcinoma is infrequently associated with the Merkel cell polyomavirus'

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To the editor: We read with great interest the recent paper by Miner *et al*[1] titled 'Cytokeratin 20-negative Merkel cell carcinoma is infrequently associated with the Merkel cell polyomavirus' published online in November 2014. They concluded that when quantitative PCR without immunohistochemistry (IHC) is used for Merkel cell polyomavirus (MCPyV) detection, cytokeratin 20 (CK20)-negative Merkel cell carcinoma (MCC) is infrequently related to MCPyV. We have evaluated the MCPyV status of their virus-related case no. 13 as virus-nonrelated MCC and have recommended additional IHC for MCPyV–large T antigen (LT; CM2B4; Santa Cruz Biotechnology, Dallas, TX, USA) to increase the accurate evaluation on the MCPyV status. However, we generally agreed and reconfirmed their argument using our data of 111 MCC cases and also added new insights on the CK20-negative or -focally weak-positive MCCs with prognostic assessment.

The diffused and strong CK20 immunopositive staining pattern is a well known specific feature of MCC.[2] However, some MCCs show a negative or focally weak expression pattern for CK20 immunostaining. We reviewed 111 cases (Japan (JPN): 69 cases (female 49, male 19, and unknown 1); United Kingdom (UK): 42 cases (female 29, male 12, and unknown 1)) and analyzed the relationship of CK20 negativity and MCPyV status. Both quantitative PCR and IHC were used to evaluate the MCPyV infection status in MCCs. Immunohistochemically, nine (JPN eight and UK one) MCCs were negative for CK20, and six (JPN one and UK five) MCCs were very focally weak-positive for CK20. In CK20-negative cases, the immunoreactivity of the following was reconfirmed: the positivity of chromogranin A, synaptophysin or CD 56 (neural cell adhesion molecule) and the negativity for thyroid transcription factor-1 to distinguish MCC from metastasis of other small cell carcinomas. Quantitative PCR revealed that 70/111 (63%) (JPN, 52 (75%) and UK, 18 (42%)) cases had the relevant levels of MCPyV–LT DNA quantity for MCPyV-related MCC. Seven (78%) of nine CK20-negative cases and three (50%) of six focally weak-positive cases were negative for MCPyV. In addition, two CK20-negative MCC cases had combined squamous cell carcinoma *in situ*. Fisher's exact test revealed that MCPyV-negative MCCs were significantly associated with negativity or focally weak positivity for CK20 ($P=0.018$).

We noticed that Miner *et al*[1] continue to make a distinction between MCPyV-positive and -negative MCCs using only quantitative PCR without IHC for MCPyV–LT (CM2B4 antibody). In case no. 13 of Miner *et al*'s[1] article (Table 3 and Figure 3), quantitative PCR data suggested very low copy number of MCPyV DNA. MCC-related MCPyV is usually detected as high viral loads, such as ~1 copy per tumor cell, because tumor-related MCPyV DNA is monoclonally integrated into the carcinoma cell genome. In our previous study,[3] we showed very low viral loads of MCPyV DNA in various non-neoplastic tissues from 29 of 41 (71%) autopsy cases without MCC, and MCPyV DNA prevalence was ~50% in the normal skin. Therefore, there is a possibility that the detected viral DNA in case no. 13 is derived from MCPyV-infected non-neoplastic background tissues and not from MCPyV-infected MCC. In this case, IHC detection of the MCPyV protein in MCC cells, such as MCPyV–LT or small T antigen is important to identify MCPyV-related MCCs.[4] Commercially, anti-MCPyV–LT antibody (CM2B4) is the only available antibody for detecting the MCPyV protein. We also performed CM2B4 immunostaining. In our eight CK20-positive cases, discrepant data between MCPyV DNA quantified data and CM2B4 immunostaining (+/−: six cases and −/+: two cases) were observed. In these cases, high viral loads detected by quantitative PCR were the gold standard for identifying MCPyV-related MCCs. Conversely, in CK20-negative MCCs, no mismatched results were observed between MCPyV quantification and CM2B4 IHC.

We also conducted the prognostic analysis using age-adjusted Cox hazard analysis; however, CK20 negativity was insignificantly related to the MCC-specific death (hazard ratio: 0.56; 95% confidence interval: 0.23–1.36).

The expression of CK20 is also known in normal Merkel cells,[2] and some types of carcinomas, such as colorectal cancer, highlight the association between the loss of CK20 expression and poorly differentiated-type carcinomas.[5] In our previous study,[6] we demonstrated that MCPyV-negative MCCs show more poorly differentiated features, such as severe nuclear atypia and pleomorphism, than MCPyV-positive MCCs. The combination of CK20 expression loss and MCPyV negativity may be associated with the poorly differentiated MCC features.

We hope that our comments on the association of CK20-negative expression with MCPyV infection status

and our data in this regard will be helpful to readers for further understanding the pathology of MCCs.

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Disclosure/conflict of interest

The authors declare no conflict of interest.

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Reply to Commentary on "Cytokeratin 20-negative Merkel cell carcinoma is infrequently associated with the Merkel cell polyomavirus"

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To the editor: We appreciate the comments by Iwasaki *et al* regarding our recent article, 'Cytokeratin 20-negative Merkel cell carcinoma is infrequently associated with the Merkel cell polyomavirus.' They report findings in an independent cohort of cytokeratin 20-negative Merkel cell carcinomas (MCCs), the majority of which are negative for Merkel cell polyomavirus (MCPyV), in agreement with our observations.

Among MCCs classified as MCPyV positive in our study, one case (#13) had relatively low MCPyV by quantitative PCR (qPCR). Although qPCR is accepted as the gold standard for MCPyV detection in MCC, there is debate about whether low levels of MCPyV represent tumorigenic virus or contamination by background wild-type virus.[1–3] In addition, the sensitivity of any given primer pair may vary dramatically from case to case.[1] Hence, currently there is no universally accepted threshold for considering a tumor MCPyV positive by qPCR. We agree with Iwasaki *et al* that immunohistochemistry for MCPyV large T antigen (LTAg), while less sensitive than qPCR, may be informative in some cases that are borderline by qPCR. We performed immunohistochemistry for LTAg expression in case #13 using CM2B4 antibody as previously described.[4] This demonstrated moderate to strong nuclear staining for LTAg in $>80\%$ of tumor cells, validating our classification of this tumor as MCPyV positive.

We agree that loss of cytokeratin-20 expression may be associated with loss of differentiation in MCC. However, further study is needed to determine the molecular similarity of these tumors to conventional (cytokeratin 20-positive) MCC.

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