Identification of Merkel cell polyomavirus in Merkel cell carcinoma tissue: case report of a Taiwanese patient

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ABSTRACT

Merkel cell carcinoma (MCC) is a rare malignancy with aggressive behavior mostly seen in the elderly and immunosuppressed patients. In 2008, the clonal integration of a new human polyomavirus, named Merkel cell polyomavirus (MCPyV), was found to be closely associated with the development of MCC. This correlation was established by subsequent reports, mostly in Caucasians. To evaluate whether this correlation is also relevant among the Taiwanese population, we used polymerase chain reaction to detect the presence of MCPyV in a formalin-fixed and paraffin-embedded MCC specimen. In addition, we used tissue with seborrheic keratosis from the same patient as a control. Three different specific primer sets, LT1, LT3 and VP1, were used to amplify characteristic MCPyV genes, and large T antigen and VP1 genes, respectively. Amplicons of the expected sizes for all three primer pairs were detected in MCC tissue and amplicons for both LT3 and VP1 were detected in seborrheic keratosis tissue. More studies are needed to quantify viral positivity of MCPyV in various tissues of patients with MCC and to establish genetic interactions between the virus and host.

KEYWORDS
Merkel cell carcinoma
Merkel cell polyomavirus

CASE REPORT

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Introduction

Merkel cell carcinoma (MCC) is a rare cutaneous neuroendocrine carcinoma. It consists of malignant small blue round cells typically expressing neuroendocrine markers and has a characteristically paranuclear dot-like expression pattern of cytokeratin (CK)-20. MCC has a high tendency for local recurrence, lymph node metastasis, and ultimately, hematogenous spread. There has been a great deal of research into the etiology of MCCs. In 2008, a breakthrough discovery of one new polyomavirus, named Merkel cell polyomavirus (MCPyV), was shown to be clonally integrated in MCC cases.¹ This phenomenon was also considered to be associated with the tumorigenesis of MCC. To assess the implications of MCPyV in MCC of the Taiwanese population, recently excised MCC tissue was examined for MCPyV positivity by polymerase chain reaction (PCR) using two specific primer pairs in the T antigen locus (LT1 and LT3) and one in the VP1 gene (VP1).

Case report

An 89-year-old woman presented with a 3-month history of a progressively enlarging nodule on the nasal bridge (Figure 1). There was no pruritus, pain or tenderness. Approximately 30 years ago, she had undergone surgical excision and radiotherapy for cervical carcinoma. She also had a history of bilateral hydronephrosis, coronary artery disease and hyperlipidemia. A physical examination revealed a mild erythematous nodule approximately 1 cm × 1 cm on the nasal bridge.

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The bilateral cervical lymph nodes were not palpable. Clinical differential diagnoses included basal cell carcinoma, MCC, and appendageal tumors. Wide surgical excision was performed. Pathologically, the dermis was composed of nests and sheets of small, round, blue cells displaying scant cytoplasm, large and round nuclei, conspicuous nucleoli, and increased mitotic figures. Immunohistochemically, these neoplastic cells were positively stained for CK-20, neuron-specific enolase and synaptophysin, and were negatively stained for S-100 and thyroid transcription factor-1 (Figure 2). Therefore, we confirmed the diagnosis of MCC.

**Methods and results of detection of MCPyV**

**DNA extraction**

Both formalin-fixed and paraffin-embedded tissues were deparaffinized with xylene and dehydrated with 100% ethanol. DNA was then extracted from each deparaffinized fixed tissue using a QIAamp DNA Mini Kit (QIAGEN, Valencia, CA, USA) according to the manufacturer’s instructions.

**PCR and DNA sequencing**

PCR reactions were performed in a 25 μL volume containing 5 μL of extracted DNA, 15 μL of 2 × GoTag Green Master Mix (Promega Co., Fitchburg, WI, USA) and 50 mol of each primer. The primer sequences from 5’ to 3’ were as follows, LT1-F: TACAAGCACTCCACCAAAGC, LT1-R: TCCAATTA-CAGCTGGCCTCT, LT3-F: TTGTCTCGCCAGCATTGTAG, LT3-R: ATATAGGGGCCTCGTCAACC, VP1-F: TTTGCCAGCTT-ACAGTGGCTT, VP1-R: TGGATCTAGGCCCTGATTTTT. The expected sizes of PCR products for LT1, LT3 and VP-1

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Figure 1  One erythematous nodule progressively enlarged on the nasal bridge for 3 months.

Figure 2  (A) Tumor cells are aggregated in the upper dermis (H&E, 100×). (B) Sheets of small blue round cells (H&E, 400×). (C) Positive staining for CK-20 (100×). (D) Negative staining for S-100 (100×).
Merkel cell carcinoma and Merkel cell polyomavirus

PCR results in tissues of MCC and seborrheic keratosis

Both of the specimens showed MCPyV (Figure 3). The fragment sizes of the amplicons of LT1, LT3 and VP1 were 440 bp, 309 bp and 352 bp, respectively. According to Feng et al., any amplicon detected by these primers indicates the presence of MCPyV.

Results of DNA sequencing for PCR products

The DNA sequences of PCR products of MCC were aligned using the National Center for Biotechnology Information Basic Local Alignment Search Tool (BLAST) to find regions of similarity between biological sequences. The results of alignment showed 100% identity to the Merkel cell polyomavirus strain Tokyo Kaposi sarcoma, complete genome (GenBank: FJ464337.1).

Discussion

Immunosuppressed transplant and AIDS patients are much more likely to develop MCC than are age-matched controls.1–3 These similarities to Kaposi’s sarcoma (KS), an immune-related tumor caused by human herpesvirus (HHV)-8, raise the possibility that MCC may also have an infectious origin. In 2008, Feng et al.1 made an important discovery of MCPyV in all 10 patients with MCC.

MCC is a rare and aggressive neuroendocrine tumor. Its mortality rate is high and its overall survival rate ranges between 25% and 75%.4 It usually appears as a solitary, violaceous, dome-shaped nodule or indurated plaque in a sun-exposed area. The most common clinical features are its asymptomatic nature, rapid expansion, immunosuppression, prevalence in older individuals, and UV-exposed site, which are abbreviated as AEIOU.5 Microscopically, the tumor is located in the dermis and infiltrates the subcutaneous tissue; the overlying epidermis is normal. A pathological examination shows a generally dense growth of small, blue cells, with immunohistochemical evidence of neuroendocrine differentiation. Its histopathological findings are similar to those of metastatic small-cell lung cancer, lymphomas, and melanoma. Immunohistochemical staining can help make the differentiation between other diseases. MCC often shows positive staining for CK-20, neuron-specific enolase, chromogranin, and synaptophysin, and negative staining for thyroid transcription factor-1 and S-100.

While several hypothesized pathogeneses of MCC have been proposed including Bcl-2, the Wnt signaling pathway, mitogen-activated protein kinase signaling cascades, the PI3K/Akt signal pathway, and tumor-suppressor genes,6 none of them can fully explain its pathogenesis. In 1994, Chang et al.7 discovered HHV-8 in KS. Since both KS and MCC occur more frequently among immunosuppressed and AIDS patients, it can be speculated that MCC might also have an infectious origin. Feng et al.1 sequenced a 5387-base pair genome called MCPyV from MCC tissue. MCPyV has a circular double-stranded DNA genome and encodes characteristic polyomavirus genes including large T antigen, small T antigen, and VP1 and VP2/3 genes.

Furthermore, Feng et al.1 found that this virus was not a casual infection since all 10 patients with MCC had MCPyV. Eight of the 10 patients with MCC were strongly positive for MCPyV infection by DNA PCR, and the other two were negative, but were positive by Southern hybridization of PCR products. In the non-MCC control group, they found MCPyV in only 5 of the 59 tissues (8%) from different organs, and 4 of the 25 skin tissues (16%). The virus can appear in the skin and in other various tissues. Of infected skin tissues, the virus is found in MCC as well as in normal or other diseased skin. However, the MCPyV positive rate in MCC is much higher than that in other skin diseases. None of the previous reports have examined the positive rate of MCPyV.
detected from normal or other diseased skin tissue in the same patient with MCC. In our study, we detected viral infection of both MCC and seborrheic keratosis from the same patient. HHV-8 may be present in KS, lymph nodes, muscle and unaffected skin in AIDS patients with KS, and it can result in tumorigenesis of KS by viral interaction and interference with host DNA cycling.7,8

According to Feng et al,1 viral integration into host DNA that affects cell cycling may be the key for causing tumorigenesis of MCC. Since our two sampled tissues were soaked in formalin, which may have caused fragmentation at some DNA segments, we were not able to further test if there was viral integration in MCC or seborrheic keratosis.

In addition to viral integration to host DNA, there may be other possible mechanisms for tumorigenesis. MCPyV has both early and late genes. The early-expressed genes encode large and small T antigens, which bind to host proteins to force the cell into the S phase and facilitate viral replication. The late genes, such as VP1 and VP2/3, encode the viral coat and enable lysis. Importantly, the large T antigen of MCPyV contains an oncogenic Leu-X-Cys-X-Glu motif that may directly bind pRb, and MCPyV may impede tumor suppression.1 Furthermore, in all published MCPyV genome sequences, premature codons are predicted within the second exon of the large T antigen.6 This phenomenon provides another mechanism to explain how MCPyV contributes to the progression of MCC. Cell-cycle progression activities are preserved via the intact amino terminus of large T antigen. Conversely, lethal genomic instability cannot occur because of the lack of replicative function of the carboxyl terminus in the truncated protein.

Becker et al9 analyzed 53 European patients with MCC and 45 patients (84.9%) presented positivity. Garneski et al10 found that 16 of the 37 (43%) MCC tumors were positive for MCPyV originating from North America and Australia. Interestingly, the proportion of tumors positive for MCPyV was much higher in tumors originating from North America (11/16, 69%) than those originating from Australia (5/21, 24%).10 Notably, MCCs that contain the viral genome display a more-aggressive behavior than their virus-negative counterparts.9 Viral prevalence in MCC seems to differ in different races. Our results showed that MCPyV is found in Taiwanese, and that MCPyV is found in MCC as well as in seborrheic keratosis. Further studies are required to establish the prevalence rate of MCPyV in the general population and patients with MCC, the viral positive rate in variable tissues from patients with MCC, and genetic interactions between virus and host. Further new discoveries might also provide new strategies to treat this aggressive tumor.

References