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Prevalence of Merkel Cell Polyomavirus among Swiss Merkel Cell Carcinoma Patients

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Key Words
Carcinogenesis · MCPyV · Merkel cell carcinoma · Polyomavirus

Abstract
Background: Merkel cell carcinoma (MCC) is a malignant neuroendocrine neoplasm which shares structural and immunohistochemical features with neuroectodermally derived cells. One hypothesis claims that it arises from Merkel cells, highly innervated neuroendocrine cells involved in mechanoreception in the skin. The incidence rate of this cancer increases with age and sun exposure as well as after immunosuppression. Recently, the clonal integration of a newly identified polyomavirus called Merkel cell polyomavirus (MCPyV) was reported in up to 80% of MCC tissue. Here we report the incidence rate of MCPyV in MCC patients in Switzerland.

Methods: We performed polymerase chain reaction in a collection of 32 samples obtained from pathology institutes around Switzerland. We used three different published primer sets. As control groups we used 7 squamous cell carcinoma samples and 11 normal skin samples. Conclusions: We detected viral DNA in 20 out of 30 cases of MCC and in 0 out of 19 control samples. The presence of viral DNA in 66.6% of our MCC tissue specimens confirms the high prevalence of MCPyV in MCC patients described in American, German, French and Hungarian patient collections.

Introduction

Merkel cell carcinoma (MCC) is a rare aggressive neuroendocrine malignancy that frequently shows local recurrence, lymph node and distant metastases. One hypothesis claims that it derives from the normal Merkel cells of the epidermis which are slow-adapting mechanoreceptors. It was first described in 1972 by Toker [1] who identified five patients with unusual skin tumors. Within the last 20 years its incidence has tripled, with an 8% annual increase rate [2], leading the American Cancer society to predict approximately 1,500 new MCC cases in 2008 [3]. It occurs on sun-exposed skin areas in the Caucasian population, predominantly males, with a mean age at the time of diagnosis of 70 years [4]. Its incidence rate rises with immunosuppression, e.g. in organ transplant recipients, as well as in those with chronic lymphocytic leukemia and HIV infection [5–7]. This link to im-
munosuppression raised the question of a possible infectious etiology of this type of cancer.

In 2008, Feng et al. [8] identified a new human polyomavirus, designated as Merkel cell polyomavirus (MCPyV) based on its detection in human MCC samples using a technique known as digital transcriptome subtraction. MCPyV is a double-stranded DNA virus of 5,387 bp that is highly homologous to the murine polyomavirus subgroup [8] and expresses an early region (small and large T antigen) and a late region containing VP1, VP2 and VP3 open reading frames. It has also been shown that the MCC harbors genes encoding for a truncated form of large T antigen (LT) mutations, a process believed to be necessary for polyomavirus-mediated carcinogenesis. This monoclonal pattern of viral integration suggests a possible role of this virus into MCC carcinogenesis. It has also been shown that the MCC harbors genes encoding for a truncated form of large T antigen (LT) mutations, a process believed to be necessary for polyomavirus-mediated carcinogenesis [11]. This study was designed to test the largest number of MCC patients so far studied in Switzerland for the presence of viral DNA using PCR.

The study was submitted to the ethical committee of Zürich (EK No. 860) and the still alive patients gave informed consent to use their material for the purposes of the study.

**DNA Extraction**

Five consecutive 5-μm paraffin sections from each specimen were used for DNA extraction. DNA was extracted using a standard procedure protocol. After deparaffinization the tissues were lysed by overnight treatment with proteinase K (56°C). DNA was purified with phenol and was precipitated with 3 M sodium acetate and absolute ethanol. The pellet was extra washed with 70% ethanol to eliminate excess salt. Dried DNA was resuspended in 60 μl of sterile TE and was stored at 4°C before it was used for PCR. Purified DNA was measured using a Nanodrop 1,000 spectrophotometer (Thermoscientific, Wilmington, Del., USA).

**Patient Population**

The medical records of 9 of the 32 patients included in the study were retrospectively reviewed. The median age of the patients at the time of diagnosis was 77 years; 45 and 55% of the cases were male and female, respectively. One patient had had a squamous cell carcinoma (SCC) 2 years before developing MCC and one patient was diagnosed with melanoma and SCC also 2 years before the diagnosis of MCC. The SCC sample from the last patient was also included in the study.

As control groups, we used 8 SCC and 11 normal skin samples selected from the archives of the Dermatopathology Institute of the University Hospital of Zürich. Control cases were matched with the examined MCC patients only according to age, gender and localization of the primary lesion.

**Polymerase Chain Reaction**

DNA integrity was tested with β-globin PCR. PCR was performed with 100–120 ng of genomic DNA using the AmpliTaQ Gold (Roche Molecular Systems, Basel, Switzerland) in a final volume of 50 μl. For detection of the MCPyV we used 3 primer sets (LT1, LT3 and VP1) as published [8]; two were directed against the LT antigen and one was directed against the VP1 gene. Water was used instead of template DNA as negative control.

**Sequence Analysis**

PCR products of 8 patients were subjected to sequence analysis. The resulting sequences were compared to the reference sequences of NCBI Entrez Nucleotide Database gb/EU375803 MCPyV isolated from MCC350 using the Blast program [12].

**Results**

Thirty out of 32 samples revealed a β-globin positive product of 268 bp and these samples were included in the study. In total, 20 out of 30 MCC samples tested positive for the virus with PCR (fig. 1). Five were positive in all primer sets, while in 10 cases no viral DNA was amplifiable. Out of 30 MCC samples, 6 tested positive for the presence of the virus by LT1 (440 bp) primer set, 19 tested positive using the LT3 (308 bp) primer set and 16 tested

![Fig. 1. Representative results of the PCR products (308 bp) with LT3 primers. m = Molecular weight marker VI; neg = negative control (H2O); 1–4 = MCC samples; pos = positive control (Merkel cell tumor sample obtained from Prof. Axel zur Hausen’s lab).](image-url)
positive using the VP1 (351 bp) primer set (fig. 2). Compared with MCPyV DNA-negative cancers, MCPyV DNA-positive cancers tended to be more often located on the extremities (65 vs. 40%, \( p = 0.193 \), two-sided \( \chi^2 \) test), which is in accordance with the findings of Sihto et al. in a set of North European patients [13]. PCR-negative controls remained negative in all experiments.

The virus was not detected in the SCC sample derived from one MCC patient. None of our samples of the control groups (8 SCC and 11 normal skin) were positive for the presence of MCPyV.

The sequenced PCR amplicons showed 96–99% homology to the NCBI Entrez Nucleotide Database gb/EU375803 MCPyV isolated from MCC350. We have not sequenced the entire viral genome for detection of further strain variations.

Clinical follow-ups of the nine patients for whom we received clinical information suggest that there is no significant difference regarding the course of disease in MCPyV-positive and -negative patients, stratified by tumor stage (table 1).

Discussion

Even though MCC is one of the most aggressive skin cancers, with a mortality rate of 33% [2], little is known about potential signalling mechanisms that drive carcinogenesis in MCC [14]. The high incidence of the recently described MCPyV in MCC, together with the monoclonal integration pattern [8] and the truncation of LT antigen in MCC [11] shed new light on a potential viral carcinogenesis of MCC.

We demonstrated the presence of viral genome in 66.6% of our cases with PCR, which is similar to the results published from other groups [8, 15–22]. In contrast to Feng et al. [8], we used DNA extracted from formalin-fixed paraffin-embedded specimens. It is known that fixation causes a decrease in amplification efficiency of larger amplicons. Accordingly, we have noticed some differences in detection of MCPyV using the different primer sets. In our lab, LT3 primer proved to be superior for the detection of MCPyV, which is also confirmed by other groups [15–17, 21]. Only 6 out of 19 cases tested positive for the presence of the virus with primer LT1 (440 bp).

We were not able to detect any viral DNA in our control groups, neither in SCC nor in normal skin. Kassem et al. [23] recently demonstrated the presence of viral DNA in 32% of sporadic non-melanoma skin cancers. They also showed that MCPyV was more frequently found in non-melanoma skin cancers of immunosuppressed patients compared to immunocompetent patients. This discrepancy may be due to the higher numbers of cases in their study than in ours.

Andres et al. reported no relevant association of MCPyV with seborrheic keratosis, basal cell carcinoma, and lentigo maligna melanoma in an age- and sex-matched non-MCC population when the virus was found in 6% (2/33) of all the investigated non-MCC skin tumors [24].

In the one patient in our set suffering from a MCPyV-positive MCC and, previously, from an SCC, we did not

Fig. 2. MCPyV PCR results using the three different primer sets LT1, LT3, VP1, and overall positivity/negativity combining all the results from the three primer sets.

Table 1. Patient characteristics and comparison of tumor stage between MCPyV-positive and -negative patients

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Gender</th>
<th>Age at diagnosis</th>
<th>MCPyV Stage at diagnosis</th>
<th>Stage at date last seen</th>
<th>Follow-up months</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>f</td>
<td>72</td>
<td>neg</td>
<td>stage I</td>
<td>stage II</td>
</tr>
<tr>
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<td>m</td>
<td>76</td>
<td>neg</td>
<td>stage I</td>
<td>stage III</td>
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<td>73</td>
<td>neg</td>
<td>stage I</td>
<td>stage I</td>
</tr>
<tr>
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<td>m</td>
<td>71</td>
<td>neg</td>
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<td>stage I</td>
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<tr>
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<td>75</td>
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<td>stage I</td>
</tr>
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<td>pos</td>
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<td>stage I</td>
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<td>83</td>
<td>pos</td>
<td>stage II</td>
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<td>9</td>
<td>f</td>
<td>84</td>
<td>pos</td>
<td>stage I</td>
<td>stage I</td>
</tr>
</tbody>
</table>

f = Female; m = male; pos = positive; neg = negative.
Merkel Cell Polyomavirus Prevalence in Switzerland

find the virus in the SCC. This may be due to a specific targeting of Merkel cells by the virus, or to a viral infection after the excision of the SCC. Foulongne et al., on the other hand, detected viral DNA in 71% of non-tumoral skin samples of patients with MCC. However, the MCPyV DNA levels appeared to be higher in MCC tumour tissue than in nontumoral skin in their set of patients [25]. Furthermore, based on the presence of LT-truncated MCPyV in approximately 15% of SCCs from immunocompetents thermore, based on the presence of LT-truncated MCPyV in in their patient set, Dworkin et al. [26] even suggested a causative role of MCPyV in SCC, in analogy to Shuda et al. [11] who showed that the LT antigen derived from MCC tumor areas is truncated and lacks the LT MCPyV helicase. This in turn leads to replication incompetence in the virus, thus suggesting an early precancerous integration of the virus into the genome.

Recently, a monoclonal antibody CM2B4 was developed against a peptide fragment of the MCV T antigen exon 2 [27]. The antibody was immunoreactive with the majority of the PCR-positive tumors (77%) [28] and showed also high specificity. Immunohistochemistry as a relatively cheap routine procedure may become the method of detection of MCPyV, but the sensitivity issues will have to be addressed.

In summary, we found MCPyV in 66.6% of our Swiss MCC patient set. This number is similar to the reported incidences in two American MCC patient sets (69% [20] and 80% [8]) and the European experience (85% [18] and 79.8% [13]), but higher than in an Australian patient set (24% [20]). It has been speculated that the differences in the virus prevalence may be due to a higher contribution of UV in the pathogenesis of MCC in Australian patients, or to the presence of virus that remained undetected in the assays which were applied, but not to the quality of DNA used in the assays, as the PCR controls were considered excellent in the Australian set with the low detection rate of MCPyV [20].

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