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The new polyomavirus (MCPyV) does not affect the clinical course in MCCs

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Abstract. Since 2008, a new polyomavirus (MCPyV) in Merkel cell carcinomas (MCC) has been described, but little is known about its impact on the clinical course. The purpose of this study was to determine the presence of MCPyV in a large sample and to correlate the results with the clinical course of the disease. 59 samples from 44 patients were analysed for the presence of MCPyV using the primers LT3, VP1 and LT1. The clinical records of these patients were evaluated and correlated with the presence of MCPyV. 58% of specimens were positive for MCPyV. Of these, LT3 was positive in 53%, VP1 in 37% and LT1 in 10%. 57% of primary tumours and 53% of metastases were positive for LT3; the numbers for VP1 and LT1 were lower. There was no correlation between the detection of MCPyV in the primary tumour and the appearance of metastases. The survival time was statistically independent from the presence of MCPyV. There is a striking occurrence of MCPyV in MCC, but whether it affects the clinical course remains unclear.

Key words: Merkel cell carcinoma; polyomavirus MCPyV; clinical course.

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The Merkel cell carcinoma (MCC) is a highly aggressive cutaneous malignancy that occurs predominantly in the older Caucasian population. One major location for the occurrence of primary MCC is the head and neck region. The cells were first described in 1875 by Friedrich Sigmund Merkel as large, pale cells in the basal layer of the epidermis forming synapse-like contacts with enlarged nerve terminals[14]. These cells, now commonly referred to as Merkel cells, function as mechanoreceptors and resemble cells of the diffuse neuroendocrine system[4]. About 100 years later, Tang & Toker suggested that 'trabecular carcinoma' originated from Merkel cells[16]. MCC is relatively rare, but the incidence has tripled during the past 20 years[4]. The median time after the first treatment to recurrence of the disease is 8–9 months; the 5-year survival rates are quite poor and range from 23% to 80% in the literature[10]. Until recently, the pathogenesis of MCC remained largely unknown[4,9]. In 2008, Feng et al. provided evidence for a possible viral oncogenesis, which resulted in the discovery of a new polyomavirus, the Merkel cell polyomavirus (MCPyV) encompassing 5387 base pairs[6]. MCPyV is not a 'passenger virus' that secondarily infects MCC tumours but is actively involved in tumorigenesis[6].

Several studies have confirmed the presence of MCPyV in MCC[1,7,8,11,12]. The prevalence of MCPyV in MCC ranges from 43% to 89%. Most reports study a small sample size. There are only a few studies on the correlation between MCPyV and the clinical course. Sihto et al. reported that MCPyV DNA was present in 80% of MCC tissue samples. Patients with MCPyV DNA-positive tumours had better overall

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survival than those with MCPyV DNA-negative tumours[15]. Bhatia et al. found that a higher viral abundance in MCC tended to be associated with longer survival, but this was not statistically significant[2]. Loyo et al. found that MCV is widespread in the human body and suggest a possible faecal–oral transmission route similar to the hepatitis A virus[13].

The purpose of this study was to determine the presence of MCPyV in a relatively large number of MCC specimens and to correlate the presence of MCPyV with the clinical outcome.

Material and methods

66 paraffin embedded, histologically proven MCCs (20 metastasis and 46 primaries) from 50 patients were tested for the presence of MCPyV. 42 of the tumours were selected from the archives of the Institute of Pathology and Clinic of Dermatology of University Hospital Düsseldorf (period of investigation 1999–2008), the other 24 MCC (primaries) were provided by Dr. Schaller (Institute of Dermatopathology, Duisburg, Germany).

For DNA extraction, an Hematoxylin and Eosin Stain (HE) section and five paraffin sections of 5 μm were taken in each case. According to the area of the tumour, 3–5 paraffin sections per case were deparaffinized using in succession xylol and ethanol (98%, denatured). For the internal cases, the paraffin sections were placed on specimen holders so that after deparaffinization the tissue of the tumour area was removed using the HE as a template. In case of the external MCCs, which had been provided as loose sections, the whole tissue of the MCC-positive section was used.

The tissue was lysed overnight by proteinase K (Qiagen, Hilden, Germany) at 56 °C, the DNA was extracted using the DNA tissue kit and the EZ1 BioRobot (Qiagen, Hilden, Germany). The concentration of the extracted DNA was measured by NanoDrop ND-1000 and each sample was diluted to a concentration of 20 ng/μl or 40 ng/μl, depending on the tumour size. Two samples had to be excluded from further analysis because their DNA concentration was too low. To prove the presence of MCPyV, a polymerase chain reaction (PCR) was carried out with LT1, LT3 and VP1 primer sets[5,6] using the following PCR-mix and program: sterile water (Aqua B Braun) 7.9 μl, dNTP-Mix 2 mM 2.0 μl (Sigma, Saint Louis, Missouri, USA), 10 x taq-buffer 2.0 μl (GE Healthcare, Amersham Place, Little Chalfont, Buckinghamshire,

Table 1. Patients and their histological findings.

Sample id	Patient id	Primarius/ metastasis	Gender	Age	β-Globin	LT3	VP1	LT1	MCPyV
1	1	M	m	65		–	–	–	Negative
2	1	P	m			–	–	–	Negative
3	2	P	f	66		+	+	–	Positive
4	2	M	f			+	+	–	Positive
5	3	M	f	84		+	+	–	Positive
6	3	M	f			+	+	–	Positive
7	3	M	f			–	–	–	Negative
8	3	M	f			–	–	–	Negative
9	4	P	f	78		+	+	–	Positive
10	5	P	f	66		–	–	–	Negative
11	5	M	f			–	–	–	Negative
12	6	P	m	76		–	–	–	Negative
13	7	P	m	77		–	–	–	Negative
14	7	M	m			–	–	–	Negative
15	8	P	m	58		+	+	–	Positive
16	8	M	m			+	+	+	Positive
17	8	M	m			+	+	+	Positive
18	8	M	m			+	+	–	Positive
19	9	M	f	62		+	+	+	Positive
20	10	M	f	78		+	+	–	Positive
21	11	P	m	83		+	–	–	Positive
22	11	M	m			–	–	–	Negative
23	11	M	m			–	–	–	Negative
24	11	M	m			–	–	–	Negative
25	11	M	m			–	–	–	Negative
26	12	P	m	57		–	–	–	Negative
27	13	M	m	71		+	+	–	Positive
28	14	P	m	93		–	–	–	Negative
29	15	P	m	73		–	+	–	Positive
30	16	P	f	65		+	+	–	Positive
31	17	P	m	70		+	–	–	Positive
32	18	P	f	78		+	–	–	Positive
33	19	P	f	96		–	–	–	Negative
34	20	P	f	68		+	–	–	Positive
35	21	P	m	56		–	–	–	Negative
36	22	P	m	84		+	–	+	Positive
37	23	P	f	62		–	–	–	Negative
38	23	M	f			+	–	+	Positive
39	24	P	f	100		+	–	–	Positive
40	25	P	f	83		+	+	–	Positive
41	26	P	m	55		+	–	–	Positive
42	27	P	m	74		+	–	–	Positive
43	28	P	m	83		–	–	–	Negative
44	29	P	f	75		+	+	–	Positive
45	30	P	m	83		+	–	–	Positive
46	31	P	m	85		+	+	–	Positive
47	32	P	f	85		–	–	–	Negative
48	33	P	f	71		–	–	–	Negative
49	34	P	f	76		–	–	–	Negative
50	35	P	f	81		+	+	–	Positive
51	36	P	m	78		+	–	–	Positive
52	37	P	f	82		–	–	–	Negative
53	38	P	m	76		+	–	–	Positive
54	39	P	f	88		+	+	–	Positive
55	40	P	f	80		–	–	–	Negative
56	41	P	f	95		–	–	–	Negative
57	42	P	f	73		+	+	+	Positive
58	43	P	f	84		–	+	–	Positive
59	44	P	f	91		–	+	–	Positive
Pos/tot						31/59	22/59	6/59	34/59
%						52.5%	37.3%	10.2%	57.6%

Abbreviations: P, primarius; M, metastasis; f, female; m, male.