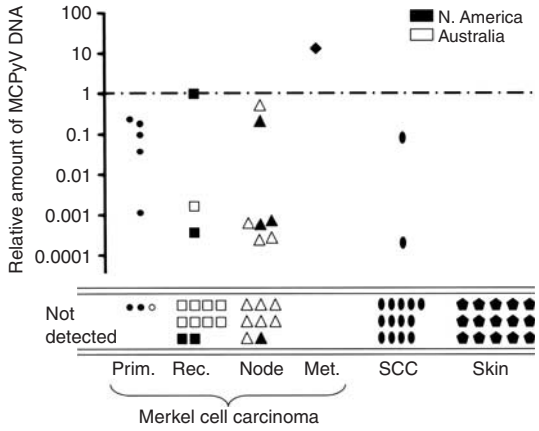

Figure 2. Marked variability of Merkel cell polyomavirus DNA quantity among MCC tumors. The amount of MCPyV DNA was compared to the amount of DNA of a control gene on chromosome 2 (*TPO*). Note the wide range in levels of MCPyV DNA. MCC primaries ($n=8$, circles), recurrences ($n=13$, squares), nodal metastases ($n=15$, triangles), and distant metastases ($n=1$, diamond) are compared. Also shown are squamous-cell carcinomas (ovals) and sun-exposed skin controls (pentagons). Tumors originating from North America are represented with closed symbols and those originating from Australia are represented with open symbols. Tumors represented as not detected had good amplification of control gene but no detectable MCPyV by both primer sets.

MCPyV represents an exciting direction for future studies in two major areas. The first area is functional characterization of the MCPyV and determination if it expresses biologically active viral proteins and whether it is in fact involved in the generation or maintenance of MCC tumors. The second major direction is an epidemiologic characterization of the prevalence of MCPyV in healthy people and in various tumors as this virus may plausibly be associated with other human diseases or malignancies.

Merkel cell carcinoma specimens were from the ''Repository of Data and Specimens for Merkel Cell Carcinoma Research'' at the Fred Hutchinson Cancer Research Center. Normal sun-exposed skin and squamous-cell carcinoma tumors were from the ''Main Biomarker Research Specimen Repository'' at the University of Washington. The relevant Institutional Review Boards approved this study, and this study was performed with strict adherence to the Declaration of Helsinki Principles.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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See related commentary on pg 9

MC Polyomavirus Is Frequently Present in Merkel Cell Carcinoma of European Patients

Journal of Investigative Dermatology (2009) **129,** 248–250; doi:10.1038/jid.2008.198; published online 17 July 2008

TO THE EDITOR

Merkel cell carcinoma (MCC) is a rare, but highly aggressive neuroendocrine cancer of the skin that occurs primarily in elderly immune-suppressed individuals. Little is known about the pathogenesis of MCC. Now Feng *et al.* (2008) have identified a new polyomavirus present in MCC (MCPyV). In this regard, viral proteins expressed by polyomaviruses are known to initiate

Abbreviations: MCC, Merkel cell carcinoma; MCPyV, Merkel cell polyomavirus

transformation and immortalization of cultured cells. Accordingly, in experimental models, polyomaviruses induced tumors upon integration of the viral genome into the host genome (Poulin and DeCaprio, 2006). Therefore, the detection of MCPyV integrated into the host genome in six out of eight MCCs (75.0%) by Feng *et al.* argues for a causative role of this polyomavirus for MCC. Although polyomaviruses have been discussed for a long time to be involved in human tumorigenesis (Poulin and DeCaprio, 2006), this observation is the first clear hint for their impact on tumor etiology in humans as the other known human-specific polyomaviruses, that is, BKPyV, JCPyV, KIPyV, and WUPyV, have not yet been found to be integrated in tumor-derived genomic DNA. Notably, the patterns of integration indicated that viral infection by MCPyV precedes clonal expansion of tumor cells consistent with MCPyV's role in MCC.

To expand the study of Feng *et al.*, we analyzed 75 samples of 53 MCC patients for the presence of MCPyV by real-time PCR. To this end, DNA was obtained from formalin-fixed patient material and samples were analyzed for the presence of MCPyV by using TaqMan technology with primers (forward: 5′-CCAAACCAAAGAATAAAGCACTGA-3′; reverse: 5′-TCGCCAGCATTGTAGTCTAAAAAC-3′) and a respective probe (TP: FAM-AGCAAAAACACTCTCCCCACGTCAGACAG-BHQ) specific for the MCPyV large T-antigen gene. The Taqman probe, for example, was designed to have at least 11 mismatches to the other known human-specific polyomaviruses. By normalization to the highly repetitive DNA elements LINE1—for which the copy number even in cancer cells is largely constant—a relative quantification of the samples could be calculated by the $\Delta\Delta C_t$ method. These analyses demonstrated that in the tumors of 45 patients (84.9%) the virus was present. The presence or absence of the virus was concordant in consecutive samples, that is, primary tumor and subsequent metastases for all patients. The clinical follow-up of a patient cohort for which the detailed clinical course was available suggests that MCC containing the viral genome display a more aggressive behavior than their virus-negative counterparts (Table 1). However, if all patients were stratified by tumor stage, that is, stage I or higher, there was no significant difference between these patient cohorts ($P = 0.4527$ in Fisher's exact test) (Figure 1). Hence, as the rate of MCPyV-negative tumors is low, the impact of the MCPyV virus on clinical course has to be revealed in a larger patient population. It should be further noted that of 24 basal cell carcinomas, another skin cancer affecting mostly elderly patients, only three samples (12.5%) harbored the viral genome; moreover, the mean relative frequency of virus DNA in the positive basal cell carcinoma samples is about 4 log lower than in MCC.

Table 1. Clinical course of a patient cohort for which detailed information was available

Patient id	Gender	Age at diagnosis[1]	Stage at diagnosis	Stage at date last seen	Follow-up[2]
Patients with MCPyV					
RF	M	91.4	I	II	64.1
RR	M	80.1	I	II	9.97
BE	F	68.7	I	II	48.8
GW	M	63.8	III	III	31.7
HW	M	80.9	I	III	6.47
KA	M	85.1	II	II	3.27
ST	F	81.4	I	I	3.5
BR	M	89.8	I	I	0.0
NR	M	72.6	II	II	15.43
KM	F	72.9	II	II	26.83
HO	M	79.6	I	I	0.00
HW	M	93.7	I	III	27.5
PH	F	78.8	I	I	43.47
RE	F	90.4	I	I	1.4
KR	M	63.1	III	III	6.57
SB	F	57.0	III	III	0.00
LH	M	72.9	I	III	1.80
LB	M	54.8	I	III	9.5
Patients without MCPyV					
SR	M	76.8	I	I	88.47
KE	M	71.4	I	Unknown	35.43
SM	F	83.4	I	I	0.00
HA	F	83.1	I	I	11.63
GH	M	84.4	I	I	10.03

F, female; M, male; MCPyV, Merkel cell polyomavirus.
[1]In years.
[2]In months. Time span between diagnosis and date last seen or date of death.

In summary, our study unambiguously confirms the association of MCPyV with MCC reported by Feng *et al.* Given the oncogenic potential of polyomaviruses, and accounting for the lack of systemic therapies improving the survival of metastatic MCC, this observation may open new therapeutic options in this disease. Antivirals such as IFNs (Borden *et al.*, 2007) or—as proteins from polyomavirus are known to be immunogeneic (Binggeli *et al.*, 2007)—active immunotherapy has to be considered.

CONFLICT OF INTEREST

The authors state no conflict of interest.

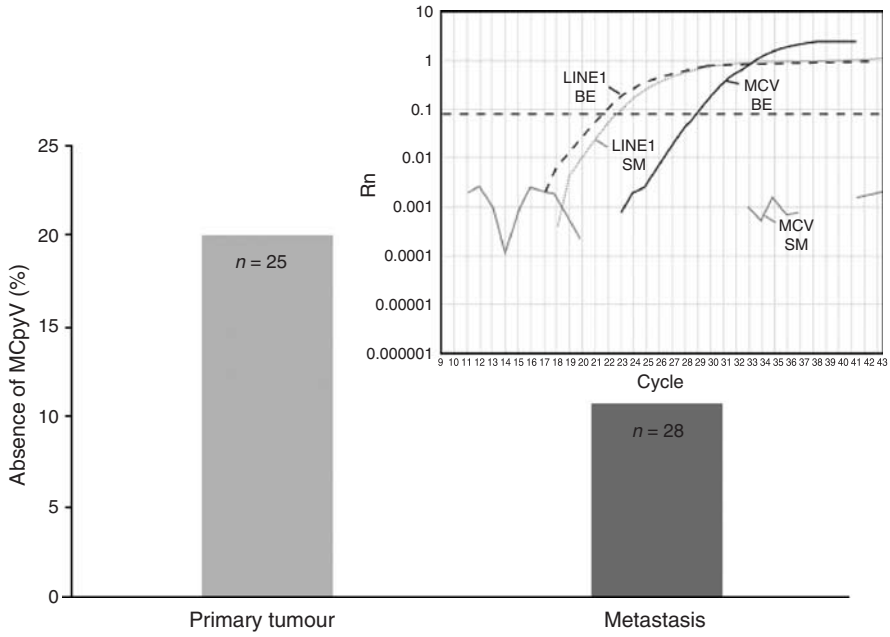

Figure 1. MCPyV is more prevalent in patients with metastatic disease. The percentage of MCPyV absence is given for patients without (primary tumor only) and with (metastasis) known metastatic disease. The inset depicts representative results for presence (patient BE; MCV: black line; LINE1: black dashed line) and absence (patient SM; MCV gray line; LINE1: gray dotted line) of MCPyV in two primary tumors. LINE1 serves as DNA control.

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The Presence of Polyomavirus in Non-Melanoma Skin Cancer in Organ Transplant Recipients Is Rare

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TO THE EDITOR

Organ transplant recipients have a 100-fold increased risk of developing cutaneous squamous cell carcinoma (SCC) compared to the general population (Euvrard *et al.*, 1993). Compared to immunocompetent patients, organ transplant recipients often develop more invasive and aggressive SCCs, with increased multiplicity, recurrence, and risk for metastasis. In addition to SCCs, organ transplant recipients develop more keratoacanthomas (KAs), Bowen's disease, and actinic keratoses. A potential reason for the development of these tumors is that posttransplant immunosuppressive medications result in enhanced susceptibility to infection. Histologically, many of the lesions in these patients demonstrate the hallmarks of viral infection such as the presence of koilocytes and verruciform epidermal hyperplasia (Euvrard *et al.*, 1993; Hsi *et al.*, 1997; Harwood *et al.*, 2006).

Study of viruses in skin tumors of transplant patients has previously focused on human papilloma virus (HPV). PCR assays for epidermodysplasia verruciformis and mucosal serotypes of HPV have revealed that high risk mucosal HPVs are relatively infrequent in these cutaneous tumors. The frequency of epidermodysplasia verruciformis HPV infection ranges from 39–92% in SCCs from transplant patients (Berkhout *et al.*, 1995; Boxman *et al.*, 1997; Hopfl *et al.*, 1997). However, the presence of epidermodysplasia verruciformis HPV can also be found in plucked hairs and forehead swabs from healthy individuals and skin tumors from immunocompetent patients (Boxman *et al.*, 1997; Hsi *et al.*, 1997; McGregor *et al.*, 1997; Hazard *et al.*, 2007). Thus, the exact etiologic role that these viruses play in skin cancer in organ transplant recipients remains unclear. Other viruses may also contribute to tumorigenesis in organ-transplant patients. Recently, the presence of polyomavirus has been detected in 80% of Merkel cell carcinomas (MCCs; Feng *et al.*, 2008). In

Abbreviations: HPV, human papilloma virus; KA, keratoacanthoma; MCC, Merkel cell carcinoma; SCC, squamous cell carcinoma