No evidence for a causal role of Merkel cell polyomavirus in keratoacanthoma

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Background: Merkel cell polyomavirus (MCPyV) is a recently discovered virus that is monoclonally integrated into the genome of approximately 80% of all Merkel cell carcinomas (MCCs). While some evidence exists that MCPyV does not play a pathogenic role in other nonmelanoma skin cancers, such as basal cell carcinoma and squamous cell carcinoma (SCC), little is known about the presence of MCPyV in keratoacanthoma (KA).

Objectives: To evaluate the prevalence, viral DNA-load, and large T(umor)-antigen expression of MCPyV in KA of immunocompetent patients and to compare the results with those found in SCC and MCC.

Methods: Paraffin-embedded tissue samples were analyzed for the presence of MCPyV-DNA by polymerase chain reaction (PCR). MCPyV-DNA load (MCPyV-DNA copies per beta-globin gene copy) was determined by using quantitative real-time PCR. Immunohistochemical analysis of the MCPyV large T-antigen was performed with the monoclonal antibody CM2B4.

Results: A total of 137 samples (42 KA, 52 SCC, and 43 MCC) were analyzed. MCPyV-DNA was found significantly more frequently in MCC (37/43, 86.0%) compared with KA (12/42, 28.6%) and SCC (14/52, 26.9%). Moreover, MCPyV-DNA loads were more than two orders of magnitude lower in KA and SCC compared with MCC (median/mean loads 0.005/0.015 [KA] vs 0.023/0.059 [SCC] vs 2.613/56.840 [MCC] MCPyV-DNA copies per beta-globin gene copy). All MCC analyzed (n = 3) expressed MCPyV large T-antigen, whereas 8 KA and 7 SCC were negative in immunohistochemistry.

Limitations: The relatively small number of samples is a limitation.

Conclusions: Our findings argue against a pathogenic role of MCPyV in KA and SCC. (J Am Acad Dermatol 2012;67:41-6.)

Key words: keratoacanthoma; Merkel cell carcinoma; Merkel cell polyomavirus; squamous cell carcinoma.

INTRODUCTION

In 2008, a novel polyomavirus called Merkel cell polyomavirus (MCPyV) was detected in Merkel cell carcinoma (MCC), an aggressive skin tumor with increasing incidence rates in recent years.[1] Since then, several studies have confirmed the presence of MCPyV in the majority of MCC (approximately 80%).[2-6] It has also been shown that a high proportion of the population is asymptomatically infected

Abbreviations used:

BCC:	basal cell carcinoma
KA:	keratoacanthoma
LNA:	locked nucleic acid
MCC:	Merkel cell carcinoma
MCPyV:	Merkel cell polyomavirus
PCR:	polymerase chain reaction
Q-PCR:	quantitative PCR
SCC:	squamous cell carcinoma

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with MCPyV and that cutaneous MCPyV infection occurs already early in life.[7-10] In MCC, integration of the viral DNA into the tumor genome is frequent. However, MCPyV was also found with lower frequencies and lower viral loads in other skin tumors, such as basal cell carcinomas (BCC) and squamous cell carcinomas (SCC).[2,6,11-13] In contrast to MCC, MCPyV large T(umor)-antigen is not expressed in BCC and SCC.[14]

Keratoacanthoma (KA) is a distinct epidermal neoplasia that originates from pilosebaceous glands. Some evidence indicates that KA is a clinical variant of well-differentiated SCC, although this is still a subject of debate.[15,16] KA shares a couple of similarities with MCC, including rapid growth, predominant localization in the sun-exposed areas of the body, frequent affection of elderly people, and more severe clinical course under immmunosuppression.[17,18] So far, only few data exist on the prevalence of MCPyV in KA. In a recent study conducted by our group, the highest rate of MCPyV-DNA positivity among benign and malignant skin tumors of immunocompetent patients was observed in 3 of 7 KA (43%).[6] This observation encouraged us to analyze a larger collective of KAs for the presence of MCPyV-DNA and to compare the results with the MCPyV prevalence of SCC and MCC. Moreover, we thought to compare the lesional MCPyV-DNA loads in KA, SCC, and MCC and to evaluate the expression of the large MCPyV T(umor)-antigen in the respective lesions.

CAPSULE SUMMARY

- Merkel cell polyomavirus most likely causes about 80% of Merkel cell carcinomas and is sporadically found in some basal and cutaneous squamous cell carcinomas.
- MCPyV-DNA was only found in about one fourth of KAs and SCCs analyzed in this study. In MCPyV-DNA-positive KA or SCC, the MCPyV-DNA loads were significantly lower than in MCC.
- Our data argue against a pathogenic role of MCPyV in keratoacanthoma development. Sporadic PCR detection of MCPyV in nonmelanoma skin cancer therefore most likely represents a coincidental infection with a widespread virus.

METHODS

Skin samples

One hundred forty-five archival paraffin-embedded samples (46 KA, 55 SCC, and 44 MCC) of immunocompetent patients were available for virologic analyses. Patients' medical files were additionally reviewed. All MCC were cytokeratin-20 positive in immunohistochemistry. Four KA, 3 SCC, and 1 MCC had to be excluded because beta-globin gene DNA was not detectable in these samples, leaving 137 biopsy specimens (42 KA, 52 SCC, and 43 MCC) for final analysis. The protocol of the study was approved by the ethics review board of the Ruhr University Bochum, and the study was conducted according to the Declaration of Helsinki principles.

Merkel cell polyomavirus DNA detection

DNA-isolation was performed as described before using the QIAamp DNA Mini kit (Qiagen, Hilden, Germany).[19] Real time beta-globin gene polymerase chain reaction (PCR) (Light Cycler Control Kit DNA, Roche, Mannheim, Germany) was carried out to demonstrate that samples contained adequate DNA and were free of substances inhibitory to PCR. For the detection of MCPyV-DNA, samples were analyzed by nested MCPyV-PCR (358 bp) (nPCR) basically as described before.[20] MCPyV_OS, _OAS, _IS and _IAS primer sequences have been published previously.[20] Hot-start PCR was performed in 50 μL containing 5 μL of purified total cellular DNA, 50 mM KCl, 20 mM TRIS-HCl (pH 8.4), 1.5 mM $MgCl_2$, deoxynucleotide triphosphates 0.2 mM each (Roche), 2.5 units Platinum Taq DNA polymerase (Invitrogen, Karlsruhe, Germany), and primers 0.5 μM each. Cycling conditions (T3 Thermocycler, Biometra, Goettingen, Germany) were as follows: 94°C for 5 minutes, followed by 31 cycles of 94°C for 45 seconds, 58°C for 45 seconds and 72°C for 60 seconds. For internal nested IS/IAS-PCR, 3 μL of OS/OAS-PCR-product were used. PCR-products (10 μL) were separated on 2% agarose gels and visualized by ethidium-bromide staining. Negative controls with water and human placental DNA instead of patient samples were included in each amplification series and never yielded positive results. The analytical sensitivity of nPCR was 10 copies of cloned MCPyV LT1-DNA per assay.[1]

Merkel cell polyomavirus DNA load determination

All samples positive in nested MCPyV-PCR were analyzed by quantitative MCPyV PCR (Q-PCR) by means of the LightCycler 480 Real-Time PCR System (Roche). Q-PCR was performed in 20 μL containing 5 μL of purified total cellular DNA and generated a 74 bp amplimer. Primer and locked nucleic acid (LNA) hydrolysis probe sequences as well as PCR-conditions have been previously described by