APMIS JOURNAL OF PATHOLOGY, MICROBIOLOGY AND IMMUNOLOGY

APMIS 128: 104–120

DOI 10.1111/apm.13019

REVIEW ARTICLE

Merkel cell polyomavirus and non-Merkel cell carcinomas: guilty or circumstantial evidence?*

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Csoboz B, Rasheed K, Sveinbjørnsson B, Moens U. Merkel cell polyomavirus and non-Merkel cell carcinomas: guilty or circumstantial evidence?. APMIS 2020; 128: 104–120.

Merkel cell polyomavirus (MCPyV) is the major causative factor of the rare but aggressive cancer, Merkel cell carcinoma (MCC). Two characteristics of MCPyV-positive MCCs are integration of the viral genome and expression of a truncated version of one of its oncogenic proteins, namely large T antigen. The strong association of MCPyV with MCC development has incited researchers to further investigate a possible role of this virus in other cancers. However, many of the examples displaying the presence of the virus in the various non-MCC cancers are not able to clearly demonstrate a direct connection between cellular transformation and the presence of the virus. The prevalence of the virus is significantly lower in non-MCC cancers compared to MCCs, with a lower level of viral load and sparse viral protein expression. Moreover, the state of the viral genome, and whether a truncated large T antigen is expressed, has rarely been investigated. Nonetheless, considering the strong oncogenic potential of MCPyV proteins in MCC, the plausible contribution of MCPyV to transformation and cancer growth in non-MCC tumors cannot be ruled out. Furthermore, the absence of MCPyV in cancers does not exclude a hit-and-run mechanism, or the oncoproteins of MCPyV may potentiate the neoplastic process mediated by co-infecting oncoviruses such as high-risk human papillomaviruses and Epstein–Barr virus. The current review is focusing on the available data describing the presence of MCPyV in non-MCC tumors, with an aim to provide a comprehensive overview of the corresponding literature and to discuss the potential contribution of MCPyV to non-MCC cancer in light of this.

Key words: Merkel cell polyomavirus; Merkel cell carcinoma; non-Merkel cell carcinomas; viral carcinogenesis; viral oncoprotein; oncogenic DNA viruses.

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Members of the *Polyomaviridae* family are naked viruses with a circular double-stranded DNA genome, typically approximately 5000 base pairs. Their genome can be divided into three functional domains. The early region is expressed at the initial phase of infection and encodes the regulatory proteins large T antigen (LT) and small T antigen (sT). The late region is transcribed after the initiation of viral DNA replication and encodes the capsid proteins, typically VP1, VP2, and VP3. Interspersed between the early and late region is the non-coding control region, with the origin of replication and transcription control region directing the expression of the early and late genes (1). Polyomaviruses (PyV) have predominantly been isolated from birds and mammals, but recently viral nucleotide sequences have also been detected in invertebrates, fish, amphibians, and reptiles (2, 3). Their name (poly = many; oma = tumors) is derived from the observation that the first isolated polyomavirus was able to induce several tumors in an animal model (4, 5). The oncogenic potential of PyV is attributed to LT and to a lesser extent sT (6–8). Later, it was shown that other polyomaviruses, including human polyomaviruses, also provoked tumors in animal models and that they could transform cells in cell culture (reviewed in Ref. (9–11)). However, thus far only two polyomaviruses cause cancer in their

Received 29 October 2019. Accepted 2 December 2019

*This article is part of the special issue on Infection and Cancer by Guest Editors Åse Bengård Andersen and Lene N. Nejsum.
This is an invited article.

natural host. Raccoon polyomavirus is associated with brain tumors in raccoons (12), while the human polyomavirus Merkel cell polyomavirus (MCPyV) is strongly correlated with the rare skin tumor Merkel cell carcinoma (MCC) (13–16). MCPyV was originally identified in 2008 by the group of Cheng and Moore (13), who showed that the viral genome was integrated into the host genome, interrupting the late region. In addition, a C-terminal truncated LT was also expressed. This deletion removed the helicase activity of LT, which is required for viral DNA replication. Worldwide studies by many groups have now shown that approximately 80% of all MCCs are positive for MCPyV and that the viral genome is integrated, and a C-terminal truncated LT is expressed (14). Virus-positive tumors usually contain >1 genome copies per cell (17–21). MCC is considered as a rare but aggressive type of skin cancer, being among the fastest growing tumors with a mortality rate of ~45% (14). The incidence has increased significantly during the last 10 years and is prospected to increase further, as its occurrence is associated with aging and exposure to the sun (22). It is generally accepted that MCPyV is a major cause of MCC (14). During the transformation process, the virus is monoclonally integrated into the genome of the tumor cells, thereby indicating that the proto-tumor cell was infected with the virus prior to its cancerous expansion (13). Furthermore, the oncogenic LT of the virus is expressed in all of the tumor cells, and when it is inhibited, MCPyV-positive cells die (23).

Seroepidemiological studies revealed that MCPyV infects most humans and establishes a life-long harmless persistent infection in healthy individuals (24–26). Antibodies against viral proteins are detected in 50%–80% of the serum from healthy adults and children (27, 28). Moreover, MCPyV is chronically shed from the skin of healthy individuals (29), thereby indicating that MCPyV is a part of the human skin microbiome. The natural host cell for MCPyV replication in the human body could be dermal fibroblasts cells, as virus could be propagated in human dermal fibroblast cell cultures (30). The replicated viral genome was measured in an extremely high copy number at approximately 12 000 copies per cell in cell cultures (30), in contrast to MCC samples in which the MCPyV genome was assessed to be present at an average copy number of 5.2 (range 0.8–14.3) per cell (21). MCPyV was also shown to be capable of expressing LT and VP1 in fibroblast cell lines originating from lung tissue (30). Hence, an active viral replication of MCPyV might be connected to fibroblast tissues in general. Besides the fact that dermal fibroblasts are primarily residing in the dermis layer of the skin, cutaneous swabs were also shown to contain MCPyV DNA (31). This suggests that viral particles can be more widespread from the site of replication and release. A study using a wide cohort of patients has also described an age-related increase in the prevalence of MCPyV DNA in the sun-exposed skin of patients (32), thus suggesting an age-related association of viral replication in the host. Increased viral activity may result from a weakened immune survey (immune senescence or immunosuppression), which is supported by the fact that a higher occurrence of MCC was observed in organ transplant patients undergoing immunosuppressive treatments (33), as well as in patients with severe T-cell leukemia (34).

MCPYV IN NON-CANCEROUS TISSUES

Despite the fact that the replication of MCPyV has so far shown to be limited to fibroblasts from the dermis and potentially from the lung (30), MCPyV DNA has been detected in various non-cancerous tissues of the body like the adrenal gland, spleen, bone mar\row, stomach, gallbladder, pancreas, heart, and aorta, although with a relatively low viral load between 0.00026 and 0.22 copies per cell (35). On average, the viral genome copy number of MCPyV was 60 times lower in healthy tissues across the body compared to MCC samples (36). A study identified the highest presence for MCPyV among different tissues in the digestive system, saliva, and in the upper aerodigestive tract (36). MCPyV DNA was also present in bodily fluids, such as the blood and urine from two patients with advanced MCC (one with immunosuppression and one without immunosuppressive side treatments). However, viral DNA was not detected in the whole blood samples of patients without MCC by using simple PCR (37). MCPyV positivity in blood was associated with monocytes, and MCPyV was shown to be selectively associated with the $CD14^{+}CD16^{-}$ 'inflammatory' monocyte subpopulation. This finding suggests that inflammation-associated monocytes might serve as potential vehicles for MCPyV, which could aid viral transmission in the body through harboring and transferring the virus to inflammation sites (37). In contrast, MCPyV DNA sequences were amplified in the buffy coats of blood, with a very low viral load of 10 to 100 molecules/100 000 cells (38). The reason that the low levels of MCPyV DNA were identified in this study might have originated from the fact that the blood samples were prefractionated by a density gradient centrifugation in order to obtain the leukocyte rich fraction. Subsequently, circulating MCPyV DNA was detected in the unfractionated sera of healthy individuals by using a more sensitive quantitative

Table 1. Incidence of MCPyV in non-Merkel cell carcinoma tumors

Tissue (n)	Prevalence (average%; range)	Viral load[1]	Method	Comments	Reference
Lymphatic system					
Tonsillar SCC (150)	48 (32.0; 21.1–35.7)	0.000064–0.0038	qPCR (LT, sT)		(41, 77, 78)
Chronic tonsillitis and tonsillar hyperplasia (497)	21 (4.2)	0.000004–0.00018			(78, 143–145)
Hypertrophy adenoid (179)	3 (1.7; 1.3%–10%)	0.00036	qPCR (LT)		(145)
Thymoma (46)	7 (15.2; 0–19.4)		qPCR (LT, sT), FISH (LT), IHC (LT)	6/7 were also positive by ISH and 3/7 were positive by IHC.	(69, 79, 80)
Carcinoid of the thymus (5)	0 (0)		qPCR (LT)		(146)
Normal healthy lymph nodes (2)	0 (0)		IHC (LT)		(17)
Nervous system					
Brain tumors (176)	3 (1.7; 0–30)	0.0000007–0.05051	qPCR (LT, sT, VP1), nPCR (LT), IHC (LT)		(62, 75, 98, 118, 146, 147)
Glioblastoma (46)	2 (4.3; 0–28.6)	<0.0001	qPCR (LT)		(115, 148)
Meningioma (12)	8 (66.7)	0.000	qPCR (LT)		(115)
Neuroblastoma (57)	0 (0)		qPCR (LT, VP1)		(65, 105)
Neurofibroma (1)	0 (0)		qPCR (sT)		(80)
Schwannoma (19)	12 (63.2; 0–78.6)			One schwannoma sample had LT transcripts.	(80, 100, 115)
Skeletal system					
Bone from patients with Langerhans cell histiocytosis (5)	4 (80)	0.002–0.027	qPCR (LT)		(149)
Chondrosarcoma (25)	0 (0)		RT-PCR (LT, VP1)		(114)
Chordoma (18)	0 (0)		RT-PCR (LT, VP1)		(114)
Ewing sarcoma (37)	1 2.7; 0–14.3)		qPCR (LT); PCR (LT, VP1)		(65, 105)
Rhabdosarcoma (5)	0 (0)		qPCR (LT)		(105)
Small-cell cancer of the bone (2)	0 (0)		IHC (LT)		(98)
Endocrine system					
Neuroendocrine carcinoma (102)	3 (2.9; 0–5)		PCR (LT), qPCR (LT, sT), IHC (LT)	None were positive by IHC (LT).	(62, 119, 150, 151)
Neuroendocrine tumor of the gastrointestinal tract (1)	0 (0)		IHC (LT)		(17)
Excretory system					
Bladder cancer (149)	6 (4.0; 0–75)	0.004	qPCR (LT, VP1), IHC (LT)		(36, 62, 65, 98)
Renal cancer (81)	3 (3.7; 0–18.9)	0.001	qPCR (LT, VP1), IHC (LT)		(36, 62)
Respiratory system					
Bronchial carcinoid (11)	0 (0)		PCR (LT)		(146)
Large-cell carcinoma (32)	1 (33.3)		PCR (LT, VP1), qPCR (sT)	RT-PCR (LT) and IHC (LT) were negative.	(69)
Extrapulmonary small-cell carcinoma (16)	3 (18.8)		qPCR (LT, sT)		(152)
Lung cancer (388)	12 (3.1; 0–35.7)	0.001	qPCR (LT, VP1), nPCR (VP1), IHC (LT)		(36, 60–62, 65, 153)
Mesothelioma (138)	1 (0.7; 0–4.2)	<0.00015	qPCR (LT, sT), IHC (LT)		(62, 69, 154)
Neuroendocrine cancer of the lung (37)	0 (0)		PCR (LT), IHC (LT)		(57, 146, 155)
NSCLC (910)	148 (16.3; 0–100)		PCR (LT, VP1), qPCR (LT, sT, VP1, VP2), RT-PCR (LT, VP1)	Integrated viral DNA in one adenosarcoma sample with expression of truncated LT. Integrated + episomal viral DNA in one	(67, 69–72, 76, 156–161)

Table 1. *(continued)*

Tissue (n)	Prevalence (average%; range)	Viral load[1]	Method	Comments	Reference
				SCC sample with expression of full-length and truncated LT.	
Pleomorphic carcinoma (3)	1 (33.3)	0.0008	PCR (LT, VP1), qPCR (sT)	RT-PCR (LT) and IHC (LT) negative.	(69)
SCLC (193)	10 (5.2; 0–38.9)	0.000005–0.026	PCR (LT, VP1), qPCR (LT, VP2), PCR (LT)/ Southern blot2, nPCR (LT, VP1), IHC (LT)	Two different LT antibodies were used.	(17, 63, 64, 66–68, 98, 146, 155, 162)
Digestive tract					
Oral cavity (including lip, cheek, tongue, throat, larynx, jaw) (531)	56 (10.5; 0–42.8)	0.00024–0.026	PCR (LT, sT, VP1), nPCR (LT), qPCR (LT, sT, VP1), IHC (LT)		(36, 60, 62, 98, 100, 110, 141, 163, 164)
Salivary gland cancer (185)	27 (14.6; 0–66.7)		qPCR (LT, sT), IHC (LT)	One positive parotid sample expressed truncated LT.	(107, 108, 165)
Esophagus cancer (156)	56 (35.9; 30–60)	0.0000054–0.000024	qPCR (LT, VP1)		(36, 106, 109)
Stomach cancer (58)	1 (1.7; 0–5)		PCR (LT, VP1)IHC (LT)		(57, 62, 65)
Liver cancer (27)	10 (37; 0–62.5)		qPCR (LT, VP1), IHC (LT)		(36, 60, 98)
Gallbladder cancer (1)	0 (0)		qPCR (LT)		(60)
Pancreas cancer (1)	0 (0)		qPCR (LT)		(60)
Intestine cancer (11)	0 (0)		nPCR (LT, VP1)		(57, 66)
Colorectal cancer (340)	12 (3.5; 0–16)		PCR (LT, VP1), nPCR (LT) qPCR (LT, VP1), FISH (LT), IHC (LT)		(41, 57, 60, 62, 65, 96, 112, 113, 166)
Appendix (4)	0 (0)		IHC (LT)		(57)
Gastrointestinal cancers (8)	0 (0)		IHC (LT)	Two different LT antibodies were used.	(17)
Reproductive system					
Prostate cancer (64)	5 (7.8; 0–18.2)	0.002	qPCR (LT, VP1)qRT-PCR (LT), NGS, IHC (LT)		(36, 98, 101, 167)
Testicular cancer (9)	1 (11.1)	0.934	qPCR (LT, VP1)		(36)
Penile intraepithelial neoplasia and acanthoma/benign papilloma (20)	6 (30; 28.6–33.3)		nPCR (LT)	HIV-positive men.	(166)
Cervical cancer (328)	129 (39.0; 0–56.4)	0.00003055–0.0015	PCR (LT, VP1), qPCR (LT, sT), RT-PCR (LT), IHC(LT)	Some samples also positive for LT transcripts and protein 140 of the examined women were HIV-positive.	(65, 98, 103, 155, 168);
Uterine cancer (4)	1 (25; 0–100)		PCR (LT), nPCR (LT, VP1)		(66, 155)
Ovarian cancer (186)	0 (0)		PCR (LT, VP1), qPCR (LT), IHC (LT)		(65, 105, 155, 169)
Cancer of the vulva (2)	0 (0)		PCR (LT)		(155)
Breast cancer (474)	20 (4.2; 0–14)		PCR (LT, VP1), qPCR (LT), qRT-PCR (LT), transcriptome sequencing	Three positive samples were also positive for LT transcripts.	(65, 102, 170–174)
Integumentary system					
Actinic keratosis (101)	15 (14.9; 0–100)	0.00014–0.068	PCR (LT, sT, VP1), nPCR (LT, sT, VP1), qPCR (LT, sT), IHC (LT)	Of the six samples tested by IHC (LT), none were positive.	(43, 47, 48, 50, 122, 175–178)
Atypical fibroxanthoma (37)	8 (21.6; 17.4–28.6)	0.0001–0.031	PCR (LT, VP1), qPCR (LT), IHC (LT)	IHC (LT) was negative.	(48, 179)
BCC (451)	135 (29.3; 0–100)	0.0001–0.662	PCR (LT, sT, VP1), nPCR (LT, sT, VP1), PCR (LT, sT)/Southern blot, qPCR (LT, sT), IHC (LT)	All IHC (LT) were negative.	(41, 48–50, 58, 91, 176, 177, 180–182)

Table 1. *(continued)*

Tissue (n)	Prevalence (average%; range)	Viral load[1]	Method	Comments	Reference
Mixed MCC-BCC (1)	1 (100)		PCR (LT, sT, VP1), qPCR (LT), IHC (LT)	Both tumors had a different, but truncated LT mutation.	(183)
Bowen's disease (110)	17 (15.5; 0–69.2)		PCR (LT, VP1), nPCR (LT, sT, VP1), qPCR (LT)		(41, 50, 175–177)
Dermato fibrosarcoma (1)	1 (100)		nPCR (LT)		(50)
Kaposi's sarcoma (39)	11 (28.2; 0–66.7)	0.00001–0.00685	PCR (LT, VP1), nPCR (LT, sT, VP1), qPCR (LT, VP1)		(13, 52, 53, 122, 184)
Keratoacanthoma (215)	25 (11.6; 0–100)	0.0001–0.10	PCR (LT, sT, VP1), nPCR (LT), qPCR (LT), IHC (LT)	IHC (LT) was negative.	(43, 48, 50, 51, 175, 176, 185)
Langerhans cell sarcoma (7)	3 (42.9)		qPCR (LT)	All samples were negative for IHC (LT).	(55)
Melanoma (189)	9 (4.8; 0–191.)	0.0016–0.082	PCR (LT, sT, VP1), nPCR (LT, sT, VP1), PCR(LT, sT)/Southern blot, qPCR (LT, sT)		(47, 49, 56, 57, 91, 122, 162, 177, 182)
Non-melanoma skin cancers (99)	31 (31.3; 6.3–36.1)		PCR (LT), nPCR (LT)	Included BCC, SCC, Bowen's disease, and actinic keratosis. Sixteen of the patients were bone marrow transplant patients (8) or CLL patients (8). Of these, the skin tumor of one bone marrow transplant was MCPyV positive.	(186, 187)
Porocarcinoma (67)	45 (67.2; 0–100	0.00022–0.212	PCR (sT), nPCR (LT), qPCR (LT, sT), ddPCR (sT)		(45, 48, 50, 54, 188)
SCC (877)	24.9 (0–75)	0.00013–0.316	PCR (LT, sT, VP1), nPCR (LT, VP1), qPCR (LT, sT, VP1, ddPCR (sT), NGS, IHC (LT)	All IHC(LT) were negative; sequencing of some LT revealed. Truncated LT.	(36, 41–43, 45–50, 57, 122, 162, 175, 176, 178, 180, 189–191)
SCC + BCC (10)	4 (40)		PCR (LT) and qPCR (LT)	Not specified how many BCC and how many SCC samples were positive.	(192)
Combined SCC and neuroendocrine carcinoma (7)	0 (0)		IHC (LT)		(153)
Fanconi anemia-associated head and neck SCC (43)	17 (39.5; 10.3–100)	0.0007–0.0359	qPCR (LT); IHC (LT, sT)	3/29 samples positive by IHC (LT) 14/14 additional samples positive by qPCR.	(193)
Mixed MCC-SCC (21)	2 (9.5; 0–100)		PCR (LT, VP1), qPCR (LT), IHC (LT)		(59, 98, 162)
Seborrheic keratosis (20)	3 (15; 0–33.3)		PCR (LT, VP1), PCR (LT,sT)/Southern nPCR (LT, sT, VP1)	One of the three patients with MCPyV seborrheic keratosis was immunosuppressed.	(91, 176, 177)
Trichoblastoma (41)	11 (26.8)		PCR (LT, VP1)		(181)
Circulatory system					
ALL (50)	0 (0)		qPCR (LT, VP1)		(99)
AML (29)	6 (20.7; 0–100)		qPCR (LT, sT, VP1), NGS		(81, 82, 194)
CBCL (180)	5.6 (3.6; 3.1–20)	0.002–12.467	PCR (LT, sT)/Southern blot, qPCR (LT, VP2), IHC (LT)	IHC (LT) was negative for all samples.	(21, 92, 95)

Table 1. *(continued)*

Tissue (n)	Prevalence (average%; range)	Viral load[1]	Method	Comments	Reference
CLL/SLL (378)	54 (14.3; 0–66.7)	0.000017–0.002	PCR (LT, sT, VP1), qPCR (LT, sT, VP1, VP2), FISH (LT), IHC (LT)	Six had truncated LT (Two of them also full-length LT).	(21, 83–87, 96, 194, 195)
CTCL (352)	13.9 (2.7; 0–88.9)	0.0012–12.467	PCR (LT, sT)/Southern blot, qPCR (LT, VP2), IHC (LT, VP1)	Two MCPyV DNA-positive samples examined by IHC (LT) were negative for LT. No truncated LT in those samples that were sequenced. 4/4 were positive for IHC (LT) and IHC (VP1); Two other studies: All examined samples were negative for IHC (LT).	(21, 88–90, 92–95, 122, 177, 184)
Lymphoma (12)	0 (0)		qPCR (LT)	Eleven were AIDS patients.	(52, 60)
Lymph nodes CLL/SLL patients (18)	6 (33.3)	<0.0004	PCR (LT, VP1), qPCR (sT)		(97)
CML (6)	0 (0)		qPCR (sT)		(82)
Follicular lymphoma (17)	0 (0)		PCR (LT, VP1), qPCR (sT)		(97)
Lymphoma (196)	13 (6.6)	0.000016–0.0027	qPCR (sT)		(80)
Mantle cell lymphoma (1)	0 (0)		FISH (LT)		(96)
Non-Hodgkin's lymphoma (10)	1 (10)		qPCR (LT)		(100)
Primary effusion lymphoma (4)	0 (0)		qPCR (LT)	From AIDS patients.	(52)
Small-cell carcinoma lymph node (4)	0 (0)		IHC (LT)		(98)
Soft tissue					
Desmoplastic tumor (24)	0 (0)		PCR (LT, VP1)		(105)

[1]Viral genome copies/cell;
[2]Southern blot of positive PCR products.

PCR and droplet digital PCR, with the prevalence of 2.6% and a low viral load of 1–5 copies/μL (39). The presence of MCPyV VP1 transcripts was also detected in the urine of both immunosuppressed and non-immunosuppressed individuals (40). This draws a picture that MCPyV could potentially be transferred to many tissues through bodily fluids. Yet, it is clear that because the viral load in these compartments is low, the virus is most likely just passively being transferred and actively replicating not in blood cells the bloodstream nor in epithelial cells in urine.

MCPYV IN NON-MCC TUMORS

Considering the role of MCPyV in the development of MCC and the widespread prevalence of the virus across the body prompted researchers to investigate a possible role and presence of MCPyV in non-MCC cancers. Malignant tissues have been examined for the presence of viral DNA, transcripts, and proteins, with the results of these studies summarized in Table 1. A more detailed overview is given in Table S1. In most of the non-MCC tumors investigated, MCPyV DNA was detected by PCR. However, in those cases in which the copy number of the viral genome per cell was determined, MCPyV copies were shown to be very low with <<1 copy/cell, that is, several logs lower compared to virus-positive MCPyV tumor cells. In those cases, in which the expression of LT was monitored by immunohistochemistry, LT could only be detected in a handful of cases, even though the viral DNA was present.

The earliest observation associating MCPyV with non-MCC was the detection of MCPyV DNA in non-melanoma cancers of the skin from immunosuppressed patients (41). MCPyV was eventually detected in many other neoplasia in non-immunocompromised individuals as well (listed in detail in Table 1 and Table S1). Among the various body sites, the integumentary system is well represented as a site for MCPyV-positive non-MCC tumors. Many non-melanoma skin cancers, including squamous cell carcinomas (41–47) and basal cell carcinomas (41, 48–50), are frequently found to contain MCPyV DNA or transcripts at a varying level. The presence of the virus was also detected in a few cases of keratoacanthoma (43, 48, 51), Kaposi's

sarcoma (52, 53), porocarcinoma (45, 54), atypical fibroxanthoma (48), and langerhans cell sarcoma (55). On the contrary, melanomas are not associated at all with MCPyV (56–58), with the exception of one case in which MCPyV LT transcripts were detected in four acral lentiginous melanomas and in five nodular melanomas, whereas superficial spreading melanomas were virus-negative (49). The viral load of MCPyV was significantly higher in these skin-related samples compared to other virus-positive non-MCC cancers, but the expression of the viral LT was solely detected in only one case of a combined MCC–squamous cell carcinoma (59). The high viral load observed in skin-related non-MCC cancers might not be a surprising phenomenon as one would expect, in that such a close proximity to the original replication of the virus would render other cells of the skin susceptible to the presence of the virus. Still, the observed tissues are showing a picture, in which the virus is not actively participating in the maintenance of the cancerous growth, as its LT is not present in the tumors or expressed at undetectable levels using immunohistochemistry.

As previously mentioned, MCPyV can also replicate in cultures of lung fibroblasts (30). Therefore, it might not be unexpected to detect MCPyV in tumors of the respiratory system. The virus was detected in 10 cases of lung carcinomas (36), but the majority of the studies demonstrated a general lack of viral presence in these tumors (60–62). The situation is similar in combined small-cell lung carcinomas, in which only a few number of tumors were found to contain MCPyV DNA (63–65), though the large majority of analyzed samples were lacking any sign of the virus (66–68). However, neither of them showed a detectable level of LT protein expression. Non-small-cell lung carcinoma was associated with the presence of MCPyV more tightly (69–71), although none of the examined non-small-cell lung carcinoma samples showed any LT protein expression (72). One exception was the detection of truncated LT in two non-small-cell lung carcinoma (69). Intriguingly, in one of these two non-small-cell lung carcinomas, a sample displayed a peculiar duality by containing both episomal and integrated MCPyV DNA, and expressing both the full-length and truncated LT protein (69). Despite the fact that this latter case has only been detected in one sample, it highlights the possibility that the episomal viral genome could possibly be maintained separately in the cytoplasm despite the integration event to the genome, as similarly observed in a few MCCs (73, 74). It is worth mentioning that healthy lung tissues are relatively well studied in this respect (36, 52, 60, 61, 75), with MCPyV transcripts detected in a similar number of cases compared to lung carcinomas and small-cell lung carcinomas. Nevertheless, compared to skin-related non-MCC tissue, tumors of the circulatory system do not have a higher frequency of MCPyV transcript-positive tissues or a higher rate of genomic integration, while a full LT protein expression could be detected in some of the tumors. LT was also expressed in 30 non-small-cell lung carcinoma (76) and in another 14 samples (69). Since the MCPyV has been successfully propagated *in vitro* in a lung fibroblast cell line (30), it is tempting to hypothesize that this high number of LT expressing lung carcinoma cells are arising from the fact that the virus could potentially propagate in the vicinity of these cells. Therefore, it could potentially integrate to some of the lung-related cancer cells.

MCPyV prevalence is low in most of the lymphatic system cancers studied, with the exception of tonsillar squamous cell carcinoma (32%; n = 150) (77, 78) and thymoma (15.2%; n = 46) (69, 79, 80). The expression of the LT was only examined in thymomas and from the seven samples that were MCPyV DNA-positive, with three containing detectable LT protein levels (79). It is important to note that benign lymph nodes also contained the transcript of the MCPyV sT to a small extent (80) and that the genome copy number in all positive examined lymphatic system tumors ranged between 0.000004 and 0.0013 (77, 78).

MCPyV also exhibited a presence in tumors of the circulatory system, as many leukemia cells were found to harbor MCPyV sequences. One acute myeloid leukemia sample was positive for MCPyV DNA (81), in contrast to other observations in which no sign of the virus was observed (82). Chronic lymphocytic leukemia cell also contained MCPyV transcripts (83–86), whereas truncated LT mRNA was also detected in six samples, of which two also harbored full-length LT mRNA (84). Although when examined, all the chronic lymphocytic leukemia samples were negative for LT protein expression (21, 87). The presence of the truncated LT transcripts may be a sign of viral genomic integration. Even so, considering that some samples also contained full-length LT, it is plausible that in these tumors the virus was initially present in the cytoplasm for a longer period before integration, but later lost its ability to express its proteins. In the one case in which the viral load was determined, it was markedly low between 0.000017 and 0.0012 viral copies per cell (86). Cutaneous T-cell lymphoma (CTCL) is a special non-Hodgkin's lymphoma, which is migrating to and resides in the skin. Therefore, it is potentially more exposed to MCPyV than other leukemia cells. The studies on the association between MCPyV and

CTCL are seemingly antagonizing each other, since some are reporting no detectable levels of MCPyV in CTCL cells (88–90), while others described a certain level of MCPyV DNA and transcripts in CTCL (91, 92). Although where the CTCL containing skin lesions were examined together with neighboring non-lesional skin tissues, the viral prevalence was similar between the two (93, 94). However, it is important to note that one of these studies has detected the expression of the VP1 protein in four and the expression of LT protein in one CTCL-related samples, which were completely absent in the controls (93). Cutaneous B-cell lymphomas (CBCL) are skin-resident, generally slowly growing B-cell lymphomas. Considering the fact that these cells are also in close proximity to the viral replication sites at the skin, the potential contribution of the virus to the development of CBCL cannot be ruled out. Yet, the available studies were only able to detect the MCPyV in CBCLs with a relatively low prevalence, without viral protein expression (21, 92, 95), and with a low viral load, with the average viral copy per cell at 0.009 copy/cell (92). No presence of MCPyV was detected in the studies examining chronic myelomonocytic leukemia cells (82), mantle cell lymphoma cells (96), follicular lymphomas (97), primary effusion lymphomas (52), small-cell carcinomas of the lymph nodes (98), and acute lymphoblastic leukemia (99). LT transcripts were found in one study, analyzing a set of non-Hodgkin's lymphomas (100).

The available reports regarding the presence of MCPyV DNA in reproductive system-related tumors are scarce, although some studies are displaying some level of occurrence of the virus in prostate cancer (36, 101), breast cancer (102), and cervical cancer (103). Surprisingly, in one case of testicular cancer, the viral load was relatively high at 0.934 copies per cell. Unfortunately, LT protein expression was not assessed in this case (36). On the other hand, MCPyV-positive prostate cancers exhibited a lower viral copy number at 0.002 copies per cell (36), with samples from cervical cancer containing an even lower level of viral copies between 0.00003055 and 0.0015 per tumor cell (103, 104). MCPyV viral transcripts were not detected in any examined ovarian cancer samples and in the cancers of the vulva (65, 105), and only a small set of breast cancer cells were shown to contain viral transcripts (65, 102). Despite the low viral genome copy number in cervical cancer ($\leq$0.0015), LT transcripts and protein were detected in cervical cancers originating from HIV-positive women (104). It is possible that the expression of LT in these tumors will have originally resulted from an HIV-related immunosuppression.

Compared to the previous examples, some of the tumor samples originating from the digestive track are harboring the MCPyV sequences, with a slightly higher frequency as in the case of esophagus cancer (45.1%) (106), liver cancer (62%) (36), or salivary gland cancer (26.2%) (107). In contrast, the maximum of detected viral genome copies per cell was 0.33 in an exceptionally outstanding case of a small-cell carcinoma of the parotid (108), followed by 0.026 in a case of oral cavity tumors (100). In other cases, the viral genome copy number per cell was even lower, between 0.0000054 and 0.0063 (44, 106, 109, 110). However, only one tumor sample, a small-cell carcinoma of the parotid showed expression of LT protein (108). In this particular case, a LT truncating mutation was also found (108). There was a significant difference between squamous cell carcinomas of the oral cavity and other oral cavity tumors, since approximately 40% of the examined oral cavity squamous cell carcinomas were positive for viral transcripts (36), even though the presence of the virus was barely detectable in other oral tumors. Only sporadically were tumors of the larynx, and in one case in the tumors of the mandible, throat and tongue (110) and one tumor of the jaw (100) MCPyV DNA-positive, but it is important to note that healthy oral tissues contained MCPyV transcripts at a relatively higher frequency of approximately 17% (111). LT protein was not detected in either of them, and the copy number of the virus per cell was low (0.00024–0.026) (100). Many other examined digestive track-associated cancers, including stomach cancer (62, 65) and colorectal cancer (41, 62, 112, 113), showed a very low or no positivity toward viral transcripts. This pattern of occurrence might draw a picture, in which the virus is present in the proximal part of the digestive system at a low level (rather in a latent from), but not in other parts.

A low prevalence of MCPyV was detected in bladder (4%; n = 147) (36, 62, 65) and renal cancer (3.7%; n = 81) (36, 62) samples, with the viral load in the tumor cells relatively low compared to MCCs, between 0.001 and 0.004 copies per cell (36). Considering that none of these tumors had a detectable level of LT protein (62, 65), it is presumed that the virus does not play a causative role in these cancers. It has to be noted that the virus was observed in the urine of healthy patients (40), though until now it is not clear whether this represents a way the body clears out the virus or whether these viral particles are originating form a potential host cell in the excretory system. In either case, the tissues of the excretory system could be potentially exposed to a higher titer of viral

particles, which could be acquainted for the detected viral DNA in the bladder and renal cancer samples.

MCPyV has sporadically been identified in tumors originating from other organ systems. MCPyV can rarely be traced in tumors of the skeletal system, including Ewing sarcomas, chordomas, chondrosarcoma, and rhabdosarcomas (105, 114). A limited number of desmoplastic tumors are the only soft tissue-related tumors examined thus far and did not harbor MCPyV DNA (105). Studies focusing on tumors of the nervous system described a limited number of cases in which MCPyV transcripts were detectable in a few schwannomas, meningiomas, glioblastomas (115), and neurofibromas (100), whereas no relation to the virus was established in neuroblastomas (65, 105, 116). Neuroblastoma is a childhood cancer, with approximately 90% of the cases occurring in children less than 5 years old (117). At this early age, children may not yet have been infected with MCPyV because seroepidemiological studies demonstrated that <20% of children aged 0–5 years displayed antibodies to the virus. This may explain the lack of involvement of MCPyV in this malignancy. Nevertheless, even in the cases of viral presence in tumors of the CNS, the assessed number of viral copies in the host cell was markedly low between 0.0000007 and 0.05 (75, 115). These results are in accordance with the study describing the lack of MCPyV in healthy brain tissues (118). The detected virus-positive samples were correspondingly low in neuroendocrine carcinomas, from which none were shown to be positive for LT protein expression (65, 119). This is particularly interesting, considering the initially proposed interaction between neuroendocrine Merkel cells and the MCPyV.

In summary, MCPyV DNA can be detected in most of the tumor tissues that have been examined. However, the role of MCPyV in tumors other than MCC remains obscure for several reasons:

1. The viral genome copy number is very low.
2. LT transcripts and protein are seldomly detected.
3. Truncated LT and viral genome integration are hallmarks for MCPyV-positive MCCs, but these characteristics have not systematically been investigated in non-MCC tumors. Only a few cases reported an expression of truncated LT and/or integration of the viral genome.
4. Not all groups studying the same type of tumor could reproduce a similar level of prevalence, viral load, or LT antigen expression.
5. Healthy adjacent non-tumor tissue was seldom tested.
6. The prevalence in healthy tissue was in several cases comparable to malignant tissue.
7. Potential pitfalls and shortcomings of the studies. They will be discussed in the next paragraph.

The prevalence of MCPyV in a specific tumor sometimes varied from study to study. Several reasons can explain the discrepancies between different studies. The detection of MCPyV DNA on PCR-based methods can be affected by the primers that were used. Indeed, many studies applied the LT primers originally used by Feng et al. (13). These primers are superior to primers directed against VP1 (41, 42, 120, 121). The quality of the samples may also affect the outcome of the PCR reaction, as several studies have reported that MCPyV DNA detection in fresh-frozen tissues is more reliable compared with detection in FFPE samples (66, 73, 122). Other factors that may influence the genoprevalence may be that the cohort that was examined (immunocompetent vs immunocompromised patients, smoking and drinking habits, age, gender, geographic differences, etc.). Furthermore, the number of specimens examined may give a wrong idea of the incidence of MCPyV in a specific tumor (e.g., case reports vs large cohorts examined). Another flaw of PCR-based studies is that DNA is extracted from a tumor biopsy. The tumor is a heterogeneous population that contains among others the tumor cells, infiltrating immune cells, endothelial cells, and cancer-associated fibroblasts (CAFs) (123). Knowing that fibroblasts can support viral replication and may be genuine host cells for the virus (30) and that infiltrating monocytes are reservoirs for the virus (37), positive PCR products may derive from viral sequences in CAFs and/or monocytes. PCR-based methods are also prone to contamination. Because MCPyV is chronically shed from the skin (29), contamination during tumor sample taking or handling cannot be excluded.

Fluorescence in situ hybridization (FISH), coupled with DNA hybridization chain reaction (HCR DNA FISH), has been shown to provide a highly sensitive approach to detect the viral genome in MCPyV-infected dermal fibroblasts in cell culture (124). This method can theoretically be adapted to detect integrated viral DNA elements in order to monitor the presence and relative copy number of the virus in non-MCC tumors. Combined with immunofluorescence, HCR DNA FISH was also proven to be useful in the detection of MCPyV proteins in cultured dermal fibroblasts, simultaneously with viral DNA (124). This combination of methods might provide an alternative solution to help identify fibroblast cells in tissues samples together with viral DNA elements.

FUTURE DIRECTIONS

To unequivocally establish a role of MCPyV in malignancies, other than MCC, additional well-controlled investigations are required, and larger cohorts should be examined for the presence of viral proteins (LT and/or sT). Several studies in cell culture and in animal models indicate that MCPyV sT, rather than LT, may be the main oncogenic protein (125–133). Hence, it may also be important to monitor the expression of sT. The genome state of the virus should be determined (episomal or integrated). Integration of the viral genome may be a prerequisite for the neoplastic process. This event is fulfilled in virus-positive MCC, but may be characteristic for polyomavirus-induced cancers, because integration of the human polyomavirus BK (BKPyV) genome seems to be an essential step in BKPyV-associated cancers (134). The gene-encoding LT should be sequenced to determine whether full-length or truncated LT is expressed. In situ hybridization against viral DNA or RNA should be done to ensure that the viral sequences are detected in tumor cells and not in other cell types in the tumor microenvironment. Matching adjacent non-neoplastic specimens should be included, and the viral load (genome copies/cell) should be determined. Moreover, fresh-frozen samples are favored over paraffin-embedded tissues. PCR-based studies on DNA extracted from tumor tissue should include a reverse transcriptase PCR control with primers against transcripts not found in the tumor cells but are exclusively expressed in cells of the tumor microenvironment to ensure that non-tumor cells of the tumor microenvironment are not the source for MCPyV DNA in the biopsy sample. A similar control can be implemented to screen for transcripts of skin cells to exclude contamination of the sample during surgery or handling. Finally, other techniques such as digital droplet PCR and deep sequencing can be considered. Tumor samples of different stages should be examined because the virus may be involved in initiating the neoplastic process but may not be required for further progression of the tumor. Indeed, a hit-and-run mechanism for MCPyV in some MCC cases has been suggested based on the observation that a knockdown of LT in the MCPyV-positive MKL-1 cell induced growth repression, whereas no impaired growth was observed in LT-deprived LoKe cells. In this scenario, MCPyV would be necessary for tumor initiation, but at later stages the virus is dispensable (135).

One potential mechanism through which the short presence of MCPyV could contribute to tumorigenic transformation is LT- and sT-induced genomic instability in the host. LT expression was shown to upregulate the host defense factors DNA cytosine deaminases, APOBEC3G, and APOBEC3B (136). APOBEC3B, a host defense factor against viruses, was described to have a high mutagenic effect on the genome and is suggested to be the main responsible factor for the majority of the mutations observed in human papillomavirus-induced cervical carcinomas and in other multiple cancers (137, 138). APOBEC3G was primarily considered as an anti-HIV factor which can restrain HIV viral DNA integration into the host genome (139). In contrast, APOBEC3G-mediated mutations were recently shown to contribute to the generation of HIV variations by introducing sublethal mutations into the virus genome (140). This feature of APOBEC3G could also potentially introduce nonlethal alterations to the genome of MCPyV hosts cells as well, which might contribute to the development of cancer in the long term. sT was also recently detected to induce genomic instability in its host genome by targeting the E3 ubiquitin ligase (131). Such features of the two viral antigens may contribute to the accumulation of mutations in the host genome and could subsequently pave the road to cancerous transformation, even in the absence of the initial causative factor. However, this hypothesis should be verified by experimental evidence by future studies.

Although MCPyV may not be the culprit, its gene products may enhance the expression of oncoproteins from another co-infecting oncovirus, which may be responsible for the tumor. As such, MCPyV can enhance the oncogenic potentials of the cohabitant virus. The co-presence of MCPyV and human papillomavirus and Epstein–Barr virus in tumors has been reported (78, 103, 141, 142). The high seroprevalence of MCPyV in the human population, the in vitro oncogenic potentials of LT and sT, and its causal role in MCC suggest that this virus may play a role in other human cancers, especially in individuals with a compromised immune system.

ABBREVIATIONS

AD: adenocarcinoma; ALL: acute lymphoblastic leukemia; AML: acute monocytic leukemia; BCC: basal cell carcinoma; CBCL: cutaneous B-cell lymphoma; CLL: chronic lymphocytic leukemia; CML: chronic myelomonocytic leukemia; CTCL: cutaneous T-cell lymphoma; ddPCR: droplet digital; FISH: fluorescence in situ hybridization; IHC: immunohistochemistry; LT: large T antigen; PCR: polymerase chain reaction; NEC: neuroendocrine

carcinoma; NGS: next generation sequencing; nPCR: nested PCR; NSCLC: non-small-cell lung cancer; qPCR: real-time PCR; RT-PCR: reverse transcriptase PCR; SCC: squamous cell carcinoma; SLL: small lymphocytic lymphoma; sT: small T antigen; VP1: viral protein 1 (capsid protein.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Prevalence of MCPyV in non-MCC tumors and non-malignant matching tissue.

