

Review Article

Human Polyomaviruses in Skin Diseases

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Polyomaviruses are a family of small, nonenveloped viruses with a circular double-stranded DNA genome of ~5,000 base pairs protected by an icosahedral protein structure. So far, members of this family have been identified in birds and mammals. Until 2006, BK virus (BKV), JC virus (JCV), and simian virus 40 (SV40) were the only polyomaviruses known to circulate in the human population. Their occurrence in individuals was mainly confirmed by PCR and the presence of virus-specific antibodies. Using the same methods, lymphotropic polyomavirus, originally isolated in monkeys, was recently shown to be present in healthy individuals although with much lower incidence than BKV, JCV, and SV40. The use of advanced high-throughput sequencing and improved rolling circle amplification techniques have identified the novel human polyomaviruses KI, WU, Merkel cell polyomavirus, HPyV6, HPyV7, trichodysplasia spinulosa-associated polyomavirus, and HPyV9. The skin tropism of human polyomaviruses and their dermatopathologic potentials are the focus of this paper.

1. Introduction

Polyomaviruses (PyV) were first described in 1953 in mice, but have since then been detected in different bird species, cattle, bats, rabbits, hamster, sea lion, monkeys from the Old and New World, and humans [1, 2]. Their name (Greek *poly* means many, and *oma* refers to cancer) is derived from the fact that the first known polyomavirus, mouse polyomavirus, was able to induce a wide range of tumours in mice (reviewed in [3]). Members of the *Polyomaviridae* family are naked viruses that are built up of circular double-stranded DNA of approximately 5,000 base pairs surrounded by an icosahedral capsid composed of the 3 structural proteins VP1, VP2, and VP3. The viral genome contains a noncoding control region (NCCR) that encompasses the origin of replication and the transcriptional control region. On the opposite sites of the NCCR are the early and late regions, which encode the early proteins and the late proteins (Figure 1). The viral life cycle can be divided in an early phase taking place before viral DNA replication and a late phase occurring after replication of the viral genome. Early in infection, the early proteins large tumour antigen (LT-ag) and small tumour antigen (st-ag) are expressed [1]. LT-ag is absolutely required for viral

replication, but is also involved in regulation of expression of both the early and late genes. The LT-ag of different polyomaviruses can transform cells from different species and induce tumours in animal models and is therefore implicated in the tumourigenic properties of polyomaviruses (see further). St-ag has auxiliary functions, and its major contribution in transformation relies on its ability to inactivate protein phosphatase 2A. The early regions of some polyomaviruses possess additional putative open reading frames and encode other early proteins due to translation of alternatively spliced transcripts [4]. The late region encodes the capsid proteins VP1–3, while the late region of BKV, JCV, and SV40 also encodes an agnoprotein. The exact function of this protein remains incompletely understood. In contrast to the other late proteins, it is not part of the viral particle, but it seems to be implicated in viral maturation and release [5, 6]. A fifth late encoded protein, referred to as VP4, was described for SV40. This protein enhances lysis of the host cell and facilitates release of mature virus particles [7]. Lymphotropic polyomavirus (LPV) and the recent described human PyV KI, WU, Merkel cell polyomavirus (MCPyV), trichodysplasia spinulosa-associated PyV (TSPyV), HPyV6, HPyV7, and HPyV9 all lack an open reading frame (ORF)

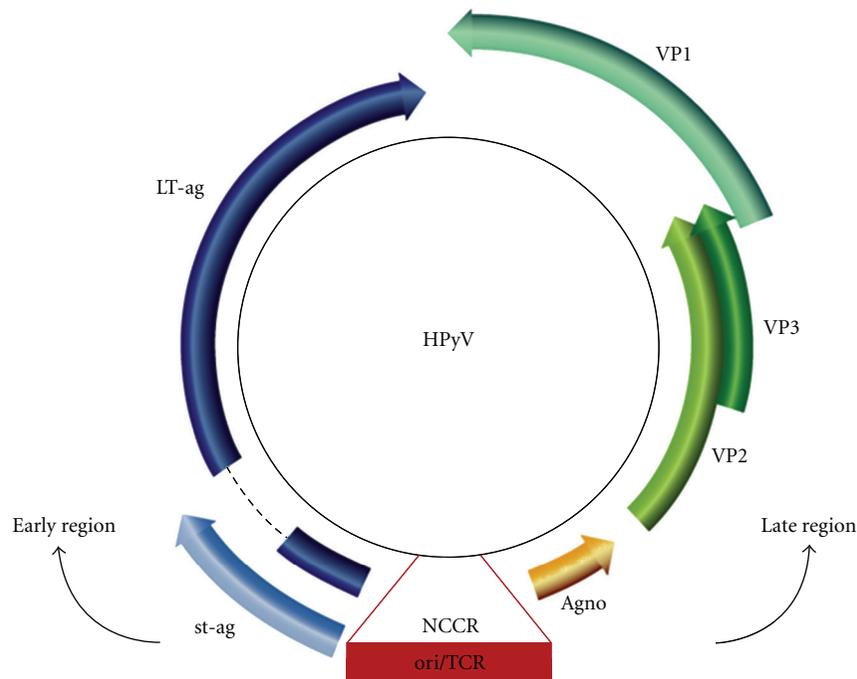


FIGURE 1: Functional organization of the HPyV genome. The viral genome consists of a circular dsDNA of ~5,000 base pairs. It can be divided into the noncoding control region (NCCR) flanked by the early and late regions. The NCCR encompasses the origin of replication and the early and late promoter elements. The early region, predominantly expressed before viral DNA replication, encodes the large T-antigen (LT-ag) and small t-antigen (st-ag). The late region is expressed late in the viral life cycle and codes for the capsid proteins VP1, VP2, and VP3. Some HPyV encode additional early proteins and the late protein, agnoprotein. The early and late genes are transcribed in opposite direction and from complementary DNA strands.

corresponding to VP4, while BKV and JCV contain a putative VP4 ORF but the expression has not yet been confirmed (Table 1). The genomes of SV40, BKV, JCV, and MCPyV encode a miRNA that downregulates LT-ag expression levels (reviewed in [8]). Cells expressing SV40 miRNA are less susceptible to cytotoxic T cells and trigger less cytokine expression than cells infected with an SV40 mutant lacking miRNA. Hence, miRNA-mediated downregulation of LT-ag levels may allow the virus to escape the immune system (reviewed in [8]). Whether BKV, JCV, and MCPyV miRNAs exert the same function remains to be tested. Viral-encoded miRNA for LPV, WUPyV, KIPyV, TSPyV, HPyV6, HPyV7, and HPyV9 has not been reported so far.

Until 2006, BK virus and JC virus, named after the initials of the patients from which they were first isolated, were the only two real human polyomaviruses (HPyV) known, while simian virus 40 (SV40) also seems to circulate in the human population. Lymphotropic polyomavirus (LPV), another monkey polyomavirus, may also infect man as specific antibodies against the major capsid protein VP1 were present in 15% of the blood samples from healthy individuals, and LPV DNA has been detected in blood of immunosuppressed and healthy subjects [9–11]. BK virus (BKV) and JC virus (JCV) are ubiquitous in the human population with up to 90% and 60%, respectively, of the adults having antibodies against these viruses [9, 12]. SV40 seems to be less common with only around 2% of the human population showing anti-SV40 antibodies [9]. In 2007, two

new human polyomaviruses were described. One virus was identified by a research group at the Karolinska Institute, Sweden and they named the virus KI [13]. The other virus was isolated by researchers from the Washington University and they referred to the new virus as WU [14]. The HPyV KI and WU are closely related, but are more different from the previously known BKV and JCV. In addition to amino acid sequence differences between the viral proteins, KI and WU lack a putative agnoprotein. Both viruses are also common in the human population with seropositivity varying between 55% and 90% [9, 15, 16]. Shortly after the discovery of HPyV KI and WU, a third new HPyV was reported. In 2008, Feng et al. identified this new polyomavirus in Merkel cell carcinoma tissue and hence named it Merkel cell polyomavirus (MCPyV) [17]. Using improved rolling circle amplification techniques resulted in the discovery of three novel HPyV. These viruses were isolated from the skin and were named HPyV-6, HPyV-7, and trichodysplasia spinulosa-associated virus [18, 19]. This year, another new human polyomavirus was isolated by PCR using degenerated VP1 primers. This virus, tentatively named HPyV9, shows highest homology with LPV [20].

2. The Oncoproteins of Human Polyomaviruses

All HPyV known to date encode proteins with oncogenic properties in cell culture and animal models. The HPyV oncoproteins include LT-ag and st-ag, while some also

TABLE 1: Comparison of the coding regions of the human polyomaviruses. The numbers refer to the number of amino acid residues, except for the genome size, which is indicated in base pairs (bp). Absent means that a putative ORF for the protein is lacking at the corresponding site in the genome. ELP: early leader protein; ORF: open reading frame.

HPyV	Accession number	Genome	LT-ag	st-ag	Agno	VP1	VP2	VP3	VP4	Alternative ORF	miRNA
SV40	J02400.1	5243	708	174	62	364	352	234	125	17 kT, SELP	Present
BKV	AB211371	5141	695	172	66	362	351	232	Putative ORF	BELP	Present
JCV	J02226	5130	688	172	71	354	344	225	Putative ORF	JELP, T' ₁₃₅ , T' ₁₃₆ , T' ₁₆₅	Present
LPV	K02562.1	5270	697	189	Absent	368	356	237	Absent	Not tested	Not tested
KI	EF127906	5040	641	191	Absent	378	400	257	Absent	Not tested	Not tested
WU	EF444549	5229	648	194	Absent	369	415	272	Absent	Not tested	Not tested
MCPyV	NC_010277	5387	818	186	Absent	423	241	196	Absent	57 kT	Present
HPyV6	HM011558	4926	669	190	Absent	387	336	215	Absent	Not tested	Not tested
HPyV7	HM011565	4949	671	193	Absent	380	329	209	Absent	Not tested	Not tested
TSPyV	GU989205	5232	698/692*	199	Absent	376	313	195	Absent	Not tested	Not tested
HPyV9	HQ696595	5026	680	189	Absent	371	352	233	Absent	Not tested	Not tested

* Two possible splice variants.

express additional early proteins and the regulatory late protein agnoprotein. The oncogenic properties will be briefly discussed in this section.

LT-ag is a multifunctional protein that is pivotal for the viral life cycle of PyV because it is indispensable for viral DNA replication and viral gene expression [1]. This protein possesses oncogenic potentials: LT-ag can induce oncogenic transformation of cells, can cause tumours in animal models, and is expressed in human cancer cells containing the virus (reviewed in [21, 22]). LT-ag is composed of several functional domains including the DnaJ domain, the retinoblastoma (pRb) binding domain, the p53 binding domain, a nuclear localization signal (NLS), a DNA binding domain, and the helicase domain (see Figure 2). LT-ag can induce oncogenic transformation through several mechanisms (reviewed in [4, 21, 22]). The DnaJ domain can transform cells through binding of HSc70, which affects the cell cycle and gene expression. Through its interactions with pRb and p53, LT-ag perturbs cell cycle regulation. Moreover, LT-ag has been shown to inhibit apoptosis, stimulate telomerase activity, modulate protein turnover, affect signalling pathways and gene expression, disturb chromosome fidelity, and induce angiogenesis. St-ag's oncogenic properties are mainly exerted by inhibiting PP2A [4, 21–23], while JCV agnoprotein was shown to bind p53, to interfere with the DNA repair protein Ku70, and to enhance transcription of the cyclin-dependent kinase inhibitor p21^{Waf1/Cip1} gene [5]. The additional early proteins expressed by some of the human polyomaviruses may also contribute to oncogenesis by dysregulating the cell cycle and influencing cellular gene expression [4, 21].

3. HPyV and Human Diseases

All known human polyomaviruses can act as oncoviruses because they all encode the oncoproteins LT-ag and st-ag. However, the role of BKV, JCV, and SV40 in human cancers remains controversial [24–27], while the role of LPV, KIPyV, WUPyV, HPyV6, HPyV7, TSPyV, and HPyV9 in

human tumorigenesis has not been studied yet. The only human PyV that is a candidate oncovirus is MCPyV, which seems to be a causal factor in Merkel cell carcinoma (see below). Human polyomaviruses seem to be harmless in immunocompetent individuals, but they can cause diseases in immunocompromised patients. Mainly BKV, but also JCV, is associated with polyomavirus nephropathy in renal transplant recipients, while BKV is also associated with hemorrhagic cystitis in bone marrow transplant patients [28]. JCV is the etiological agent of progressive multifocal leukoencephalopathy, while TSPyV may be the etiological agent in trichodysplasia spinulosa [28–30]. The role, if any, of LPV, KIPyV, WUPyV, HPyV6, HPyV7, and HPyV9 in human disease is not known [31]. In the next part of this paper, we will discuss the tropism of human polyomaviruses for the skin and elaborate their role in skin pathologies.

4. Human Polyomaviruses: Skin Tropism and Their Role in Skin Pathologies

4.1. BKV, JCV, SV40. Earlier studies had demonstrated that SV40 can infect and transform fibroblasts derived from human skin, human keratinocytes, and human melanocytes and that transgenic mice expressing SV40, LT-ag in pigment cells developed melanomas [32–36]. BKV DNA could be amplified in 25/33 (76%) of healthy skin tissue and BKV and JCV, but not SV40 LT-ag DNA sequences were detected in Kaposi's sarcoma skin lesions [37]. In a recent report, BKV DNA was detected in skin lesions from a bone marrow transplant patient that developed hemorrhagic cystitis. The skin lesions resolved after 5 days of treatment with cidovir, an antipolyomavirus drug, suggesting that a polyomavirus was involved as a causal agent [38]. Furthermore, epidemiological studies have indicated that immunosuppressed renal transplant patients, a condition that favours reactivation of BKV, have ~20-fold higher incidence of developing cancer, including skin cancer ([39], reviewed in [28]). These observations suggest that SV40, BKV, and JCV can infect the skin and may even be involved in human skin cancer. However,

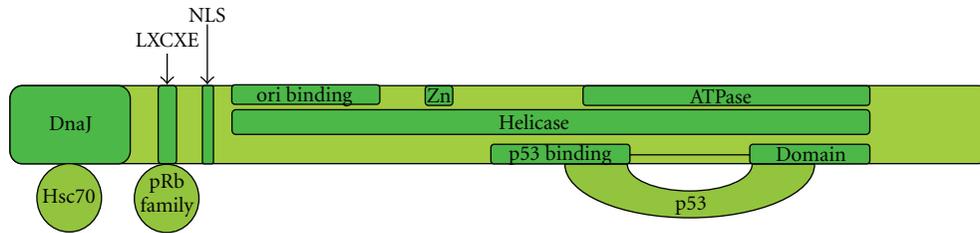


FIGURE 2: Functional domains of PyV LT-ag. The DnaJ domain (DnaJ) binds Hsc70, while the pRb pocket with the motif LXCXE binds the retinoblastoma family members pRb, p107, and p130. NLS: nuclear localization signal; Zn: Zn-finger.

several reports decline the skin as a natural site for replication and an etiological role for these viruses in skin malignancies. An early study using Southern blotting had failed to detect BKV DNA in one normal skin, in 3 skin tumours, and in two melanoma cell lines [40]. Tumour biopsies from eight patients with lymphoepithelioma-like carcinoma confined to the skin (face, scalp, or retroauricular area) were all negative for SV40 [41], and Gellrich and co-workers failed to detect BKV, JCV, and SV40 LT-ag DNA by PCR in skin biopsies from 20 primary cutaneous B cell lymphoma patients [42]. Thirty-eight paraffin-embedded samples prepared from formalin fixed melanomas collected from body surfaces completely sheltered from the sun, the so called extracutaneous melanomas, were investigated for the presence of SV40, BKV, and JCV LT-antigen DNA by nested PCR. The extracutaneous melanomas were derived from the anal canal ($n = 12$), anus-rectum ($n = 4$), nasal cavity ($n = 6$), vulva ($n = 6$), vagina-cervix ($n = 4$), tongue (1), penis (1), subungual site (2), skin (1), and oral cavity (1). All samples were negative for all three viruses [43]. Another group also failed to detect BKV and JCV in skin carcinomas obtained from 27 patients [44]. Taken together, these findings jeopardize a role for the skin as a natural habitat for SV40, BKV, and JCV.

4.2. LPV. Lymphotropic polyomavirus (LPV) was originally identified as a monkey virus, but recent reports indicate that this virus may infect humans. Indeed, 15–20% of adults are seropositive for LPV antibodies, while <10% of the blood samples obtained from healthy individuals and immunosuppressed patients were positive for LPV DNA [9–11, 45–47]. So far, no reports have been published on the presence of LPV in skin and its possible association with skin diseases has not been addressed. However, the high LPV seroprevalence may indicate that LPV has a serologically related counterpart [48]. This could be the new HPyV9 isolated by Scuda and coworkers (see Section 4.7; [20]).

4.3. KIPyV and WUPyV. Serological studies using VP1 capsomere-based ELISA have shown that infections with the polyomaviruses KI and WU are very general in the human population. Seropositivity varies between 55 and 90% and between 64 and 97.5% for KIPyV and WUPyV, respectively (reviewed in [47]). The genuine host cells for both viruses have not been established, but viral DNA has been detected in blood, brain, central nervous system, lungs, and

tonsils (reviewed in [47]). No KIPyV or WUPyV DNA was detected in one case of skin with neuroendocrine tumour [49], while all 36 examined melanoma biopsies were also negative for these two viruses [50]. Moreover, using an improved rolling circle amplification technique, Schowalter and colleagues were able to isolate MCPyV and the new human polyomaviruses HPyV6 and HPyV7 in skin samples, but the authors did not report the finding of KIPyV or WUPyV [18]. Although a small number of individuals have been screened so far, KIPyV and WUPyV do not seem to possess skin tropism.

4.4. MCPyV

4.4.1. Merkel Cell, Merkel Cell Carcinoma, and MCPyV. Merkel cells, originally described by Friedrich Merkel in 1875, are primarily located in the basal layer of the epidermal cells near the end of axons. These cells are especially dense in the skin of the limbs and face and around hair follicles. Their role remains incompletely understood, but they may function as mechanoreceptors or chemoreceptors (reviewed in [51, 52]). Merkel cell carcinoma (MCC) is a rare but aggressive skin cancer of neuroendocrine origin [53–56]. MCC is extremely unusual before the age of 50 (~5% of the cases) and people at risk include those with fair skin, excessive UV light exposure, and immunosuppressed patients [55–57]. The incidence rate is 0.44 cases per 100,000 individuals in the USA and 0.15 cases per 100,000 individuals in Japan. The number of MCC cases has, however, tripled the last 15 years [58, 59]. In 2008, Feng and colleagues detected sequences resembling LT-ag of polyomavirus in Merkel cell carcinoma transcriptomes consisting of >380,000 cDNA sequences obtained by pyrosequencing [17]. The authors succeeded in cloning and sequencing the entire genome of this virus, which turned out to be a novel polyomavirus. This new polyomavirus was commonly found in Merkel cell carcinoma (MCC) samples and therefore named Merkel cell polyomavirus (MCPyV) [17]. After the original isolation of this virus in MCC, a large number of studies have been performed worldwide to look for MCPyV DNA in MCC. Results from these studies show that approximately 80% of the examined MCC samples contain MCPyV DNA, strongly suggesting that MCPyV is a candidate etiological agent in the development of MCC (see further). Although MCC occurs most commonly in the skin, it has also been reported in sun-protected sites such as the oral and nasal mucosa, vulva, and

penis. In a case report, MCPyV DNA and LT-ag expression were detected in a patient with nasopharyngeal MCC, both in the primary tumour and in a lymph node metastasis, showing that MCPyV may also be an etiological factor in these types of MCC [60].

4.4.2. MCPyV and Cell Tropism. MCPyV infection is probably not restricted to Merkel cells because these cells make up <1% of the cells in the epidermis, yet MCPyV virions are being shed from the skin at high numbers [18]. This strongly suggests that more common cells in the epidermis like keratinocytes and/or melanocytes are involved in the production of virions [18]. Transient transfection studies in the human keratinocyte cell line HaCaT with a reporter plasmid encoding *luciferase* under control of the MCPyV NCCR showed that the MCPyV promoter/enhancer region is functional in these cells, but the presence of MCPyV in keratinocytes has not been investigated, while no MCPyV DNA was detected in melanocytes [50, 61]. Hence, a role for these two cell types as reservoirs for MCPyV remains to be established. In two independent studies, MCPyV DNA was detected in eyebrow hairs in 50% of the tested subjects [19, 62]. This high prevalence in eyebrow hairs may suggest that hair bulbs are a reservoir for MCPyV. MCPyV DNA was also detected in warts of the skin from immunocompetent and immunosuppressed patients, as well as in various skin lesions from a renal transplant patient. However, only a minority of the patients ($\leq 10\%$) were MCPyV DNA positive and no LT-ag expression was found [44]. Numerous studies have addressed the cell tropism of MCPyV mainly by performing PCR with MCPyV specific primers on different cell types and tissues. Using this technique, MCPyV DNA was found in nasal swabs, oral cavity, oesophagus, blood, tonsil biopsies, gall bladder, appendix, liver, lung, lymphoid, and intestine tissue of healthy individuals ([17, 63–70], reviewed in [47]). In blood of one psoriasis patient who was diagnosed with MCC and of one renal transplant recipient who had developed actinic keratoses, seborrheic keratoses, and nonmelanoma skin cancer patient, MCPyV DNA was exclusively detected in inflammatory monocytes, suggesting that MCPyV may persist in and spread through these cells [71]. However, for all studies, the number of MCPyV-DNA-positive samples was very low and a limited number of samples were investigated, making conclusions as to whether these cells represent initial sites of infection or sites of persistence preliminary.

Erickson and colleagues set out to identify the cellular receptor for MCPyV in order to better understand the cell tropism of this virus. The authors demonstrated that purified MCPyV VP1 protein could assemble into pentamers, but not into virus-like particles. Such VP1 pentamers bound to liposomes containing ganglioside GT1b, but not GM1 (i.e., the receptor for SV40) or GD1a (i.e., the mouse polyomavirus receptor), while a very weak binding to GD1b (i.e., the receptor for BKV) was observed [72]. This result indicates that ganglioside GT1b is a putative receptor for MCPyV. The findings suggest that MCPyV has different host cell targets than the other HPyV. The binding of MCPyV

to its target receptor may be more complicated. The studies were done with pentamers and not intact virions, which may have a different conformation and hence different binding requirement. Moreover, a coreceptor may be necessary for optimal binding. These aspects may explain why the authors found that MCPyV pentamers could bind to HeLa (human cervical cancer carcinoma cells) and HEK293T (adenovirus-transformed human embryonal kidney cells that express SV40 LT-ag) cells, which may not be genuine target cells for MCPyV. Indeed, MCPyV has not been detected in cervical or kidney tissue so far and MCPyV viruria is a rare event, indicating that the kidneys may not be an in vivo reservoir [17, 49, 50, 73, 74]. Moreover, GT1b is particularly abundant in neuronal cell membranes, but MCPyV DNA has not been amplified in cerebrospinal fluid, brain tumours, or tumours of the central nervous system [17, 43, 75, 76], indicating that these cell types lack the appropriate coreceptor for MCPyV.

4.4.3. MCPyV as the Causal Factor for MCC. The finding that 80% of the tested MCC are positive for MCPyV DNA raises the possibility that MCPyV is a candidate etiological agent in the development of MCC (reviewed in [47]). On the other hand, several groups have demonstrated the presence of MCPyV DNA in skin of healthy subjects, but also in skin biopsies adjacent to MCC [17, 44, 62, 69, 77, 78]. Moreover, serological studies demonstrated that MCPyV infection occurs early in life and that up to 84% of healthy adults have antibodies against this virus, indicating that MCPyV infection is common in the human population [9, 15, 18, 46, 79, 80]. The high seroprevalence of MCPyV in healthy subjects is underscored by the high incidence with which MCPyV DNA, complete genomes, or virus particles have been found in skin swabs, skin biopsies, and plucked eyebrow hairs of healthy subjects [18, 19, 62]. Two skin swabs collected approximately 3 months apart from 16 volunteers all tested MCPyV DNA positive and contained $\sim 2 \times 10^6$ genome equivalents/mL of gradient material obtained from the skin swab specimen. These findings indicate that healthy individuals are chronically infected with MCPyV and that the virus is continuously released from the skin [18]. Skin to skin contact may therefore be one way to horizontally transmit the virus and may explain the high seroprevalence in the human population. So, what argues for a role of MCPyV in MCC besides the high incidence of MCPyV positive MCC? First, small cell lung carcinoma (SCLC) is morphologically similar to MCC, yet no MCPyV DNA could be detected in this tumour [49, 81, 82]. However, two groups have succeeded in amplifying MCPyV DNA from SCLC, but the frequency of positive samples is much lower than in MCC (7.5%; $n = 30$ and 39%; $n = 18$) [83, 84]. Secondly, the reported numbers of virus genome copies per MCC tumour cell are much higher than the highest value obtained for other MCPyV DNA positive tissues.

Loyo and her colleagues estimated the relative viral copy number in different tissues by arbitrary assuming that MCC had an average of 10 MCPyV genome copies per cell (range 175 to 0.05). Based on this assumption, saliva had an average of 0.13 genome copies per cell, while lung, kidney,

bladder, and prostate had <0.001 genome copies per cell [69]. Nonmelanoma skin cancer samples had on average 5-fold lower viral load than MCPyV-positive MCC [85]. Shuda and colleagues confirmed these results as they found that MCPyV-positive MCC contained $\sim 1\text{--}15$ genome copies/cell, while the copy numbers in lymphoid cells from different patient groups that did not have MCC were 100- to 10,000-fold lower [67]. Other groups reported numbers of genome copies per MCC tumour cell or MCC cell line varying between 3×10^{-4} and 4×10^3 genome copies per cell, and 0.02 and 10 for MCC cell lines [73, 74, 86–91]. Despite extensive variation, which could be due to the detection method used, non-MCC tissues always contained less genome copies per cell than MCC samples in the corresponding study. It should be noted that the numbers estimated may actually be higher because the MCC tissue samples also contain nontumour cells, making accurate determination of the virus genome copy number per tumour cell difficult. Another indication that MCPyV may be involved in MCC is that the viral genome is clonally integrated in MCC tumour cells and the primary tumours as well as metastatic cells carry the same integration pattern, while viral DNA is episomal in other MCPyV-positive cells [17, 74, 89, 92]. MCPyV DNA seems to be randomly integrated at a single integration site in the different MCC [74]. Another strong clue pointing to a causal role of MCPyV in the development of MCC is that knockdown of the early proteins in MCPyV-positive MCC cell lines by RNA interference initiates growth arrest and cell death [93]. Finally, the antibody titres against MCPyV VP1 are on average ~ 60 -fold higher in MCC patients compared to healthy controls. This is not the result of VP1 expressed on the cell surface of the tumour cells, as MCC tumour cells did not express detectable levels of VP1, but rather by an unusually immunogenic MCPyV infection [46]. It is unlikely that the MCPyV-positive MCC patients newly acquired an infection because the MCPyV IgM antibodies were not significantly different from the other subjects [79]. Serum antibodies recognizing MCPyV capsid protein are detectable at high titre in nearly all MCC patients as well as in more than half of the general population. Accordingly, they provide limited value as a diagnostic marker for MCPyV-positive MCC. Paulson and colleagues found that antibodies against MCPyV LT-ag and st-ag are more specifically associated with MCC. These antibodies were present in only 0.9% of 530 control serum samples tested, whereas among 205 MCC cases 40.5% of the samples were positive. Additionally, they found that titres of LT-ag and st-ag antibodies dropped rapidly in patients whose cancer did not reoccur whereas they rose in cases that experienced progressive disease [94]. Taken together, MCPyV is now considered as a causative factor in MCC.

4.4.4. Mechanisms by Which MCPyV Induces MCC. MCPyV-positive MCC possess specific traits compared to other MCPyV-positive cells. As mentioned in the previous section, the MCPyV genome is integrated in the host genome of MCC cells. MCPyV genomes in non-MCC tissues are wild-type genomes, while the integrated genomes in MCC carry

deletions in the VP1 gene and specific mutation patterns in the LT-ag gene resulting in expression of truncated LT-ag [17, 71, 74, 91]. This C-terminal-truncated LT-ag retains the regions with predicted oncogenic potentials such as the DnaJ domain and the retinoblastoma binding pocket, but fails to support viral DNA replication [92]. Despite the expression of replication-deficient LT-ag in MCC, Wetzels and her colleagues could demonstrate the presence of viral particles in one of their MCC samples by electron microscopy [82]. This suggests the expression of a functional LT-ag since LT-ag is indispensable for viral DNA replication and discloses that not all MCPyV-positive MCC express truncated LT-ag. Interestingly, Sastre-Garau and coworkers characterized one MCC case where the tumour cells contained both integrated and episomal MCPyV DNA. The episomal DNA did not carry mutations that could lead to truncated LT-ag protein, while the integrated viral genome encoded a C-terminal truncated LT-ag [74]. A similar scenario might have been the case for the patient studied by Wetzels et al., hence explaining the presence of viral particles in MCPyV-positive MCC cells of this patient. Yet, another group reported sequences encoding full length LT-ag in MCC, jeopardizing the role of truncated LT-ag in the development of MCC [95]. In conclusion, MCPyV-positive MCC can express truncated LT-ag or/and full-length LT-ag as a result of integrated MCPyV genome encoding mutated LT-ag or/and episomal genome copies encoding wild-type LT-ag, respectively. The discrepancy in the finding of viral sequences encoding truncated LT-ag or complete LT-ag may be a result of the limitation of the PCR method, which will selectively amplify the most abundant template. Numerous MCC samples should be investigated with methods distinguishing between integrated and episomal DNA to establish whether coexistence of integrated and episomal MCPyV DNA is common or exceptional.

Mutated MCPyV genomes have also been isolated from non-MCC tissues. One full-length genome isolated from a Kaposi's sarcoma specimen encoded a truncated LT-ag that retained the DnaJ and pRb domains, but lacked the helicase region [73]. Schowalter and her colleagues used a rolling circle amplification strategy to isolate full-length MCPyV DNA shed from the skin of healthy volunteers [18]. Dworkin et al. sequenced LT-ag in MCPyV-positive squamous cell carcinoma (SCC) samples. All SCC samples (19 out of 19) displayed the same missense mutation in LT-ag (Glu259 into stop codon) which resulted in a truncated protein that still contained the DnaJ- and Rb-binding domains, but lacked the complete helicase domain. Seven out of nine normal adjacent skin samples also carry this mutation, while a MCPyV-positive mouthwash sample did not possess this mutation [96]. Fourteen out of 35 (40%) subjects yielded complete MCPyV genomes, and sequence analysis revealed that they were wild-type genomes, except for one isolate which had a frame shift in the coding sequence of st-ag resulting in a truncation of carboxy terminal 15 amino acids. The functional consequences are not known, but the corresponding residues in SV40 st-ag are not necessary for the interaction with PP2A, suggesting that this MCPyV mutant st-ag still can interact with PP2A [97].

MCC cells also express higher levels of LT-ag than other MCPyV-positive cells [67]. This is the consequence of the higher genome copy number in MCC, but may also result from mutations in the NCCR which enhance the transcriptional activity of the MCPyV promoter and of the integration of the genome. The latter event may place the MCPyV promoter in a chromosomal environment which is more favourable for transcription than the MCPyV in its natural state, that is, as part of the episomal viral genome. Studies that test these assumptions are lacking so far.

Since not all MCC are positive for MCPyV, virus-dependent and -independent oncogenic pathways may be operational in the tumourigenesis of MCC [15, 98]. Bhatia and coworkers showed that MCPyV-positive MCC with relatively high MCPyV DNA levels (i.e., 0.06–1.2 genome copies/cell) had significantly higher retinoblastoma and terminal deoxynucleotide transferase, but significantly lower p53 protein levels than MCC that had low viral genome copy numbers (i.e., 0.0005–0.0035 genome copies/cell) or were MCPyV negative. This may indicate that MCC may arise through different oncogenic pathways [88]. Another explanation may be that additional modifications, most probably mutations in host genes, may be more important or are necessary for MCC development in addition to the common event of MCPyV infection. In favour of this assumption is that there is a long time interval in the development of MCC (median age = 70 years) and the initial exposure to MCPyV (early childhood) [15, 80, 99]. The contribution of other, yet unknown factors is further supported by the findings that there is a female predominance of MCPyV-positive MCC patients. Kassem et al. found that 13 out of 20 (65%) of their male MCC patients were MCPyV positive, while 17 out of 19 (89%) female MCC patients contained MCPyV DNA in the tumour tissue [100]. Another study reported that 19 out of 33 (74%) female MCC patients were MCPyV DNA-positive patients, while 7 out of 14 (50%) of the male MCC patients were MCPyV DNA positive [101]. This indicates a female dominance, but the total number of MCPyV-positive patients in these studies is relative low.

4.4.5. MCPyV and Other Skin Diseases. MCPyV DNA could also be amplified in specimens of other malignant and non-malignant skin tissues, including Kaposi's sarcoma, malignant melanoma, basal cell carcinoma, atypical fibroxanthoma, keratoacanthoma, squamous cell carcinoma, Bowen's disease, psoriasis, actinic porokeratosis, trichoblastoma, and seborrheic keratoses. However, MCPyV DNA was found significantly less often in these non-MCC skin cancers than in MCC and the viral DNA load was also significantly lower [44, 62, 68, 73, 78, 85, 96, 101–107].

Not all studies could confirm the presence of MCPyV in some of these other skin diseases. Three groups failed to detect MCPyV DNA in different non-MCC skin malignancies [74, 101, 107]. Based on the low incidence of MCPyV DNA in basal cell carcinoma (12.5% in [102]), the limited number of samples tested by Andres and collaborators ($n = 11$), by Sastre and colleagues ($n = 13$), and by Murakami and coworkers ($n = 10$) could explain why these researchers did not detect MCPyV DNA. Immunohistochemical staining

of 7 combined primary skin tumours with mixed features of squamous and neuroendocrine carcinoma with a specific MCPyV LT-ag monoclonal was negative for all samples [76]. This may indicate that MCPyV is not implicated in this skin cancer, but definitive conclusions cannot be drawn from such a small sample size.

4.5. HPyV-6 and HPyV-7. Schowalter and coworkers identified the two new polyomaviruses HPyV6 and HPyV7 in skin swabs. Complete HPyV6 (resp., HPyV7) genomes were cloned from a total of 5/35 (resp., 4/35 individuals). Similar to MCPyV, virions seemed to be shed continuously in HPyV6- and HPyV7-positive individuals; however, at a much lower number than MCPyV, approximately 5,000 HPyV6 genome equivalents/mL of gradient material were obtained from the skin swab material, and a number too low to allow confident interpretation for HPyV7, while $\sim 2 \times 10^6$ MCPyV genome equivalents/mL of gradient material were obtained. Seroprevalence was 69% for HPyV6 and 35% for HPyV7 ($n = 95$) [18]. Thus, both HPyV6 and HPyV7 are two new human polyomaviruses with skin tropism that seem to be common in the human population and are shed continuously in infected persons. So far, no clinical symptoms have been associated with these viruses.

4.6. Trichodysplasia Spinulosa-Associated Virus. Trichodysplasia spinulosa (TS) is a rare skin disease exclusively found in immunocompromised patients and characterized by the development of follicular papules and keratin spines known as spicules. Electron microscopy studies showed the presence of polyomavirus-like particles in skin biopsies of TS patients, suggesting an etiological role polyomavirus for this disease ([108–112], reviewed in [30]). In 2010, van der Meijden and her colleagues succeeded in cloning and completely sequencing the viral genome from spicules obtained from a 15-year-old male transplant patient with TS [19]. Sequence analysis confirmed that this virus is a new member of the *Polyomaviridae*. The authors provisionally called it TS-associated polyomavirus (TSPyV). The authors investigated plucked eyebrows from 69 immunocompetent renal transplant recipients and were able to obtain TSPyV PCR products in three of the patients (4%). The estimated copy number of viral genomes was much lower than in their TS patient (<1 genome copy/cell versus $\sim 10^4$ genome copies/cell). Although a limited number of patients were tested, TSPyV may represent an occasional infection in immunocompromised patients with no signs of TS. Another recent case report confirmed the presence of TSPyV, but not MCPyV in skin biopsies from a 7-year-old girl with Down syndrome and pre-B-acute lymphoblastic leukaemia who also suffered from TS [30]. Both findings indicate that TSPyV may be implicated in TS. However, viral particles are not always detected in specimens of TS patients [113]. This negative finding could be the result of low sensitivity of the method used, poor quality of the sample, or indeed the lack of TSPyV. On the other hand, conformation of TSPyV DNA by PCR in a specimen does not imply that infectious virus particles are present. Therefore, additional studies on a larger number of TS patients, as well as healthy controls and other

patient groups are required to establish a possible relation to the occurrence of TSPyV and its causal role in TS and other skin diseases.

4.7. HPyV9. The last member to join the family of human polyomaviruses was isolated from the serum of a kidney transplant patient under immunosuppressive treatment using degenerated primers against conserved regions in the VP1 genes of known PyV [20]. Sequencing of the total genome revealed that this new virus, HPyV9, bears closest similarity to LPV. The 597 clinical samples in which HPyV9 was originally detected with the degenerated primers were then tested with HPyV9-specific primers and HPyV9 DNA was amplified in only 4 other samples (serum of a patient with PML, serum of a kidney transplant patient, whole blood of an acute myeloid leukaemia patient, and urine of a kidney transplant patient). Hence, the prevalence of this virus in the human population seems to be low. Further studies are required to identify the sites of HPyV9 replication and persistence and to determine the pathogenic potentials of this virus.

5. Conclusions and Future Perspectives

The human polyomaviruses HPyV6, HPyV7, and MCPyV seem to be common in the skin and even show a high tropism for the skin. Their continuous shedding from the skin facilitates their transmission to other individuals and may explain the high seroprevalence, at least for MCPyV. Seroepidemiological studies on HPyV6 and HPyV7, two dermatotropic polyomaviruses, have not been performed, but as these two viruses are also chronically shed from the skin, they may be highly common in the human population as well. While a strong causal link between MCPyV and Merkel cell carcinoma has been established in recent years and TSPyV may be a causal factor in TS, diseases associated with HPyV6 and HPyV7 infection remain unidentified. To confirm an etiological role of TSPyV in TS, large cohorts of TS patients should be examined. It is also possible that TSPyV is latently infecting healthy individuals and that the immunosuppressed conditions in TS patients trigger reactivation of this virus in these patients. Whether active TSPyV replication occurs in other immunocompromised patients such as organ transplant recipients, AIDS patients, and autoimmune disease patients remains to be examined. The development of new techniques such as deep sequencing and the improvement of existing techniques such as rolling circle amplification have led and may lead to the identification of several new polyomaviruses and generated a resurgence of interest in these viruses and their pathogenic potentials, including skin diseases. Once again, the medical world may be challenged with the development of efficient therapies against human viruses causing health problems.

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