Detection of Merkel Cell Polyomavirus in Respiratory Tract Specimens

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Keywords

Merkel cell polyomavirus · Nested polymerase chain reaction · Respiratory diseases · Lung cancer

Abstract

Objective: The role of Merkel cell polyomavirus (MCPyV) as a respiratory pathogen is controversial. The aim of this study was to determine the prevalence of MCPyV in patients with acute respiratory diseases and chronic lung diseases, including lung cancer, in order to evaluate the association between MCPyV infection and respiratory diseases. **Methods:** This study included 221 specimens (133 nasopharyngeal swabs and 88 lung biopsy specimens) obtained from patients with acute respiratory diseases and chronic lung diseases, including lung cancer. The detection of MCPyV was performed via nested polymerase chain reaction. **Results:** MCPyV positivity was 4.3% on average. All nasopharyngeal specimens were obtained from patients with acute respiratory diseases, and 8.2% of them were MCPyV DNA positive. There were no statistically significant differences in MCPyV prevalence according to age or gender. All specimens from nonmalignant chronic lung diseases and lung cancer were MCPyV negative. **Conclusions:** MCPyV was observed in specimens from patients with acute respiratory diseases, indicating that

there may be a relationship between the virus and these diseases. We were not able to detect MCPyV in samples from patients with chronic lung diseases, including lung cancer, suggesting no association with MCPyV infection and no involvement of this polyomavirus in lung cancerogenesis.

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Introduction

Merkel cell polyomavirus (MCPyV) was identified in 2008 by digital transcriptome subtraction in Merkel cell carcinoma (MCC), an aggressive form of skin cancer of neuroendocrine origin [1]. Since then, studies from different regions of the world have shown that the MCPyV prevalence in MCC ranges between 24 and 100%, with viral DNA clonally integrated in MCC [2, 3]. The virus has been implicated in the etiology of MCC and is the only human polyomavirus considered a causative agent of human malignancies [4]. The integrated viral DNA in MCC encodes the typical polyomavirus large (LT-ag) and small (st-ag) tumor antigens [5]. The LT-ag is, however, carboxy-terminally truncated due to a nonsense mutation in its gene. RNA interference studies and transgenic mice models have shown that both LT-ag and st-ag pos-

Table 1. Summary of the demographic data and prevalence of MCPyV DNA in the clinical samples

Samples	Patients, <i>n</i>	Age range (mean), years	β-Globin positive, <i>n</i> (%)	MCPyV positive, <i>n</i> (%)	<i>p</i> value
NPS, total	133		97 (72.9)	8 (8.2)	1.000
M	49	1–75 (24)	39 (79.6)	3 (7.7)	
F	84	3–82 (31.9)	58 (69)	5 (8.6)	
Lung, total	88				
Malignant, total	53		53 (100)	0 (0)	n.a.
M	37	30–82 (59.9)	37 (100)	0 (0)	
F	16	35–83 (61.2)	16 (100)	0 (0)	
Nonmalignant, total	35		35 (100)	0 (0)	n.a.
M	21	47–68 (56.6)	21 (100)	0 (0)	
F	14	56–82 (63.2)	14 (100)	0 (0)	
Total clinical samples	221	1–83 (41.3)	185 (83.7)	8 (4.3)	

MCPyV, Merkel cell polyomavirus; NPS, nasopharyngeal swabs; M, male; F, female; n.a., not applicable.

sess oncogenic potential [reviewed in 5]. The oncogenic action of LT-ag is attributed to its interaction with the tumor suppressor retinoblastoma family members. One consequence of this is the activation of survivin, an important mediator for cancer cell proliferation [reviewed in 6]. The role of st-ag in MCC may depend on its interaction with several cellular proteins. SCF^{Fbw7} is an E3 ubiquitin ligase [7]. Binding of st-ag to this protein leads to stabilization of SCF^{Fbw7} substrates such as LT-ag, c-Myc, and cyclin E and this can contribute to carcinogenesis [reviewed in 6]. Furthermore, the interaction with the eukaryotic translation regulator 4E-BP1 and the protein phosphatase 4 affects cellular translation and NF-κB-dependent transcription, respectively, and may also play a role in MCPyV-induced MCC [6, 8, 9].

MCPyV is widespread and most MCPyV infections are asymptomatic. Up to 80% of the adult population has serum antibodies to MCPyV [10, 11]. Seropositivity to MCPyV becomes widely prevalent in children in their first decade of life and continues to increase with age. Therefore, it was hypothesized that the primary infection occurs early in childhood. However, the route of MCPyV transmission and the sites of the initial and latent infections have not yet been identified. Many studies have demonstrated the existence of MCPyV DNA in diverse specimens, including respiratory tract specimens [12–15]. MCPyV DNA has been detected in a variety of non-MCC cancers, including chronic lymphocytic leukemia, malignant tonsillar tissues, cervical carcinomas, nonmelanoma skin cancers, and lung cancer [16–21]. At the same time, a wide variation in the MCPyV prevalence rate in

non-MCC malignancies was observed in different geographical regions.

It has been shown that MCPyV can infect the upper and lower respiratory tract [12–15, 22]. Sequence data have indicated that MCPyV found in respiratory secretions is similar to the virus identified in MCC [23]. There is no information concerning the MCPyV prevalence in Bulgaria. Hence, we aimed to evaluate the distribution of MCPyV in patients with respiratory diseases – acute and chronic, including lung cancer.

Materials and Methods

Clinical Samples

This study included 221 specimens: 133 nasopharyngeal swabs (NPS) and 88 lung biopsy specimens (LBS). They were obtained from patients (107 males and 114 females) diagnosed with respiratory diseases, aged between 1 and 83 years (mean age 41.3 years; Table 1). NPS specimens were provided by the National Reference Laboratory Influenza and Acute Respiratory Diseases. LBS were obtained from the Specialized Hospital for Active Treatment in Oncology, Sofia, Bulgaria. This study was approved by the Ethical Committee of the National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria.

Detection of MCPyV by Nested Polymerase Chain Reaction

DNA extraction was performed using a GeneJET Genomic DNA Purification Kit (Thermo Scientific™) or a PureLink® Genomic DNA Mini Kit (Invitrogen™) according to the manufacturer's instructions. The quality of the extracted DNA of each specimen was evaluated by β-globin gene amplification with a GH20/PC04 primer set as previously described [24]. For amplification of MCPyV DNA, newly designed primers targeting the highly vari-

Fig. 1. Detection of Merkel cell polyomavirus in nasopharyngeal swabs by nested polymerase chain reaction (second polymerase chain reaction). Amplification of a 307-bp fragment in Merkel cell polyomavirus-positive samples (lanes 3 and 4). Merkel cell polyomavirus-negative samples (lanes 2, 5, and 6). Negative (lane 1) and positive (lane 7) controls. Left lane: MW marker, 50-bp DNA ladder.

able BC loop of VP1 of the virus genome were used. The first round of amplification was performed using the primer pair 5'-TG-CAAATCCAGAGGTTCTCC-3' and 5'-AAAACACCCAAAA-GGCAA TG-3', generating a 494-bp segment. The second polymerase chain reaction (PCR) was performed using the primers 5'-ATAT TGCCTCCCACATCTGC-3' and 5'-TGCCCTAATGTTGCCTCAGT-3', producing a fragment of 307 bp. The reaction volume was 15 μ L and included AmpliTaq Gold[®] 360 Master Mix (Applied Biosystems[™]), 10 pmoL of each primer, nuclease-free water, and template. The first PCR was performed with 4 μ L of eluted DNA, while 1 μ L of the first PCR product was transferred in the second PCR. The plasmid pMCV-R17a containing the complete genome of MCPyV (catalog No. 24729; Addgene, Cambridge, MA, USA) was used as a positive control and distilled water as a negative control. By serial dilutions of plasmid DNA it was determined that the reactions permit the detection of less than 200 copies/mL of sample. Amplifications were performed on a DNA Engine Opticon 2 system (MJ Research). The PCR conditions included denaturation for 5 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 60°C, and 30 s at 72°C and final extension step of 7 min at 72°C.

Ten microliters of amplification products were analyzed by electrophoresis in 2% agarose gels, stained with ethidium bromide, and observed under UV light. All MCPyV positive specimens were retested.

Statistical Analysis

Statistical analysis was performed with SPSS for Windows v.10.0 using Fisher's exact test to calculate *p* values. *p* < 0.05 was considered statistically significant.

Results

Of all 221 specimens, 185 were β -globin positive and were further tested for the presence of MCPyV DNA. Of these, 8 (4.3%) were positive for MCPyV and amplification of a 307-bp fragment in the second PCR was observed (Fig. 1; Table 1). Only NPS samples were positive for MCPyV DNA. Of the 97 β -globin-positive NPS, MCPyV DNA could be amplified in 8 (8.2%). NPS were obtained from patients with acute respiratory diseases of

the higher and lower respiratory tracts (pharyngitis, tonsillitis, common cold, bronchitis, pneumonia, etc.). Five of the MCPyV-positive patients had flu-like symptoms, and 2 were diagnosed with pneumonia and 1 with tonsillopharyngitis. Among children aged 12 years or younger, the MCPyV positivity was 7.4% (2 out of 27), while 8.6% (6 out of 70) of the NPS from patients older than 12 years were positive (*p* = 1.0000). The MCPyV prevalence in NPS collected from men and women was 7.7% (3 of 39) and 8.6% (5 of 58), respectively (*p* = 1.0000).

We also investigated the presence of MCPyV in LBS. LBS were collected from 53 patients with histologically proven lung cancer and from 35 cases with noncancer chronic lung diseases (pulmonary fibrosis, hamartoma, chronic obstructive pulmonary disease, inflammatory pseudotumors, and sarcoidosis). All of the LBS (*n* = 88) gave amplifiable DNA as they were PCR positive for β -globin. However, we were not able to detect MCPyV in any of them.

Discussion

We analyzed 185 β -globin DNA amplifiable clinical samples (97 NPS and 88 LBS) from patients with respiratory diseases for the presence of MCPyV DNA and observed an overall positivity of 4.3%. However, all MCPyV DNA-positive detections were in NPS samples obtained from patients with acute respiratory diseases of the upper and lower respiratory tracts. In this group, the MCPyV prevalence rate reached 8.2%, which is higher than the results of previous studies in other countries. The frequency of detection of MCPyV DNA in NPS obtained from patients with respiratory symptoms ranges from 1.3% (Australia) to 4.2% (Sweden) [12–15, 22]. It has been previously shown that the variation in MCPyV prevalence may be partially explained by methodological differences. In our

study, we used for MCPyV testing nested PCR with newly designed primers targeting the highly variable BC loop of VP1 of the virus genome. It is possible that this could result in a higher sensitivity of PCR and accordingly a higher frequency of virus detection. Using primers targeting the large T-antigen gene, Abedi Kiasari et al. [15] determined their PCR sensitivity to be 1,000 copies/mL. This is more than 5 times less sensitive than our VP1 PCR (<200 copies/mL). The human polyomaviruses KI and WU are also frequently detected in respiratory specimens [25]. Because we used primers that were specific for MCPyV DNA and that did not show homology with the genomes of these 2 human polyomaviruses, we can exclude that our amplified sequences represent these viruses. Another explanation for the prevalence variability could be the geographical factors or/and the number of samples examined. We examined a relatively small number ($n = 97$) of NPS, while Kantola et al. [14], Abedi Kiasari et al. [15], Bialasiwicz et al. [12], and Goh et al. [13] examined 246, 305, 526, and 635 NPS and nasopharyngeal aspirates, respectively. A study on 65 transbronchial biopsies of 26 lung transplant patients revealed that 22 (33.8%) of the specimens were MCPyV DNA positive with a VP2 primer-based PCR [26]. These samples were derived from 16 different patients, i.e. 61.5% of the patients had one or more MCPyV-positive transbronchial biopsies. A longitudinal study on 3,851 NPS obtained from 56 children aged <18 months demonstrated that 13 children (23%) had 1 positive sample for MCPyV DNA [27]. Both studies illustrate that MCPyV is not uncommon in the respiratory tract.

We did not observe a statistically significant difference in MCPyV prevalence according to age or gender. Previous studies have shown higher rates of MCPyV positivity among respiratory samples obtained from adults compared to pediatric cases. For example, in Sweden significantly more adults than children were found to be MCPyV positive (8.5% vs. 0.5%) [13]. At the same time, it should be considered that in our case the group of children aged 12 years or younger was quite smaller compared to the group of patients older than 12 years ($n = 27$ vs. 70).

An MCPyV positivity of about 17% was detected in lower respiratory tract samples [23]. A number of studies have shown MCPyV presence in lung tissue and lung cancer. In lung cancer samples, MCPyV has been detected in a range of 4.7–39% [28–32]. Moreover, a study in Japan provided a demonstration of not only the detection of MCPyV DNA but also the expressions of both LT RNA transcripts and LT antigen in lung cancer specimens [33]. Therefore, we tested for the presence of MCPyV DNA in LBS collected from patients with histologically proven lung cancer and noncancer chronic lung diseases (pulmonary fibrosis, hamartoma, chronic obstructive pulmonary disease, inflammatory pseudotumors, and sarcoidosis). We were not able to detect MCPyV in these specimens. The possibility of poor quality of the DNA extracted from these samples is unlikely because all LBS were β -globin positive. Our results are in support of previous studies that failed to detect MCPyV in lung cancer specimens [34–39]. In addition, several seroepidemiologic studies have not found an association between MCPyV seropositivity and lung cancer [40, 41].

In summary, we found a relatively high prevalence of MCPyV in NPS obtained from patients with acute respiratory diseases, indicating a potential role of MCPyV infection in these diseases. In addition, these results support the hypothesis of virus transmission and shedding from the respiratory tract. We were not able to detect MCPyV in chronic lung diseases, including lung cancer, suggesting no association with MCPyV infection and no involvement of this polyomavirus in lung cancerogenesis.

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Disclosure Statement

The authors have no conflict of interests to declare.

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