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ORIGINAL ARTICLE

Merkel cell polyomavirus and human papillomavirus infections in cervical disease in Iranian women

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Abstract Human papillomavirus (HPV) infection is a necessary cause of cervical neoplasia. Concomitant infection with other infectious agents has been demonstrated to be a cofactor for HPV-related cervical carcinogenesis. The present investigation aimed to determine the prevalence of HPV and Merkel cell polyomavirus (MCPyV) infections and to evaluate the role of MCPyV as a co-factor for HPV-related cervical carcinogenesis in Iranian women. From 2011 to 2013, a total of 112 cervical samples were examined. Forty-five samples (40.2 %) were positive for HPV. MCPyV was found in 37 samples (33 %). Both HPV and MCPyV were present in 14 samples (12.5 %). MCPyV was seen in 30 % of squamous cell carcinomas, 37.5 % of adenocarcinomas, and 16.7 % of undifferentiated carcinomas. The MCPyV large T antigen (LT-Ag) DNA load was determined as the viral copy number per cell. The median MCPyV LT-Ag copy number in positive women was 0.049×10^{-3} per cell (range 0.0006×10^{-3}-4.558×10^{-3} copies per cell). In comparison with other types of cervical cancer, the MCPyV LT-Ag load was higher in adenocarcinomas (0.1024×10^{-3} copies per cell). A logistic regression model adjusted to HPV positivity and age revealed no statistically significant association between MCPyV infection and cervical cancer (OR, 1.12; 95 % CI, 0.07-16.83). More studies should be conducted to clarify the role of MCPyV in cervical carcinogenesis.

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Introduction

Worldwide, cervical cancer is the third most common malignancy and the fourth leading cause of cancer death in women [1]. It is well established that persistent infection with human papillomavirus (HPV) is a necessary cause of cervical cancer [2, 3]. Over 120 different HPV types now have been identified [4]. Nearly 15 high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68, and 73) are carcinogenic and are associated with the development of cervical cancer [5, 6]. Epidemiological studies have indicated that oncogenic HPV-16 and HPV-18 are responsible for nearly 70 % of cervical cancers worldwide [7, 8]. However, the vast majority of infections are transient, and only a small fraction of infected women progress to cervical intraepithelial neoplasia (CIN), which may develop into cervical cancer. This fact indicates that there must be additional poorly understood viral, host or environmental cofactors affecting HPV carcinogenesis. Concomitant

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infections with other infectious agents have been reported in several studies as a cofactor in the development of cervical cancer [9, 10].

Merkel cell polyomavirus (MCPyV) belongs to family *Polyomaviridae* and is the only human polyomavirus for which a strong correlation with a human cancer, Merkel cell carcinoma (MCC), has been demonstrated [11]. The MCPyV large T antigen (LT-Ag) can bind tumor suppressor protein pRb, a key regulator of cell cycle progression and is a potential oncogene. Moreover, truncation mutations in the viral LT-Ag gene result in replication incompetence and tumorigenesis [12]. Merkel cell polyomavirus T antigen expression induces anchorage- and contact-independent growth in rodent cell lines and serum-independent growth of human cell lines [13].

MCPyV commonly infects the human population, and the virus has been detected at several anatomical sites including the skin [14], respiratory tract secretions [15], saliva [16], oral and anogenital mucosa [17, 18], and the cervix [19].

Since MCPyV is a tumor virus and can infect cervical tissue, we hypothesize that MCPyV may act as a cofactor for high-risk HPV to enhance cellular malignant transformation and development of cervical cancer. To examine our hypothesis, a pilot study was performed to determine the prevalence of HPV and MCPyV infections and to evaluate the association between MCPyV and development of cervical cancer in Iranian women with different stages of cervical disease.

Materials and methods

Clinical samples

In this cross-sectional study, a total of 112 cervical tissue biopsies were retrieved from women presenting with histologically confirmed cervical abnormality between 2011 and 2013 to the Cancer institute of Imam Hospital Complex affiliated to Tehran University of Medical Sciences. Except for one patient with human immunodeficiency virus type 1 (HIV-1) infection, all of the participants were immunocompetent. All experiments were performed according to the relevant laws and guidelines in accordance with the ethical standards of the Declaration of Helsinki. This study was approved by the Ethical Committee of Tehran University of Medical Sciences, and written informed consent was taken from all participants.

DNA extraction

Cervical biopsies were collected in 0.1 M PBS solution. DNA was isolated from the samples using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. The quantity and quantity of the extracted DNA was estimated using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, USA) at the end of the extraction process.

HPV detection and typing

HPV detection and typing were carried out using the reverse-hybridization-based INNO-LiPA HPV Genotyping Extra assay (Innogenetics NV, Ghent, Belgium) according to the manufacturer's instructions. This assay can identify 28 different types of HPV: HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 as high-risk types, HPV types 26, 53 and 66 as probable high-risk types, HPV types 6, 11, 40, 43, 44, 54 and 70 as low-risk types, and HPV types 69, 71 and 74 as unknown-risk types [5]. CaSki cell line DNA extract was used as a positive control for HPV testing.

Cloning of human RNase P and Merkel cell polyomavirus large T antigen amplicons

A 65-bp fragment of the human RNase P gene (RPP30) was amplified from whole-blood genomic DNA by PCR as described previously [20]. A 309-bp fragment of MCPyV LT-Ag (571-879) was amplified from MCC tumor genomic DNA by PCR as described by Feng et al. [11]. To construct the plasmids pRNase P and pMCPyV LT-Ag, both human RNase P and MCPyV LT-Ag amplicons were TA-cloned into pTZ57R/T PCR cloning vector (InsTAclone^{TM} PCR Cloning Kit, Fermentas, MD, USA) according to the manufacturer's instructions. To confirm the accuracy of the cloning process, plasmids were sent to Bioneer Corporation (Daedeok-gu, Daejeon, South Korea) for sequencing. Purified plasmids were used to generate standard curves in the real-time PCR assay for the quantification of human RNase P and MCPyV LT-Ag amplicons.

Quantitative real-time polymerase chain reaction

Quantitative real-time PCR was conducted using a Rotor-Gene® Q real-time PCR system (QIAGEN GmbH, Hilden, Germany) with the primer sets and TaqMan probe specified for the human RNase P gene and MCPyV LT-Ag gene [19, 21]. Real-time PCR was performed in a 25-µl reaction mixture containing 500 ng of extracted DNA, 12.5 µl of Maxima Probe qPCR Master Mix (Fermentas, Glen Burnie, MD, USA), 0.3 µM each primer and 0.2 µM dual-labeled probe. Genomic DNA extracted from an MCC tumor was used as a positive control. To make standard curves, real-time PCR was conducted on a ten-fold dilution series of each purified plasmid (pMCPyV LT-Ag and pRNase P) ranging from 2×10^1 to 2×10^6

copies/μl. Viral gene copy numbers per cell were computed by dividing the virus copy number by half of the RNase P copy number, since each diploid cell has two copies of RNase P gene [22]. To exclude the possibility of contamination and false positive results, a number of control specimens, including whole blood and serum from healthy subjects, in which the presence of MCPyV was not suspected, were analyzed.

Real-time reverse transcription PCR

Total cellular RNA was extracted from MCPyV-positive cervical samples using TRIzol Reagent (Invitrogen, USA) according to manufacturer's instructions. The presence of MCPyV LT-Ag transcripts was investigated by using qualitative real-time reverse transcription PCR (real-time RT-PCR). Real-time RT-PCR was performed using a Rotor-Gene® Q Real Time PCR System (QIAGEN GmbH, Hilden, Germany) with the primer sets specified for the MCPyV LT-Ag transcripts as described previously [23]. Each reaction consisted of 100 ng of total RNA, 12.5 μl of One Step SYBR® PrimeScript™ RT-PCR Buffer (Takara Bio, Shiga, Japan), 1 μl of PrimeScript 1 Step Enzyme Mix 2 (Takara Bio, Shiga, Japan), and 10 μM each primer in a 25-μl total reaction volume. Total RNA extracted from an MCC tumor was used as a positive control. To confirm amplification specificity, melting curve analysis was performed.

Statistical analysis

The chi-square test was used to compare the distribution of HPV and MCPyV infections according to stage of cervical disease. A binary logistic regression model was applied to investigate the relationship between viral infection and the stage of cervical disease. The median MCPyV DNA loads in different cervical lesion grades and various types of cervical cancer were compared with Kruskal-Wallis test. Stata software, version 12 (Stata Corp, College Station, TX, USA) was used for data analysis. Two sided p-values ≤ 0.05 were considered statistically significant.

Results

Among the 112 enrolled patients (mean age, 44.8 ± 13.5 years; range, 18-75 years), 30 (26.8 %) had CIN-1, 42 (37.5 %) had CIN-2/3, and 40 (35.7 %) had cervical cancer. Out of 40 patients with cervical cancer, 20 (50 %) had squamous cell carcinoma (SCC), 8 (20 %) had adenocarcinoma (AC) and 12 (30 %) had undifferentiated carcinoma.

The results from HPV detection and genotyping revealed the presence of HPV infection in a total of 45 (40.2 %) out of the 112 tested samples, of which 39 (86.7 %) were infected with a single HPV type and six (13.3 %) were infected with two or more HPV types (multiple-type infection).

A total of 14 different HPV genotypes were identified, including 10 high-risk types, two probable high-risk types, and two low-risk types. Out of 45 HPV-positive patients, 40 (88.9 %) were infected with high-risk types, of which 31 (77.5 %) and 5 (12.5 %) had HPV-16 and HPV-18, respectively. The less common high-risk types included HPV-39 (in 3 patients, 7.5 %) and HPV-31, HPV-45, HPV-51, HPV-56, HPV-58, HPV-68, and HPV-82 (each of them in one patient, 2.5 %).

In the current study, cervical biopsies were examined for the presence of the MCPyV LT-Ag sequence using quantitative real-time PCR. Of the 112 samples tested, the MCPyV LT-Ag sequence was found in 37 (33 %). To rule out the possibility of contamination and false positive results, a number of control specimens were investigated for MCPyV infection, and all MCPyV-positive samples were analyzed for MCPyV LT-Ag transcripts. All of the control samples tested negative for the MCPyV LT-Ag DNA sequence, and MCPyV LT-Ag transcripts were detected in all of the samples that were positive for MCPyV LT-Ag DNA.

Table 1 shows the frequency of HPV and MCPyV infection according to the stage of cervical disease. The frequency of HPV infection and increased with high-risk types infection with the severity of cervical lesions ($p < 0.001$ and $p = 0.011$, respectively). However, no significant difference was found between MCPyV positivity and the grade of cervical lesions ($p = 0.230$). Concomitant infection with HPV and MCPyV was seen in 14 cases (12.5 %). There was no significant association between concomitant infection with HPV and MCPyV, and the grade of cervical lesions ($p = 0.202$).

Regarding the type of cervical cancer, MCPyV LT-Ag was quantified in 30 % (6/20) of squamous cell carcinoma, 37.5 % (3/8) of adenocarcinoma, and 16.7 % (2/12) of undifferentiated carcinoma. However, this difference was not statistically significant ($p = 0.557$)

As shown in Table 2, the frequency of MCPyV infection was slightly higher in HPV-negative (34.3 %) than in HPV-positive women (31.1 %), but this difference was not statistically significant (OR, 0.88; 95 % CI, 0.38-2.02; $p = 0.723$). Similarly, MCPyV positivity was not significantly associated with high- and low-risk HPV types or single-type or multiple-type HPV infections (Table 2).

Table 3 shows the relationship between cervical disease stage, HPV and MCPyV infection. The odds of HPV positivity were notably higher in high-grade lesions (CIN-2/3) and cervical cancers when compared to low-grade

Table 1 Prevalence of HPV and MCPyV infections stratified by grade of cervical lesion

Cervical lesion	Total	Number HPV positive (%)	Number positive for HPV high-risk types (%)	Number MCPyV positive (%)
CIN-1	30	3 (10 %)	3 (10 %)	8 (26.7 %)
CIN-2/3	42	21 (50 %)	18 (42.9 %)	18 (42.9 %)
CC	40	21 (52.5 %)	18 (45 %)	11 (27.5 %)
P-value		<0.001	0.004	0.230

Table 2 Association between MCPyV and HPV infections in cervical samples

		Total cases (N = 112) N (%)	MCPyV-positive cases (N = 37) N (%)	OR[a] (95 % CI)	*P*-value
HPV infection	HPV−	67 (59.8)	23 (34.3 %)	0.88 (0.38-2.02)	0.723
	HPV+	45 (40.2 %)	14 (31.1 %)		
HPV risk category	High-risk types	39 (34.8 %)	11 (28.2 %)	0.75 (0.31-1.87)	0.516
	Low-risk types	6 (5.4 %)	3 (50 %)	2.12 (0.38-11.56)	0.385
	Single-type infection	39 (34.8 %)	11 (28.2 %)	0.79 (0.33-1.89)	0.606
	Multiple-type infection	6 (5.4 %)	3 (50 %)	1.75 (0.32-9.48)	0.515

[a] Odds Ratio adjusted for age

lesions (CIN-1) (age-adjusted OR > 9), and odds ratios of HPV infection increased with increasing cervical lesion grade.

Although MCPyV infection was positively associated with CIN-2/3 and cervical cancer, this association was not statistically significant (Table 3). To evaluate the effect of MCPyV infection in HPV-positive cases, a logistic regression model was adjusted to HPV positivity as well as age. Among HPV positive women MCPyV infection was positively associated only with cervical cancer; although this association was not statistically significant (OR, 1.12; 95 % CI, 0.07-16.83).

The MCPyV LT-Ag load was determined as the viral copy number per cell using a proven single-copy gene, RNase P. The amplification of this cellular gene also could be a marker for the presence of adequate amplifiable DNA. The median MCPyV LT-Ag copy number in positive women was 0.049×10^{-3} per cell (range, 0.0006×10^{-3}-4.558×10^{-3} copies per cell). In MCPyV-positive cases, the median MCPyV copy number was higher in the patients with CIN-1 (0.0579×10^{-3} copies per cell) and cervical cancer (0.578×10^{-3} copies per cell) compared to CIN-2/3 (0.0236×10^{-3} copies per cell); however, this difference was not statistically significant ($p = 0.468$) (Fig. 1A).

With respect to the type of cancer, the median MCPyV LT-Ag copy number was higher in ACs (0.1024×10^{-3} copies per cell) in comparison with SCCs (0.0617×10^{-3} copies per cell) and undifferentiated carcinomas (0.03055×10^{-3} copies per cell); however, this difference was not statistically significant ($p = 0.325$) (Fig. 1B).

Discussion

Despite the fact that HPV infection is the main factor leading to cervical cancer, the presence of HPV infection alone is unlikely to be enough for cervical cancer development, and other infections may elevate the risk of cervical carcinogenesis, together with HPV infection [9, 10].

Table 3 HPV and MCPyV as risk factors for cervical disease among women in Iran

	HPV OR[a] (95 % CI)	MCPyV OR (95 % CI)	
		All women, OR[a]	HPV DNA-positive women, OR[b]
CIN-1	1	1	1
CIN-2/3	9.09 (2.33-35.39)	1.76 (0.62-4.98)	0.86 (0.06-12.08)
Cervical cancer	9.80 (2.47-38.95)	1.40 (0.44-4.41)	1.12 (0.07-16.83)

[a] Odds Ratio adjusted for age

[b] Odds Ratio adjusted for HPV positivity and age

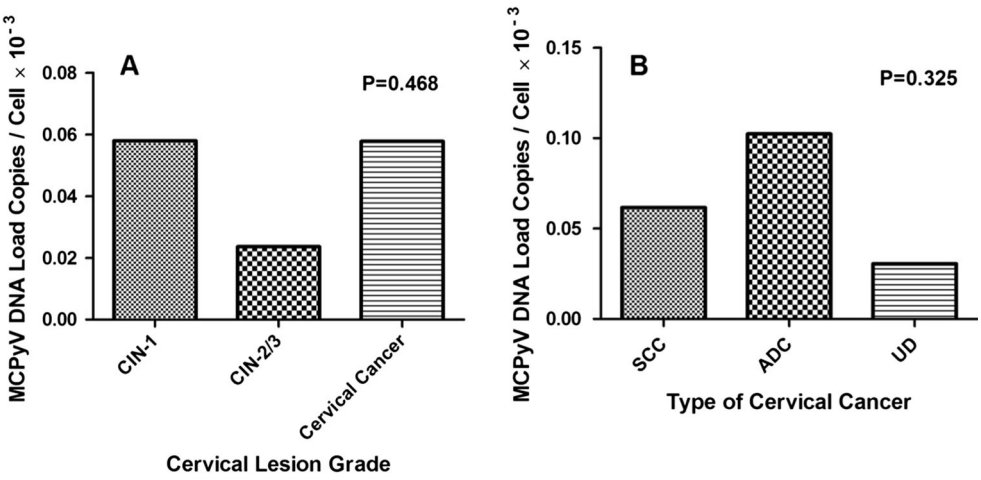

Fig. 1 Median MCPyV DNA load according to cervical lesion grade (A), and type of cervical cancer (B). The *P*-value was determined by the Kruskal-Wallis test

In the current investigation, a total of 112 cervical samples with different grades of cervical disease were examined for HPV and MCPyV infections.

The route of MCPyV transmission remains to be determined. However, considering that MCPyV has been detected in anogenital mucosa [17, 18] and cervical tissue [19], it is conceivable that this virus might be transmitted during sexual activity. In addition, the MCPyV LT-Ag is considered to be an oncoprotein that is responsible for MCPyV-related oncogenesis [12, 24–26]. Therefore, expression of the MCPyV LT-Ag in cervical cells infected with high-risk HPVs could be a cofactor with oncogenic HPVs for initiation and/or progression of cervical cancer. Importantly, the cervix is a frequent site of neuroendocrine carcinomas, which are histologically similar to MCCs.

The frequency of HPV infection was 45.4 %. In total, 14 different HPV types, including 10 high-risk, two probable high-risk and two low-risk types, were identified. In accordance with previous reports, the most commonly detected type was HPV-16 [27–29]. As expected, there was a significant correlation between the severity of cervical disease and HPV infection as well as infection with high-risk types.

In the present study, MCPyV infection was detected in all stages of cervical disease. In addition, MCPyV was found in women with or without HPV infection. The results suggest that MCPyV has no role as a cofactor in HPV-related cervical carcinogenesis; however, a role of MCPyV in cervical disease pathogenesis cannot be completely ruled out due to the fact that MCPyV is an oncogenic virus and persistent infection with this virus may lead to cell transformation.

The presence of MCPyV in cutaneous SCCs has been confirmed in several previous studies from distinct geographic regions: The frequency of MCPyV was reported to be 13 % among Japanese patients [30], 25 % in German patients [31], and 13-15 % in North American patients [32, 33]. In the present study, the frequency of MCPyV in cervical SCCs was 30 %, a rate that is higher than in previous reports about cutaneous SCCs. In addition, Imajoh et al. showed that 18.75 % of cervical SCCs and 25 % of cervical ACs were positive for MCPyV [19]. In this study, in accordance with Imajoh et al., MCPyV was detected in both SCCs (30 %) and ACs (37.5 %), with higher prevalence in our study.

In this work, the MCPyV LT-Ag oncogene was quantified as copies per cell, which is more valuable when determining viral loads in tumors than determining copies per microliter [22]. The existence of more than one DNA copy per tumor cell, which is generally seen in MCCs, suggests a carcinogenic process and tumor-viral clonality [34]. In a good agreement with Imajoh et al. [19], low copy numbers of MCPyV LT-Ag per cell were found in cervical cancer tissues, suggesting that there was no direct association with MCPyV in cervical carcinogenesis. Low copy numbers of MCPyV LT-Ag may be the reason for persistence of MCPyV as a passenger virus in the cervical tissues without obvious pathological outcome, and a role of MCPyV in tumor initiation is possible through a "hit and run" strategy [19].

The results of this study should be interpreted cautiously, since there were no matching normal cervical tissues as controls, and further investigations with a case-control or cohort design may shed more light on the role of MCPyV in the pathogenesis of cervical disease.

In conclusion, this study investigated the role of MCPyV infection in HPV-related cervical carcinogenesis among Iranian women. The current investigation suggests that MCPyV can infect cervical tissues at a low viral copy number, either alone or together with HPV. More worldwide epidemiological investigations should be done to ascertain the role of MCPyV in cervical carcinogenesis.

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Conflict of interest None to declare.

References

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61(2):69–90. doi:10.3322/caac.20107
2. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S (2007) Human papillomavirus and cervical cancer. Lancet 370(9590):890–907. doi:10.1016/S0140-6736(07)61416-0
3. Sankaranarayanan R, Nene BM, Shastri SS, Jayant K, Muwonge R, Budukh AM, Hingmire S, Malvi SG, Thorat R, Kothari A, Chinoy R, Kelkar R, Kane S, Desai S, Keskar VR, Rajeshwarkar R, Panse N, Dinshaw KA (2009) HPV screening for cervical cancer in rural India. N Engl J Med 360(14):1385–1394. doi:10.1056/NEJMoa0808516
4. Bernard HU, Burk RD, Chen Z, van Doorslaer K, zur Hausen H, de Villiers EM (2010) Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology 401(1):70–79. doi:10.1016/j.virol.2010.02.002
5. Munoz N, Bosch FX, de Sanjose S, Herrero R, Castellsague X, Shah KV, Snijders PJ, Meijer CJ (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med 348(6):518–527. doi:10.1056/NEJMoa021641
6. de Villiers EM, Fauquet C, Broker TR, Bernard HU, zur Hausen H (2004) Classification of papillomaviruses. Virology 324(1):17–27. doi:10.1016/j.virol.2004.03.033
7. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM (2007) Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 121(3):621–632. doi:10.1002/ijc.22527
8. Clifford GM, Smith JS, Plummer M, Munoz N, Franceschi S (2003) Human papillomavirus types in invasive cervical cancer worldwide: a meta-analysis. Br J Cancer 88(1):63–73. doi:10.1038/sj.bjc.6600688
9. Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV (2002) The causal relation between human papillomavirus and cervical cancer. J Clin Pathol 55(4):244–265
10. Golijow CD, Abba MC, Mouron SA, Laguens RM, Dulout FN, Smith JS (2005) *Chlamydia trachomatis* and human papillomavirus infections in cervical disease in Argentine women. Gynecol Oncol 96(1):181–186. doi:10.1016/j.ygyno.2004.09.037
11. Feng H, Shuda M, Chang Y, Moore PS (2008) Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science 319(5866):1096–1100. doi:10.1126/science.1152586
12. Shuda M, Feng H, Kwun HJ, Rosen ST, Gjoerup O, Moore PS, Chang Y (2008) T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus. Proc Natl Acad Sci USA 105(42):16272–16277. doi:10.1073/pnas.0806526105
13. Shuda M, Kwun HJ, Feng H, Chang Y, Moore PS (2011) Human Merkel cell polyomavirus small T antigen is an oncoprotein targeting the 4E-BP1 translation regulator. J Clin Invest 121(9):3623–3634. doi:10.1172/JCI46323
14. Spurgeon ME, Lambert PF (2013) Merkel cell polyomavirus: a newly discovered human virus with oncogenic potential. Virology 435(1):118–130. doi:10.1016/j.virol.2012.09.029
15. Babakir-Mina M, Ciccozzi M, Lo Presti A, Greco F, Perno CF, Ciotti M (2010) Identification of Merkel cell polyomavirus in the lower respiratory tract of Italian patients. J Med Virol 82(3):505–509. doi:10.1002/jmv.21711
16. Loyo M, Guerrero-Preston R, Brait M, Hoque MO, Chuang A, Kim MS, Sharma R, Liegeois NJ, Koch WM, Califano JA, Westra WH, Sidransky D (2010) Quantitative detection of Merkel cell virus in human tissues and possible mode of transmission. Int J Cancer 126(12):2991–2996. doi:10.1002/ijc.24737
17. Wieland U, Mauch C, Kreuter A, Krieg T, Pfister H (2009) Merkel cell polyomavirus DNA in persons without merkel cell carcinoma. Emerg Infect Dis 15(9):1496–1498. doi:10.3201/eid1509.081575
18. Wieland U, Kreuter A (2011) Merkel cell polyomavirus infection and Merkel cell carcinoma in HIV-positive individuals. Curr Opin Oncol 23(5):488–493. doi:10.1097/CCO.0b013e3283495a5b
19. Imajoh M, Hashida Y, Nemoto Y, Oguri H, Maeda N, Furihata M, Fukaya T, Daibata M (2012) Detection of Merkel cell polyomavirus in cervical squamous cell carcinomas and adenocarcinomas from Japanese patients. Virol J 9:154. doi:10.1186/1743-422X-9-154
20. Sadeghi F, Salehi-Vaziri M, Ghodsi SM, Alizadeh A, Bokharaei-Salim F, Saroukalaei ST, Mirbolouk M, Monavari SH, Keyvani H (2014) Prevalence of JC polyomavirus large T antigen sequences among Iranian patients with central nervous system tumors. Arch Virol. doi:10.1007/s00705-014-2230-0
21. Becker JC, Houben R, Ugurel S, Trefzer U, Pfohler C, Schrama D (2009) MC polyomavirus is frequently present in Merkel cell carcinoma of European patients. J Invest Dermatol 129(1):248–250. doi:10.1038/jid.2008.198
22. McNees AL, White ZS, Zanwar P, Vilchez RA, Butel JS (2005) Specific and quantitative detection of human polyomaviruses BKV, JCV, and SV40 by real time PCR. J Clin Virol 34(1):52–62. doi:10.1016/j.jcv.2004.12.018
23. Sastre-Garau X, Peter M, Avril MF, Laude H, Couturier J, Rozenberg F, Almeida A, Boitier F, Carlotti A, Couturaud B, Dupin N (2009) Merkel cell carcinoma of the skin: pathological and molecular evidence for a causative role of MCV in oncogenesis. J Pathol 218(1):48–56. doi:10.1002/path.2532
24. Sihto H, Kukko H, Koljonen V, Sankila R, Bohling T, Joensuu H (2011) Merkel cell polyomavirus infection, large T antigen, retinoblastoma protein and outcome in Merkel cell carcinoma. Clin Cancer Res 17(14):4806–4813. doi:10.1158/1078-0432.CCR-10-3363
25. Houben R, Adam C, Baeurle A, Hesbacher S, Grimm J, Angermeyer S, Henzel K, Hauser S, Elling R, Brocker EB, Gaubatz S, Becker JC, Schrama D (2012) An intact retinoblastoma protein-binding site in Merkel cell polyomavirus large T antigen is required for promoting growth of Merkel cell carcinoma cells. Int J Cancer 130(4):847–856. doi:10.1002/ijc.26076
26. Houben R, Shuda M, Weinkam R, Schrama D, Feng H, Chang Y, Moore PS, Becker JC (2010) Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. J Virol 84(14):7064–7072. doi:10.1128/JVI.02400-09
27. Bosch FX, Burchell AN, Schiffman M, Giuliano AR, de Sanjose S, Bruni L, Tortolero-Luna G, Kjaer SK, Munoz N (2008) Epidemiology and natural history of human papillomavirus infections and type-specific implications in cervical neoplasia. Vaccine 26(Suppl 10):K1–16. doi:10.1016/j.vaccine.2008.05.064
28. Mendoza LP, Arbiza J, Paez M, Kasamatsu E, Castro A, Gimenez G, Basiletti J, Gonzalez J, Mongelos P, Picconi MA (2011) Distribution of human papillomavirus genotypes in Paraguayan women according to the severity of the cervical lesion. J Med Virol 83(8):1351–1357. doi:10.1002/jmv.22112
29. Clifford GM, Rana RK, Franceschi S, Smith JS, Gough G, Pimenta JM (2005) Human papillomavirus genotype distribution in

low-grade cervical lesions: comparison by geographic region and with cervical cancer. Cancer Epidemiol Biomarkers Prev 14(5):1157–1164. doi:10.1158/1055-9965.EPI-04-0812

30. Murakami M, Imajoh M, Ikawa T, Nakajima H, Kamioka M, Nemoto Y, Ujihara T, Uchiyama J, Matsuzaki S, Sano S, Daibata M (2011) Presence of Merkel cell polyomavirus in Japanese cutaneous squamous cell carcinoma. J Clin Virol 50(1):37–41. doi:10.1016/j.jcv.2010.09.013
31. Kassem A, Technau K, Kurz AK, Pantulu D, Loning M, Kayser G, Stickeler E, Weyers W, Diaz C, Werner M, Nashan D, Zur Hausen A (2009) Merkel cell polyomavirus sequences are frequently detected in nonmelanoma skin cancer of immunosuppressed patients. Int J Cancer 125(2):356–361. doi:10.1002/ijc.24323
32. Garneski KM, Warcola AH, Feng Q, Kiviat NB, Leonard JH, Nghiem P (2009) Merkel cell polyomavirus is more frequently present in North American than Australian Merkel cell carcinoma tumors. J Invest Dermatol 129(1):246–248. doi:10.1038/jid.2008.229
33. Dworkin AM, Tseng SY, Allain DC, Iwenofu OH, Peters SB, Toland AE (2009) Merkel cell polyomavirus in cutaneous squamous cell carcinoma of immunocompetent individuals. J Invest Dermatol 129(12):2868–2874. doi:10.1038/jid.2009.183
34. Moore PS, Chang Y (2010) Why do viruses cause cancer? Highlights of the first century of human tumour virology. Nat Rev Cancer 10(12):878–889. doi:10.1038/nrc2961