Limited detection of human polyomaviruses in Fanconi anemia related squamous cell carcinoma

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Abstract

Fanconi anemia is a rare genome instability disorder with extreme susceptibility to squamous cell carcinoma of the head and neck and anogenital tract. In patients with this inherited disorder, the risk of head and neck cancer is 800-fold higher than in the general population, a finding which might suggest a viral etiology. Here, we analyzed the possible contribution of human polyomaviruses to FA-associated head and neck squamous cell carcinoma (HNSCC) by a pan-polyomavirus immunohistochemistry test which detects the T antigens of all known human polyomaviruses. We observed weak reactivity in 17% of the HNSCC samples suggesting that based on classical criteria, human polyomaviruses are not causally related to squamous cell carcinomas analyzed in this study.

Introduction

Fanconi anemia (FA) is a rare DNA repair deficiency syndrome where inactivating germline mutations in FA genes confer susceptibility to bone marrow failure, leukemia and solid tumors, particularly squamous cell carcinoma (SCC) [1–6]. 22 genes encoding FA complementation group (FANC) proteins that contribute to DNA repair have been identified. Analysis of 754 individuals with FA in the International Fanconi Anemia Registry revealed that the cumulative incidence for developing squamous cell carcinoma of the head and neck (HNSCC) is 14% by 40 years of age, compared to 0.038% in the general population [7]. In early studies, the possibility that human papillomavirus (HPV) infection may be associated with SCC was
proposed because FA individuals have impaired immune responses that might increase susceptibility to viral tumorigenesis [8–10].

High-risk human papillomavirus (HPV), particularly HPV16, is an established cause of sporadic anogenital and oropharyngeal SCC in healthy and immunosuppressed individuals [11]. HPV replication is limited by the FA pathway and FANCA or FANCD2 deficiency can lead to HPV-associated hyperplastic growth in 3D engineered epidermis [12]. Although several groups have detected the presence of HPV in SCC [13–15], as well as seropositivity and increased prevalence of oral HPV in FA patients [16], a causal association for the high-risk HPV types 16/18 in FA HNSCC remains controversial [17–20].

Since the epidemiology of FA HNSCC suggests a possible viral etiology, other previously unexamined epidermotropic viruses may be viable candidates to cause this tumor type. Human polyomaviruses (HPyV) are a family of DNA tumor viruses distantly related to papillomaviruses. These ~5 kb double-stranded DNA genome viruses encode for tumor (T) antigens that are potential oncoproteins. Only one HPyV, Merkel cell polyomavirus (MCV), is currently established to cause human cancer. MCV is responsible for ~80% of all Merkel cell carcinoma (MCC), a highly aggressive neuroendocrine skin tumor [21]. In all virus-associated tumors, the MCV genome is clonally integrated generally at >1 copies in each tumor cell [22, 23] and express oncogenic viral T antigen proteins. The remaining ~20% of MCC are thought to arise from UV-induced genomic damage and tend to have high mutation burdens [24]. While MCV DNA has been reported to be variably detectable in these latter group of tumors, the calculated viral copy numbers are exceedingly low and are most consistent with coincidental infection by an epidermotropic virus. Several studies have detected low copy numbers (0.00024–0.026 copy/cell) of MCV DNA in oral cavity tumors of immunocompetent patients [25], and in saliva and oral samples collected from healthy individuals [26]. Occasional MCC cases originating from the oral cavity have been reported [27].

Here we investigated whether any of the known HPyVs are commonly detected in FA associated cancers at a level consistent with an etiologic association. We used a pan-polyomavirus immunohistochemistry test (P-PIT) which is a three antibody cocktail that detects T antigens of BK virus, JC virus, WI virus, KI virus, MCV, HPyV6, HPyV7, Trichodysplasia spinulosa virus, HPyV9, HPyV10, HPyV11, HPyV12 and NJPyV [28]. P-PIT is a robust assay which not only determines whether viral T antigen is expressed, but also provides critical localization information to aid in evaluating whether viral detection is associated with tumor causation or merely represents a passenger infection. Recently, we used P-PIT to identify a new rat polyomavirus (RatPyV2) in an immunocompromised rat colony [29] reinforcing the potential of this assay for novel polyomavirus detection and discovery.

### Materials and methods

#### Human tissue samples

The Cincinnati Children’s Hospital Medical Center Institutional Review Board (IRB) reviewed the collection of de-identified human tissue samples described here (Study #2011–2934, Epithelial Tumors in Fanconi Anemia). The IRB determined that this study does not meet the regulatory criteria for research involving human subjects, and that ongoing IRB oversight is not required. De-identified fixed and paraffin-embedded FASC tissue samples were provided by the National Disease Research Interchange (NDRI), USA (FASC 1–2), the Deutsche Fanconi-Anämie-Hilfe d.V., Germany (FASC 3–4), the Department of Pathology at Heinrich Heine University, Germany (FASC 5–16), the Department of Genetics and Microbiology at the University Autonoma de Barcelona, Spain (FASC 17–20), the Department of
Otolaryngology at the VU University Medical Center, Netherlands (FASCC 21–28), and Cincinnati Children’s Hospital (FASCC 29).

**Immunohistochemistry**

Cell-pellet microarrays were constructed from HEK293 cells separately expressing the T antigens of each of the known HPyVs as previously described [28]. Cell pellet microarray, tissue sections from FASCC patient and MCV positive and negative MCC control sections were stained according to a previously published protocol using the P-PIT cocktail comprised of primary antibodies PAb416 (1:100), 2t2 (4 μg/ml concentrated mAb), xt7 (4 μg/ml concentrated mAb) or CM2B4 (1 μg/ml mAb) for 1 h at room temperature [28].

**DNA extraction and quantitative PCR analysis**

DNA was extracted from tissue samples using QIAmp DNA FFPE Tissue kit according to manufacturer’s recommendations (Qiagen). 100 ng genomic DNA of each sample was used for quantitative PCR analysis performed in triplicates as previously described [22]. Primers amplifying MCV LT antigen (1052–1131 nt; forward: 5’-ctcgggtatgggtccttc tca-3’, reverse: 5’-catggtgttcgggaggtatatcg-3’) and internal probe (5’-ccaggcttcagactcc-3’) labeled with FAM and MGB-NFQ quencher (Applied Biosystems) were used. Copy numbers were calculated by generating standard curves of Ct values obtained from serial dilutions of known concentrations of MCV DNA amplified by PCR. RNaseP (Applied Biosystems) was used to determine cell number. qPCR reactions were performed on the Bio-rad CFX96 Real-Time PCR system (Bio-rad) with UNG (+) TaqMan Universal PCR Master Mix II (Applied Biosystems). Amplification reactions of all target genes were performed with the following condition: 50˚C for 2 min, denaturing at 95˚C for 10 min, 40 cycles of 95˚C for 15 sec and 60˚C for 1 min. Results were expressed as numbers of viral copies per cell calculated from Ct values of viral and cellular gene standards.

**Results**

We analyzed twenty-nine FA squamous cell carcinomas by P-PIT. MCV positive and negative MCC tissues along with a cell-pellet microarray comprised of HEK293 cells expressing T antigens of all thirteen known HPyVs were used as controls. A score for each case was assigned as high positive (+++), positive (++), low positive (+), inconclusive (+/-) or negative (-) (Table 1). Out of 29 FASCC samples, three (FAHNSCC-1, 3, and 17) showed weak nuclear staining (+) with the P-PIT antibody-cocktail in tumor cells (Table 1, Fig 1A, left). Staining of these tissues with each antibody alone (pAB416, 2t2, xt7) showed weak reactivity with 2t2, an MCV specific antibody. We verified these IHC results using another MCV LT-specific antibody, CM2B4 (Fig 1B and 1C). Whereas MCV T antigen-positive MCC control tissue showed robust nuclear reactivity in the tumor cells (Fig 1C), reactivity in FAHNSCC tissues was much weaker and inconstantly scattered within the tumor. Some tissues showed cytoplasmic background (cyto bg) staining most likely due to different fixation methods and/or non-specific binding to keratin (Table 1). In FAHNSCC-5, FAHNSCC-8, and FAHNSCC-25 we observed a few cells with strong nuclear staining (1–5 cells/ section) suggestive of passenger infection.

Due to limited tissue availability, we were only able to obtain DNA from twelve FFPE samples (FASCC5-16), two frozen tumor tissues (FAHNSCC-2 and FAHNSCC-4) and one early passage cell line derived from the FAHNSCC-1 sample (FAHNSCC-1*) (Fig 1D). The FAHNSCC-1* cell line and four FA samples (FAHNSCC6, FAHNSCC7, FAHNSCC15, FAHNSCC16), had MCV copy numbers of >0.0023 copies/cell, which is comparable to copy number determinations from MCV negative MCC control cases. Established FA cell lines.
(VU-1131 and VU-1365) [19], and all other FA tumor sample DNA had MCV copy numbers below 0.0023 copies/cell. In the majority of FA tumor samples (FASCC-2 through -16) MCV-specific qPCR results were within the expected range for the oral cavity as previously described (an average of 0.026 copies/cell) [26]. Three log higher MCV DNA copy numbers (2–9 MCV genome copies/cell) were detected in positive control DNA samples extracted from MCV-positive MCC FFPE tissues. DNA from the FAHNSCC-1 line showed a relatively higher copy number of MCV (0.203 copies/cell).

P-PIT and CM2B4 staining were weakly reactive in FAHNSCC-3, however we were not able to obtain sufficient amounts of DNA to pursue further analysis. For most of the tissues

Table 1. Summary of T antigen expression status, localization, and tumor collection year of FA-associated tumor samples used in this study.

| Tissue samples | Tumor site | Year | IHC staining |
|----------------|------------|------|--------------|
| FAHNSCC-1      | Tongue     | 2010 | +            |
| FAHNSCC-2      | Oral cavity| 2015 | (+/-)        |
| FAHNSCC-3      | Larynx     | 2015 | +            |
| FAHNSCC-4      | Tongue     | 2016 | (+/-)        |
| FAHNSCC-5      | Metastatic lymph node (Hypopharynx) | 2015 | cyto bg/ few nuc |
| FAHNSCC-6      | Tongue     | 2007 | -            |
| FAHNSCC-7      | Oral cavity| 2014 | (+/-)        |
| FAHNSCC-8      | Oral cavity| 2015 | cyto bg/ few nuc |
| FASCC-9        | Clitoris   | 2008 | -            |
| FASCC-10       | Soft tissue metastasis | 2015 | - |
| FAHNSCC-11     | Hypopharynx| 2015 | cyto bg/ few nuc |
| FASCC-12       | Anus       | 2011 | cyto bg     |
| FASCC-13       | Vulva      | 2011 | cyto bg     |
| FAHNSCC-14     | Hypopharynx| 2016 | (+/-) |
| FAHNSCC-15     | Oral cavity| 2015 | (+/-) |
| FAHNSCC-16     | Oral cavity| 2009 | - |
| FAHNSCC-17     | Buccal mucosa| 1997 | + |
| FAHNSCC-18     | Oral Cavity| 1997 | - |
| FAHNSCC-19     | Hypopharynx| 2000 | - |
| FASCC-20       | Vulva      | 2003 | - |
| FAHNSCC-21     | Tongue     | 2011 | - |
| FASCC-22       | NA         | NA   | cyto bg     |
| FAHNSCC-23     | Esophagus  | 2004 | cyto bg     |
| FAHNSCC-24     | Buccal mucosa| 1998 | cyto bg     |
| FAHNSCC-25     | Esophagus  | 2003 | cyto bg/ few nuc |
| FAHNSCC-26     | Oral cavity| NA   | cyto bg     |
| FAHNSCC-27     | Metastatic lymph node (Epiglottis) | 2014 | - |
| FAHNSCC-28     | Oral cavity| 2011 | cyto bg     |
| FAHNSCC-29     | Oral cavity (site 1) | 2016 | cyto bg |
|                | Oral cavity (site 2) | 2016 | - |
| MCV(-) MCC 1   | Skin       | 2014 | - |
| MCV(-) MCC 2   | Skin       | 2014 | - |
| MCV(+) MCC 1   | Skin       | 2016 | +++         |
| MCV(+) MCC 2   | Skin       | 2015 | ++          |

NA: not available, ND: not determined, cyto bg: cytoplasmic background, nuc: nuclear staining, (+/-): inconclusive, -: negative, +: positive IHC staining.

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HPyVs are unlikely to be causally linked to Fanconi anemia SCC
Fig 1. T antigen expression in FAHNSCC. Formalin-fixed paraffin embedded tissue sections from FAHNSCC samples were immunostained with (A) P-PIT antibody-cocktail comprised of PAb416, x7, or 2t2 or with (B) CM2B4. Black arrows indicate weak nuclear signal detected in the tumor area of these tissues. (C) Left, IHC-staining with CM2B4 of MCV positive (MCV(+) MCC) and negative (MCV(-) MCC) control tissues. Right, IHC-staining with CM2B4 of FAHNSCC-6 and FASCC-10 cases as representative examples for inconclusive (+/-) and negative (-) staining pattern respectively. (D) Detection of MCV genome with quantitative PCR in various FASCC tumor tissues and cell lines. Standard RNaseP copy number was divided by two to determine the cellular equivalent of DNA. FAHNSCC-1” DNA was extracted from the cell line corresponding to the tissue analyzed in Fig 1A and Table 1.

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analyzed, we were unable to perform PCR analysis using overlapping primers to walk the genome due to insufficient DNA quality, a limitation for DNA extracted from formalin fixed, paraffin-embedded tissues. We did not observe any correlation with CM2B4 staining and MCV copy numbers detected in FA tumor cases other than FAHNSCC-1.

Discussion

FA is characterized by extreme susceptibility to bone marrow failure and SCC of the head and neck, esophagus, anogenital tract and skin. HPV, and more recently polyomavirus infections, have been linked to these tumor types in the general population. The association of HPV with HNSCC in FA remains controversial due to variable results in the detection of HPV in these tumors by different laboratories. The goal of the current study was to determine whether human polyomaviruses are causally associated with FASCC by using immunohistochemical detection and localization of polyomavirus T antigens which is correlated, in cases with available DNA, by PCR analysis.

Seventeen percent of the FAHNSCC showed weak reactivity using pan-PyV antibodies. The weak positivity of some of the specimens may suggest a hit-and-run mechanism for carcinogenesis similar to a scenario proposed for HPV in FA [20]. This scenario was based on key studies by the Lambert laboratory where sustained SCC growth in Fancd2 knockout mice occurred even in the absence of the viral E7 oncogene, perhaps due to virally induced DNA damage accumulation and rapid selection for oncogene-independent tumor growth [30]. However, technical reasons including variations in preservation/processing of tissues might also explain these observations. Serological studies might reveal a distinct pattern or level of antibody reactivity for certain polyomaviruses in FASCC patients, similar to what is seen with the high anti-VP1 mean titer levels in MCV positive MCC patients compared with MCV negative MCC patients [31]. However, serologic assays have not been developed for all human polyomaviruses underscoring the utility of P-PIT. Moreover polyomavirus infection tends to be ubiquitous [32]. For example, MCV infection occurs early in childhood and is widespread [32] with a seroprevalence of 35–50% in children <10–15 years-old [32, 33]. Therefore serological profiling alone may not be sufficient to establish a direct link between a particular human polyomavirus and tumorigenesis in FA patients.

To our knowledge, this is the first study investigating a potential causal association between HPyVs and FAHNSCC patients. By the classical model of viral tumorigenesis, viruses associated with human cancers effect a tumor phenotype through the expression of one or a combination of viral products. The corollary to this is that the viral genome or a part of the viral genome that elaborates these viral onco-molecules must be in each and every tumor cell. Because FA is a rare syndrome, our data is limited to a small number of patient samples that were collected across multiple institutions within the past 21 years. If MCV is etiologically linked to a small subset of FAHNSCCs, then this study may be underpowered to detect such an association. Nevertheless, MCV genome copies detected with qPCR and the immunostaining pattern with P-PIT and MCV specific CM2B4 suggest that based on conventional criteria, neither MCV nor other known human polyomaviruses are causal pathogens for FA-related SCCs.

Author Contributions

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References

1. Kottemann MC, Smogorzewska A. Fanconi anaemia and the repair of Watson and Crick DNA cross-links. Nature. 2013; 483(7432):356–63. Epub 2013/01/18. https://doi.org/10.1038/nature11863 PMID: 23325218.
2. Kutler DI, Patel KR, Auerbach AD, Kennedy J, Lach FP, Sanborn E, et al. Natural history and management of Fanconi anemia patients with head and neck cancer: A 10-year follow-up. Laryngoscope. 2016; 126(4):870–9. Epub 2015/10/21. https://doi.org/10.1002/lary.25726 PMID: 26484938.
3. Sumpter R Jr., Levine B. Emerging functions of the Fanconi anemia pathway at a glance. J Cell Sci. 2017; 130(16):2657–62. Epub 2017/08/16. https://doi.org/10.1242/jcs.204909 PMID: 28811338.
4. Gueiderikh A, Rosselli F, Neto JBC. A never-ending story: the steadily growing family of the FA and FA-like genes. Genet Mol Biol. 2017; 40(2):398–407. Epub 2017/05/31. https://doi.org/10.1590/1678-4685-GMB-2016-0213 PMID: 28558075.
5. Knies K, Inano S, Ramirez MJ, Ishiai M, Surrallés J, Takata M, et al. Biallelic mutations in the ubiquitin ligase RWD3 cause Fanconi anemia. J Clin Invest. 2017; 127(8):3013–27. Epub 2017/07/12. https://doi.org/10.1172/JCI92069 PMID: 28691929.
6. Rodriguez A, D’Andrea A. Fanconi anemia pathway. Curr Biol. 2017; 27(18):R986–R8. Epub 2017/09/28. https://doi.org/10.1016/j.cub.2017.07.043 PMID: 28950089.
7. Kutler DI, Auerbach AD, Satagopan J, Giampietro PF, Batish SD, Huvos AG, et al. High incidence of head and neck squamous cell carcinoma in patients with Fanconi anemia. Arch Otolaryngol Head Neck Surg. 2003; 129(1):106–12. Epub 2003/01/15. PMID: 12525204.
8. Myers KC, Sauter S, Zhang X, Blesing JJ, Davies SM, Wells SI, et al. Impaired immune function in children and adults with Fanconi anemia. Pediatr Blood Cancer. 2017; 64(11):Epub 2017/05/31. https://doi.org/10.1002/pbc.26599 PMID: 2857197.
9. Myers KC, Blesing JJ, Davies SM, Zhang X, Martin LJ, Mueller R, et al. Impaired immune function in children with Fanconi anemia. Br J Haematol. 2011; 154(2):234–40. Epub 2011/05/06. https://doi.org/10.1111/j.1365-2141.2011.08721.x PMID: 21542827.
10. Castello G, Gallo C, Napolitano M, Ascierto PA. Immunological phenotype analysis of patients with Fanconi’s anemia and their family members. Acta Haematol. 1998; 100(1):39–43. Epub 1998/08/06. https://doi.org/10.1159/000040861 PMID: 9691145.
11. Zur Hausen H. The search for infectious causes of human cancers: where and why. Virology. 2009; 392(1):1–10. Epub 2009/09/02. https://doi.org/10.1016/j.virol.2009.06.001 PMID: 19720295.
12. Hoskins EE, Morreale RJ, Werner SP, Higginbotham JM, Laimins LA, Lambert PF, et al. The fanconi anemia pathway limits human papillomavirus replication. J Virol. 2012; 86(15):7831–8. Epub 2012/05/22. https://doi.org/10.1128/jvi.00408-12 PMID: 22623765.
13. de Araujo MR, Rubira-Bullen IR, Santos CF, Dionisio TJ, Bonfim CM, De Marco L, et al. High prevalence of oral human papillomavirus infection in Fanconi’s anemia patients. Oral Dis. 2011; 17(6):572–6. Epub 2011/02/22. https://doi.org/10.1111/j.1601-0825.2011.01803.x PMID: 21332606.
14. Winer RL, Huang CE, Cherne S, Stern JE, Butsch Kovacic MS, Mehta PA, et al. Detection of human papillomavirus in the oral cavities of persons with Fanconi anemia. Oral Dis. 2015; 21(3):349–54. Epub 2014/08/28. https://doi.org/10.1111/odi.12286 PMID: 25158861.

15. Katzenellenbogen RA, Carter JJ, Stern JE, Butsch Kovacic MS, Mehta PA, Sauter SL, et al. Skin and mucosal human papillomavirus seroprevalence in persons with Fanconi Anemia. Clin Vaccine Immunol. 2015; 22(4):413–20. Epub 2015/02/06. https://doi.org/10.1128/CVI.00665-14 PMID: 25651924.

16. Sauter SL, Wells SI, Zhang X, Hoskins EE, Davies SM, Myers KC, et al. Oral human papillomavirus is common in individuals with Fanconi anemia. Cancer Epidemiol Biomarkers Prev. 2015; 24(5):864–72. Epub 2015/03/27. https://doi.org/10.1158/1055-9965.EPI-15-0097-T PMID: 25808963.

17. Alter BP, Giri N, Savage SA, Quint WG, de Koning MN, Schiffman M. Squamous cell carcinomas in patients with Fanconi anemia and dyskeratosis congenita: a search for human papillomavirus. Int J Cancer. 2013; 133(6):1513–5. Epub 2013/04/06. https://doi.org/10.1002/ijc.28157 PMID: 23558727.

18. Kutler DI, Wreesmann VB, Gobberdhan A, Ben-Porat L, Satagopan J, Ngai I, et al. Human papillomavirus DNA and p53 polymorphisms in squamous cell carcinomas from Fanconi anemia patients. J Natl Cancer Inst. 2003; 95(22):1718–21. Epub 2003/11/20. PMID: 14625263.

19. van Zeeburg HJ, Snijders PJ, Pals G, Hermens MA, Rooimans MA, Bagby G, et al. Generation and molecular characterization of head and neck squamous cell lines of Fanconi anemia patients. Cancer Res. 2005; 65(4):1271–6. Epub 2005/03/01. https://doi.org/10.1158/0008-5472.CAN-04-3665 PMID: 15735012.

20. Khoury R, Sauter S, Butsch Kovacic M, Nelson AS, Myers KC, Mehta PA, et al. Risk of Human Papillomavirus Infection in Cancer-Prone Individuals: What We Know. Viruses. 2018; 10(1). Epub 2018/01/25. https://doi.org/10.3390/v10010047 PMID: 29361695.

21. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science. 2008; 319(5866):1096–100. Epub 2008/01/19. https://doi.org/10.1126/science.1152586 PMID: 18202256.

22. Shuda M, Arora R, Kwun HJ, Feng H, Sarid R, Fernandez-Figuera MT, et al. Human Merkel cell polyomavirus infection I. MCV T antigen expression in Merkel cell carcinoma, lymphoid tissues and lymphoid tumors. Int J Cancer. 2009; 125(6):1243–9. Epub 2009/06/06. https://doi.org/10.1002/ijc.24510 PMID: 19499546.

23. Fischer N, Brandner J, Fuchs F, Moll I, Grundhoff A. Detection of Merkel cell polyomavirus (MCPyV) in Merkel cell carcinoma cell lines: cell morphology and growth phenotype do not reflect presence of the virus. Int J Cancer. 2010; 126(9):2133–42. Epub 2009/09/10. https://doi.org/10.1002/ijc.24877 PMID: 19739110.

24. Starrett GJ, Marcelus C, Cantalupo PG, Katz JP, Cheng J, Akagi K, et al. Merkel Cell Polyomavirus Exhibits Dominant Control of the Tumor Genome and Transcription in Virus-Associated Merkel Cell Carcinoma. MBio. 2017; 8(1). Epub 2017/01/04. https://doi.org/10.1128/mBio.02079-16 PMID: 28049147.

25. Tania S, Matsuishi M, Kuwamoto S, Horie Y, Kodani I, Murakami I, et al. Low prevalence of Merkel cell polyomavirus with low viral loads in oral and maxillofacial tumours or tumour-like lesions from immunocompetent patients: Absence of Merkel cell polyomavirus-associated neoplasms. Mol Clin Oncol. 2015; 3(6):1301–6. Epub 2016/01/26. https://doi.org/10.3892/mco.2015.629 PMID: 26807237.

26. Loyo M, Guerrero-Preston R, Braun M, Hoque MO, Chuang A, Kim MS, et al. Quantitative detection of Merkel cell virus in human tissues and possible mode of transmission. Int J Cancer. 2010; 126(12):2991–6. Epub 2009/07/10. https://doi.org/10.1002/ijc.24737 PMID: 19588496.

27. Yom SS, Rosenthal DI, El-Naggar AK, Kies MS, Hessel AC. Merkel cell carcinoma of the tongue and head and neck oral mucosal sites. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2006; 101(6):761–8. Epub 2006/05/30. https://doi.org/10.1016/j.tripleo.2005.10.068 PMID: 16731397.

28. Toptan T, Yousem SA, Ho J, Matsushita Y, Stabile LP, Fernandez-Figuera MT, et al. Survey for human polyomaviruses in cancer. JCI Insight. 2016; 1(2). Epub 2016/04/02. https://doi.org/10.1172/jci.insight.85562 PMID: 27034991.

29. Rigatti LH, Toptan T, Newsome JT, Moore PS, Chang Y. Identification and Characterization of Novel Rat Polyomavirus 2 in a Colony of X-SCID Rats by P-PIT assay. mSphere. 2016; 1(6). Epub 2016/12/29. https://doi.org/10.1128/mSphere.00334-16 PMID: 28028546.

30. Park S, Park JW, Pitot HC, Lambert PF. Loss of Dependence on Continued Expression of the Human Papillomavirus 16 E7 Oncogene in Cervical Cancers and Precancerous Lesions Arising in Fanconi Anemia Pathway-Deficient Mice. MBio. 2016; 7(3). Epub 2016/05/18. https://doi.org/10.1128/mBio.00628-16 PMID: 27190216.

31. Tolstov YL, Arora R, Scudiere SC, Busam K, Chaudhary PM, Chang Y, et al. Lack of evidence for direct involvement of Merkel cell polyomavirus (MCPyV) in chronic lymphocytic leukemia (CLL). Blood. 2010; 115(23):4973–4. Epub 2010/06/12. https://doi.org/10.1182/blood-2010-03-273177 PMID: 20538816.
32. Tolstov YL, Pastrana DV, Feng H, Becker JC, Jenkins FJ, Moschos S, et al. Human Merkel cell polyomavirus infection II. MCV is a common human infection that can be detected by conformational capsid epitope immunoassays. Int J Cancer. 2009; 125(6):1250–6. Epub 2009/06/06. https://doi.org/10.1002/ijc.24509 PMID: 19499548.

33. Viscidi RP, Rollison DE, Sondak VK, Silver B, Messina JL, Giuliano AR, et al. Age-specific seroprevalence of Merkel cell polyomavirus, BK virus, and JC virus. Clin Vaccine Immunol. 2011; 18(10):1737–43. Epub 2011/09/02. https://doi.org/10.1128/CVI.05175-11 PMID: 21880855.