Chapter 15
Merkel Cell Polyomavirus: Epidemiology and Clinical Features of Related Cancer

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Abbreviations

CLL	Chronic lymphocytic leukemia
EV	Epidermodysplasia-verruciformis
HPV	Human papillomavirus
IgG	Immunoglobulin G
LT	Large T antigen
MCC	Merkel cell carcinoma
MCPyV	Merkel cell polyomavirus
SCC	Squamous cell carcinoma
UV	Ultraviolet light
VLP	Virus-like particle

Epidemiology of Merkel Cell Polyomavirus and Involvement in Human Cancer

Merkel cell polyomavirus (MCPyV) is the so far only human polyomavirus that is linked to the etiology of a human cancer, namely to Merkel cell carcinoma (MCC). MCPyV was first described in 2008, when a previously unidentified polyomavirus

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was found in 8 of 10 of MCC tumours [1]. The discovery of MCPyV was made using a novel methodology called *digital transcriptome subtraction*, developed by the same research team [2]. MCPyV was demonstrated to be monoclonally integrated in the human genome in MCC [1].

Presence of the genome of MCPyV in MCC tumors from around the world has been confirmed by several independent research groups (Table 15.1). Most studies have found an MCPyV detection rate in MCC of around 70–80 %, similar to the original report by Feng et al. [1]. MCPyV infection is also common on healthy human skin [3–5] and can be found all over the body. However, on skin sites other than the MCC tumors, the viral loads of MCPyV are typically more than 60 times lower [6]. The detection rate of MCPyV DNA in tissue samples depends not only on the study population's geographical location but also on the tissue sample storage conditions and PCR type and primer-set involved. The detection rate is higher, when fresh-frozen samples are used rather than formalin-fixed paraffin-embedded samples [7–11].

The seroprevalence of MCPyV, as measured using ELISA or Luminex assays detecting MCPyV-specific IgG antibodies, is uniformly high (ranging from 46 to 88 %) in the populations studied [12–17]. The serological assays are not sufficiently standardized to allow conclusions on whether variability in seroprevalences are attributable to methodological aspects of the serology or reflect true epidemiological differences between the populations studied. However, it is clear that MCPyV infection occurs already in early childhood [14, 16, 18]. Kean et al. reported a 20.5 % MCPyV seroprevalence among 1–5-year-old children [14], Tolstov et al. a 43 % MCPyV seroprevalence among children 2–5 years [16], whereas we have found a 32 % MCPyV seroprevalence among Swedish children 1–12 years [19]. Early school age seems to be an important age for McPyV antibodies (seroconversion). Chen et al. found that while children 1–4 years of age had a seroprevalence of 9 %, the seroprevalence was 35 % among children age 4–13 years of age. Also, 33 % of MCPyV-seronegative children at the age up to 3 years were found to have seroconverted when tested again 5–8 years later [18]. Rates of acquisition appear to be substantial also among adults. In a cohort of 117 MCPyV-seronegative males, 31 seroconverted over a 4-year follow-up period, corresponding to a 6.6 % annual seroconversion rate. Once seroconversion has taken place, the MCPyV IgG levels remain detectable up to 25 years after the exposure. No signs, symptoms, or routine diagnostic test results were associated with MCV infection, indicating that the primary infection is mostly asymptomatic [20].

The exact mode of MCPyV transmission is unclear. Intrauterine transmission does not appear to occur, as no MCPyV DNA was detectable in miscarried or aborted fetuses [21]. MCPyV DNA is common on the human skin [3, 7] and frequent presence of MCPyV DNA on environmental surfaces that has been in contact with human skin suggests that virus may be shed from the surface of infected skin. In a study of 60 environmental surface samples, 45 (75.0 %) were positive for MCPyV DNA and in a few of these samples the viral DNA was even protected from DNase degradation, suggesting that it represented viral DNA encapsidated inside infectious virus particles [22]. Apart from the skin, MCPyV DNA has also been

Table 15.1 Merkel cell polyomavirus DNA in Merkel cell carcinomas and in control tissues

Study	Year	Country	MCPyV in MCC	MCPyV in normal tissue	MCPyV in other cancers
Feng et al. [1]	2008	USA	8/10 (80 %)	4/25 (16 %) skin, 5/59 (8 %) other body sites	NT
Garneski et al. [64]	2009	North-America	11/16 (69 %)	0/15 skin	2/15 (13 %) SCC of skin
Ridd et al. [65]	2009	USA	7/13 (54 %)	NT	NT
Paulson et al. [66]	2009	USA	13/22 (59 %)	NT	NT
Duncavage et al. [67]	2009	USA	22/29 (76 %)	NT	NT
Bhatia et al. [57]	2010	USA	17/23 (74 %)	Included to the box in right	1/52 (2 %) of non-MCC sites
Loyo et al. [6]	2010	USA	6/7 (87 %)	21/82 (26 %)	66/192 (34 %)
Busam et al. [47]	2009	USA	15/17 (88 %)	NT	NT
Carter et al. [12]	2009	USA	24/31 (77 %)	NT	NT
Kassem et al. [52]	2008	Germany	30/39 (77 %)	0/45 PBMC	NT
Becker et al. [68]	2009	Germany	45/53 (85 %)	NT	3/24 (13 %) BCC of skin
Helmbold et al. [69]	2009	Germany	90/98 (92 %)	3/18 (17 %) skin 1/26 (4 %) blood	NT
Andres et al. [70]	2010	Germany	21/33 (64 %)	NT	2/12 (17 %) SK, 0/11 BCC, 0/10 melanoma
Houben et al. [71]	2010	Germany	43/50 (86 %)	NT	NT
Wieland et al. [5]	2009	Germany	30/34 (88 %)	8/34 (24 %) skin	10/61 (16 %) skin tumors
Handschel et al. [60]	2010	Germany	34/59 (58 %)	NT	NT
Schrama et al. [61]	2011	Germany	116/136 (85 %)	NT	NT
Sastre-Garau et al. [72]	2009	France	10/10 (100 %)	NT	0/1,241 of tumors
Touze et al. [11]	2009	France	21/32 (66 %)	NT	0/9 of neuroendocrine carcinomas
Foulongne et al. [7]	2010	France	9/11 (82 %)	0/15 skin	NT
Martel-Jantin et al. [10]	2012	France	FF 34/36 (94 %) FFPE 30/77 (39 %)	FF skin 26/32 (81 %) 0/10 buffy coats	FF Kaposi's sarcomas 11/21 (52 %)
Laude et al. [9]	2010	France	41/43 (95 %)	NT	NT

(continued)

Table 15.1 (continued)

Study	Year	Country	MCPyV in MCC	MCPyV in normal tissue	MCPyV in other cancers
Wetzels et al. [73]	2009	Netherlands	2/5 (40 %)	NT	0/10 small-cell lung cancer
Sihto et al. [59]	2009	Finland	91/114 (80 %)	NT	NT
Varga et al. [74]	2009	Hungary	5/6 (83 %)	NT	0/29
Mangana et al. [75]	2010	Switzerland	20/30 (67 %)	0/11 skin	0/8 SCC of skin
Paolini et al. [76]	2011	Italy	8/9 (89 %)	NT	NT
Faust et al. [19]	2012	Sweden	7/14 (50 %)	NT	NT
Garneski et al. [64]	2009	Australia	5/21 (24 %)	NT	NT
Schrama et al. [61]	2011	Australia	33/38 (87 %)	NT	NT
Nakajima et al. [77]	2009	Japan	11/14 (79 %)	NT	NT
Katano et al. [78]	2009	Japan	6/11 (55 %)	NT	3/49 (6 %) Kaposi's sarcomas 0/192 other diseases
Kuwamoto et al. [48]	2011	Japan	20/26 (77 %)	NT	NT
Woo et al. [79]	2010	South-Korea	7/7 (100 %)	NT	NT
Jung et al. [80]	2011	South-Korea	12/14 (86 %)	NT	9/24 (38 %) small-cell carcinoma 0/36 other cancers

NT not tested, *FF* fresh-frozen tissue sample, *FFPE* formalin-fixed paraffin-embedded sample

found at high levels in the upper aerodigestive tract, in the digestive system, and in the saliva, but was less frequently found in lung and genitourinary system samples [6]. However, MCPyV has been found in the lower respiratory tract [23] as well as on the tonsils [18]. Presence of MCPyV DNA appears to accumulate with age, being more frequent in adults than in children [24]. MCPyV can also persist in inflammatory monocytes and spread along the monocyte migration routes [25]. MCPyV has also been found in the lymphatic system [26]

The mechanism whereby MCPyV can cause MCC has been suggested to be a 2-step process involving a random viral integration into the host genome and a mutation in the Large T antigen (LT) region that eliminates viral replication capacity but retains the Rb-binding capacity [27, 28]. As a majority of the population has been infected with MCPyV, but MCC is very rare disease, events in addition to infection itself (e.g., UV-exposure or immunosuppression) must be essential for the development of MCC. Patients with MCPyV-positive MCC generally have higher levels of serum IgG antibodies specific for the MCPyV virion than do healthy individuals [15, 16, 29] and elevated MCPyV virion antibodies are detectable many years before the MCC tumor is diagnosed [19]. Presence of high MCPyV-specific antibody levels is strongly associated with high MCPyV viral loads [13, 30], suggesting that a persistent presence of a high-viral load MCPyV infection is an important step in the carcinogenic pathway [9, 16]. Serum IgG levels against the MCPyV T antigens are elevated in 40.5 % of patients with MCC, but the levels of antibodies to the T antigen appear to follow the extent of the growing tumor [31]. Although this implies that antibodies to the T antigen are more an effect of the tumor than a marker of tumor etiology, MCPyV T antigen antibodies could represent a clinically useful tumor marker to indicate disease status. In contrast, the MCPyV-neutralizing antibodies to the virion are, especially in females, elevated already decades before the tumor arises, indicating that the neutralizing antibodies are markers of an etiologic factor on the causal pathway to the MCC tumor [19].

Epidemiology of MCC and Other MCPyV-Related Diseases

MCC is a rare but highly aggressive neuroendocrine skin malignancy that affects elderly and immunosuppressed individuals. The incidence of primary MCC in the USA is 3.4 per million person-years [32]. The number of MCC cases has increased about threefold during the past 20 years, attributable mostly to the aging population [33, 34]. In Denmark, the MCC incidence between 1995 and 2006 was 2.2 cases per million person-years [35] and in Japan it was1.45 per million [36]. The incidence rates of MCC in Denmark had increased 5.4-fold over the 18 year period from 1986 until 2003 [37]. There is a strong association between MCC and white/fair-skinned individuals, advanced age, and sun exposure. At least in Scandinavia, MCC occurs more often among females than males [35, 38]. MCC incidence is increased in both AIDS and posttransplantation populations and tends to develop at a younger age in these populations [32]. The prognosis is poor and dependent on stage at diagnosis,

with 5-year survival rates of patients with localized, regional, and distant disease reported to be 64 %, 39 %, and 18 %, respectively [39]. Clinically, MCC typically presents as a fast growing, nontender, red to violet papule or nodule on sun-exposed areas of the skin, especially in the head and neck region. As also other skin malignancies present with such features, histological examination of biopsy specimens is required for the diagnosis [40]. MCC is not only a skin malignancy but can appear also in numerous other sites such as lymph nodes, oral cavity, breast, vaginal walls, and salivary glands. It has been demonstrated that also extracutaneous MCC harbor polyomavirus DNA [41].

Patients diagnosed with MCC are at increased risk of a second cancer, particularly for other skin cancers [35, 38, 42, 43]. Some studies have also found increased risks for and for chronic lymphocytic leukemia (CLL) and multiple myeloma [42, 43], but this has not been confirmed in all populations [35, 38, 42, 43]. MCC has also been found to be increased as secondary cancer after CLL, Hodgkin, and non-Hodgkin lymphomas [35].

Squamous cell carcinoma (SCC) of the skin can occur simultaneously with MCC [44–46]. MCC arising in association with a concomitant SCC of the skin seem to be negative for MCPyV DNA [47]. One study found MCPyV DNA in pure MCC, but none of four tumors with a combined MCC + SCC morphology were positive for MCPyV ($P = 0.001$) [48]. However, another study of two mixed MCC–SCC lesions found MCPyV DNA in both of them [49]. Approximately 15 % of SCCs of the skin from immunocompetent individuals have been reported to contain MCPyV DNA [13, 50, 51]. After immunosuppression, MCPyV is more reactivated among patients with non-melanoma skin cancers compared to healthy individuals [52]. MCPyV DNA has also been detected in SCC tumor tissues. Rollison et al. found that 55 (38 %) of 145 SCC tumors were MCPyV DNA positive and that MCPyV DNA-positive SCC cases were more frequently MCPyV seropositive [53].

MCPyV DNA has also been reported to be associated with skin cancers developing among subjects with the rare hereditary immunodeficiency disease epidermodysplasia-verruciformis (EV). These tumors also contain human papillomaviruses (HPV) and it was suggested that MCPyV and HPV may act as synergistic factors in the development of these tumors [54].

There are inconclusive data on MCPyV in other diseases. MCPyV was detected in 5/19 CLL cases, but only in 13.4 % of healthy controls ($P < 0.04$) [55]. In situ hybridization studies have found MCPyV DNA in CLL cells [56]. A very low level of MCPyV DNA in was found in 33 % of CLL/ small lymphocytic lymphomas [57].

It is clear that not all MCC cases contain MCPyV DNA, suggesting that MCC may exist as two etiologically distinct subtypes: virus-related and virus-unrelated [27]. MCPyV-negative MCC have more irregular nuclei ($P < 0.001$) and more abundant cytoplasm ($P = 0.001$) than MCPyV-positive MCC, which have uniform, round nuclei and scant cytoplasm [48]. MCPyV-positive and -negative MCCs also have different prognosis. MCC patients with high-viral load MCPyV DNA have a better prognosis than MCC tumor with little or no viral DNA [9, 58, 59]. However, two reports did not find any association between MCPyV status and prognosis of MCC [60, 61].

Therapeutics Against MCPyV-Associated Cancer and Prevention of Infection

MCPyV infection activates the *BIRC5* gene, which encodes the survivin oncoprotein, and is highly upregulated by LT sequestration of Rb. A small molecule survivin inhibitor (YM155) has been tried as therapy for MCPyV-MCC and found to prolong survival of mice bearing MCC tumors [62].

A candidate prophylactic vaccine based on MCPyV virus-like particles (VLPs) has been found to, in mice, elicit antibody responses that robustly neutralize MCV reporter vectors in vitro, suggests that a VLP-based vaccine could be effective for preventing MCPyV infection [15]. There is also a candidate DNA vaccine containing the MCPyV Large T antigen gene (aa1-258) (pcDNA3-LT) that has been found to generate antitumor effects against a transplantable MCPyV LT carrying murine melanoma cell line in vaccinated C57BL/6 mice [63].

Conclusion

In just 5 years since the discovery of MCPyV, intensive research has established a basic knowledge regarding the epidemiology, immunology, and pathogenesis of the virus and clarified that this virus has an etiological role in a majority of MCC cases. The list of infections known to cause cancer in man has been continuously expanding and the MVPyV demonstrates that modern research technology now can enable discovery, validation, and basic characterization in just a few years.

References

1. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. Science. 2008;319(5866):1096–100.
2. Feng H, Taylor JL, Benos PV, Newton R, Waddell K, Lucas SB, et al. Human transcriptome subtraction by using short sequence tags to search for tumor viruses in conjunctival carcinoma. J Virol. 2007;81(20):11332–40.
3. Schowalter RM, Pastrana DV, Pumphrey KA, Moyer AL, Buck CB. Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. Cell Host Microbe. 2010;7(6):509–15.
4. Foulongne V, Kluger N, Dereure O, Mercier G, Moles JP, Guillot B, et al. Merkel cell polyomavirus in cutaneous swabs. Emerg Infect Dis. 2010;16(4):685–7.
5. Wieland U, Mauch C, Kreuter A, Krieg T, Pfister H. Merkel cell polyomavirus DNA in persons without merkel cell carcinoma. Emerg Infect Dis. 2009;15(9):1496–8.
6. Loyo M, Guerrero-Preston R, Brait 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.
7. Foulongne V, Dereure O, Kluger N, Moles JP, Guillot B, Segondy M. Merkel cell polyomavirus DNA detection in lesional and nonlesional skin from patients with Merkel cell carcinoma or other skin diseases. Br J Dermatol. 2010;162(1):59–63.

8. Foulongne V, Kluger N, Dereure O, Brieu N, Guillot B, Segondy M. Merkel cell polyomavirus and Merkel cell carcinoma, France. Emerg Infect Dis. 2008;14(9):1491–3.
9. Laude HC, Jonchere B, Maubec E, Carlotti A, Marinho E, Couturaud B, et al. Distinct merkel cell polyomavirus molecular features in tumour and non tumour specimens from patients with merkel cell carcinoma. PLoS Pathog. 2010;6(8):e1001076.
10. Martel-Jantin C, Filippone C, Cassar O, Peter M, Tomasic G, Vielh P, et al. Genetic variability and integration of Merkel cell polyomavirus in Merkel cell carcinoma. Virology. 2012;426(2): 134–42.
11. Touze A, Gaitan J, Maruani A, Le Bidre E, Doussinaud A, Clavel C, et al. Merkel cell polyomavirus strains in patients with merkel cell carcinoma. Emerg Infect Dis. 2009;15(6): 960–2.
12. Carter JJ, Paulson KG, Wipf GC, Miranda D, Madeleine MM, Johnson LG, et al. Association of Merkel cell polyomavirus-specific antibodies with Merkel cell carcinoma. J Natl Cancer Inst. 2009;101(21):1510–22.
13. Faust H, Pastrana DV, Buck CB, Dillner J, Ekstrom J. Antibodies to merkel cell polyomavirus correlate to presence of viral DNA in the skin. J Infect Dis. 2011;203(8):1096–100.
14. Kean JM, Rao S, Wang M, Garcea RL. Seroepidemiology of human polyomaviruses. PLoS Pathog. 2009;5(3):e1000363.
15. Pastrana DV, Tolstov YL, Becker JC, Moore PS, Chang Y, Buck CB. Quantitation of human seroresponsiveness to Merkel cell polyomavirus. PLoS Pathog. 2009;5(9):e1000578.
16. 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.
17. Touze A, Gaitan J, Arnold F, Cazal R, Fleury MJ, Combelas N, et al. Generation of Merkel cell polyomavirus (MCV)-like particles and their application to detection of MCV antibodies. J Clin Microbiol. 2010;48(5):1767–70.
18. Chen T, Hedman L, Mattila PS, Jartti T, Ruuskanen O, Soderlund-Venermo M, et al. Serological evidence of Merkel cell polyomavirus primary infections in childhood. J Clin Virol. 2011; 50(2):125–9.
19. Faust H, Andersson K, Ekström J, Hortlund M, Robsahm TE, Dillner J. Prospective study of Merkel cell polyomavirus and risk of Merkel cell carcinoma. Int J Cancer. 2013. doi:10.1002/ijc.28419 [Epub ahead of print].
20. Tolstov YL, Knauer A, Chen JG, Kensler TW, Kingsley LA, Moore PS, et al. Asymptomatic primary Merkel cell polyomavirus infection among adults. Emerg Infect Dis. 2011;17(8): 1371–80.
21. Sadeghi M, Riipinen A, Vaisanen E, Chen T, Kantola K, Surcel HM, et al. Newly discovered KI, WU, and Merkel cell polyomaviruses: no evidence of mother-to-fetus transmission. Virol J. 2010;7:251.
22. Foulongne V, Courgnaud V, Champeau W, Segondy M. Detection of Merkel cell polyomavirus on environmental surfaces. J Med Virol. 2011;83(8):1435–9.
23. Babakir-Mina M, Ciccozzi M, Lo Presti A, Greco F, Perno CF, Ciotti M. Identification of Merkel cell polyomavirus in the lower respiratory tract of Italian patients. J Med Virol. 2010;82(3):505–9.
24. Kantola K, Sadeghi M, Lahtinen A, Koskenvuo M, Aaltonen LM, Mottonen M, et al. Merkel cell polyomavirus DNA in tumor-free tonsillar tissues and upper respiratory tract samples: implications for respiratory transmission and latency. J Clin Virol. 2009;45(4):292–5.
25. Mertz KD, Junt T, Schmid M, Pfaltz M, Kempf W. Inflammatory monocytes are a reservoir for Merkel cell polyomavirus. J Invest Dermatol. 2010;130(4):1146–51.
26. Toracchio S, Foyle A, Sroller V, Reed JA, Wu J, Kozinetz CA, et al. Lymphotropism of Merkel cell polyomavirus infection, Nova Scotia, Canada. Emerg Infect Dis. 2010;16(11):1702–9.
27. Kuwamoto S. Recent advances in the biology of Merkel cell carcinoma. Hum Pathol. 2011;42(8):1063–77.
28. Moore PS, Chang Y. Why do viruses cause cancer? Highlights of the first century of human tumour virology. Nat Rev Cancer. 2012;10(12):878–89.

29. Touze A, Le Bidre E, Laude H, Fleury MJ, Cazal R, Arnold F, et al. High levels of antibodies against merkel cell polyomavirus identify a subset of patients with merkel cell carcinoma with better clinical outcome. J Clin Oncol. 2011;29(12):1612–9.
30. Pastrana DV, Wieland U, Silling S, Buck CB, Pfister H. Positive correlation between Merkel cell polyomavirus viral load and capsid-specific antibody titer. Med Microbiol Immunol. 2012;201(1):17–23.
31. Paulson KG, Carter JJ, Johnson LG, Cahill KW, Iyer JG, Schrama D, et al. Antibodies to merkel cell polyomavirus T antigen oncoproteins reflect tumor burden in merkel cell carcinoma patients. Cancer Res. 2010;70(21):8388–97.
32. Agelli M, Clegg LX, Becker JC, Rollison DE. The etiology and epidemiology of merkel cell carcinoma. Curr Probl Cancer. 2010;34(1):14–37.
33. Albores-Saavedra J, Batich K, Chable-Montero F, Sagy N, Schwartz AM, Henson DE. Merkel cell carcinoma demographics, morphology, and survival based on 3870 cases: a population based study. J Cutan Pathol. 2010;37(1):20–7.
34. Hodgson NC. Merkel cell carcinoma: changing incidence trends. J Surg Oncol. 2005;89(1):1–4.
35. Kaae J, Hansen AV, Biggar RJ, Boyd HA, Moore PS, Wohlfahrt J, et al. Merkel cell carcinoma: incidence, mortality, and risk of other cancers. J Natl Cancer Inst. 2010;102(11):793–801.
36. Symposium of the Japanese Society for Ultrastructural Cutaneous Biology, Suzuki H, Ono T. Merkel cells, Merkel cell carcinoma, and neurobiology of the skin. Proceedings of the 1st Symposium of the Japanese Society for Ultrastructural Cutaneous Biology held in Tokyo, Japan, 24–25 November 1999. Elsevier; 2000.
37. Lyhne D, Lock-Andersen J, Dahlstrom K, Drzewiecki KT, Balslev E, Muhic A, et al. Rising incidence of Merkel cell carcinoma. J Plast Surg Hand Surg. 2011;45(6):274–80.
38. Bzhalava D, Bray F, Storm H, Dillner J. Risk of second cancers after the diagnosis of Merkel cell carcinoma in Scandinavia. Br J Cancer. 2011;104(1):178–80.
39. Sarnaik AA, Lien MH, Nghiem P, Bichakjian CK. Clinical recognition, diagnosis, and staging of merkel cell carcinoma, and the role of the multidisciplinary management team. Curr Probl Cancer. 2010;34(1):38–46.
40. Sondak VK, Messina JL, Zager JS. Merkel cell carcinoma: a multidisciplinary approach. Cambridge: World Scientific Publishing Company, Incorporated; 2010.
41. de Biase D, Ragazzi M, Asioli S, Eusebi V. Extracutaneous Merkel cell carcinomas harbor polyomavirus DNA. Hum Pathol. 2012;43(7):980–5.
42. Koljonen V, Kukko H, Tukiainen E, Bohling T, Sankila R, Joensuu H, et al. Second cancers following the diagnosis of Merkel cell carcinoma: a nationwide cohort study. Cancer Epidemiol. 2010;34(1):62–5.
43. Howard RA, Dores GM, Curtis RE, Anderson WF, Travis LB. Merkel cell carcinoma and multiple primary cancers. Cancer Epidemiol Biomarkers Prev. 2006;15(8):1545–9.
44. Al-Ahmadie HA, Mutasim DF, Mutema GK. A case of intraepidermal Merkel cell carcinoma within squamous cell carcinoma in-situ: Merkel cell carcinoma in-situ? Am J Dermatopathol. 2004;26(3):230–3.
45. Walsh NM. Primary neuroendocrine (Merkel cell) carcinoma of the skin: morphologic diversity and implications thereof. Hum Pathol. 2001;32(7):680–9.
46. Iacocca MV, Abernethy JL, Stefanato CM, Allan AE, Bhawan J. Mixed Merkel cell carcinoma and squamous cell carcinoma of the skin. J Am Acad Dermatol. 1998;39(5 Pt 2):882–7.
47. Busam KJ, Jungbluth AA, Rekthman N, Coit D, Pulitzer M, Bini J, et al. Merkel cell polyomavirus expression in merkel cell carcinomas and its absence in combined tumors and pulmonary neuroendocrine carcinomas. Am J Surg Pathol. 2009;33(9):1378–85.
48. Kuwamoto S, Higaki H, Kanai K, Iwasaki T, Sano H, Nagata K, et al. Association of Merkel cell polyomavirus infection with morphologic differences in Merkel cell carcinoma. Hum Pathol. 2011;42(5):632–40.
49. Mitteldorf C, Mertz KD, Fernandez-Figueras MT, Schmid M, Tronnier M, Kempf W. Detection of merkel cell polyomavirus and human papillomaviruses in merkel cell carcinoma combined with squamous cell carcinoma in immunocompetent European patients. Am J Dermatopathol. 2012;34(5):506–10.

50. Dworkin AM, Tseng SY, Allain DC, Iwenofu OH, Peters SB, Toland AE. Merkel cell polyomavirus in cutaneous squamous cell carcinoma of immunocompetent individuals. J Invest Dermatol. 2009;129(12):2868–74.
51. Murakami M, Imajoh M, Ikawa T, Nakajima H, Kamioka M, Nemoto Y, et al. Presence of Merkel cell polyomavirus in Japanese cutaneous squamous cell carcinoma. J Clin Virol. 2011; 50(1):37–41.
52. Kassem A, Technau K, Kurz AK, Pantulu D, Loning M, Kayser G, et al. Merkel cell polyomavirus sequences are frequently detected in nonmelanoma skin cancer of immunosuppressed patients. Int J Cancer. 2009;125(2):356–61.
53. Rollison DE, Giuliano AR, Messina JL, Fenske NA, Cherpelis BS, Sondak VK, et al. Case-control study of Merkel cell polyomavirus infection and cutaneous squamous cell carcinoma. Cancer Epidemiol Biomarkers Prev. 2012;21(1):74–81.
54. Mertz KD, Schmid M, Burger B, Itin P, Palmedo G, Scharer L, et al. Detection of Merkel cell polyomavirus in epidermodysplasia-verruciformis-associated skin neoplasms. Dermatology. 2011;222(1):87–92.
55. Pantulu ND, Pallasch CP, Kurz AK, Kassem A, Frenzel L, Sodenkamp S, et al. Detection of a novel truncating Merkel cell polyomavirus large T antigen deletion in chronic lymphocytic leukemia cells. Blood. 2010;116(24):5280–4.
56. Haugg AM, Speel EJ, Pantulu ND, Pallasch C, Kurz AK, Kvasnicka HM, et al. Fluorescence in situ hybridization confirms the presence of Merkel cell polyomavirus in chronic lymphocytic leukemia cells. Blood. 2011;117(21):5776–7.
57. Teman CJ, Tripp SR, Perkins SL, Duncavage EJ. Merkel cell polyomavirus (MCPyV) in chronic lymphocytic leukemia/small lymphocytic lymphoma. Leuk Res. 2011;35(5):689–92.
58. Bhatia K, Goedert JJ, Modali R, Preiss L, Ayers LW. Immunological detection of viral large T antigen identifies a subset of Merkel cell carcinoma tumors with higher viral abundance and better clinical outcome. Int J Cancer. 2010;127(6):1493–6.
59. Sihto H, Kukko H, Koljonen V, Sankila R, Bohling T, Joensuu H. Clinical factors associated with Merkel cell polyomavirus infection in Merkel cell carcinoma. J Natl Cancer Inst. 2009;101(13):938–45.
60. Handschel J, Muller D, Depprich RA, Ommerborn MA, Kubler NR, Naujoks C, et al. The new polyomavirus (MCPyV) does not affect the clinical course in MCCs. Int J Oral Maxillofac Surg. 2010;39(11):1086–90.
61. Schrama D, Peitsch WK, Zapatka M, Kneitz H, Houben R, Eib S, et al. Merkel cell polyomavirus status is not associated with clinical course of Merkel cell carcinoma. J Invest Dermatol. 2011;131(8):1631–8.
62. Arora R, Shuda M, Guastafierro A, Feng H, Toptan T, Tolstov Y, et al. Survivin is a therapeutic target in merkel cell carcinoma. Sci Transl Med. 2012;4(133):133ra56.
63. Zeng Q, Gomez BP, Viscidi RP, Peng S, He L, Ma B, et al. Development of a DNA vaccine targeting Merkel cell polyomavirus. Vaccine. 2012;30(7):1322–9.
64. Garneski KM, Warcola AH, Feng Q, Kiviat NB, Leonard JH, Nghiem P. Merkel cell polyomavirus is more frequently present in North American than Australian Merkel cell carcinoma tumors. J Invest Dermatol. 2009;129(1):246–8.
65. Ridd K, Yu S, Bastian BC. The presence of polyomavirus in non-melanoma skin cancer in organ transplant recipients is rare. J Invest Dermatol. 2009;129(1):250–2.
66. Paulson KG, Lemos BD, Feng B, Jaimes N, Penas PF, Bi X, et al. Array-CGH reveals recurrent genomic changes in Merkel cell carcinoma including amplification of L-Myc. J Invest Dermatol. 2009;129(6):1547–55.
67. Duncavage EJ, Zehnbauer BA, Pfeifer JD. Prevalence of Merkel cell polyomavirus in Merkel cell carcinoma. Mod Pathol. 2009;22(4):516–21.
68. Becker JC, Houben R, Ugurel S, Trefzer U, Pfohler C, Schrama D. MC polyomavirus is frequently present in Merkel cell carcinoma of European patients. J Invest Dermatol. 2009;129(1):248–50.
69. Helmbold P, Lahtz C, Enk A, Herrmann-Trost P, Marsch W, Kutzner H, et al. Frequent occurrence of RASSF1A promoter hypermethylation and Merkel cell polyomavirus in Merkel cell carcinoma. Mol Carcinog. 2009;48(10):903–9.

70. Andres C, Belloni B, Puchta U, Sander CA, Flaig MJ. Prevalence of MCPyV in Merkel cell carcinoma and non-MCC tumors. J Cutan Pathol. 2010;37(1):28–34.
71. Houben R, Schrama D, Alb M, Pfohler C, Trefzer U, Ugurel S, et al. Comparable expression and phosphorylation of the retinoblastoma protein in Merkel cell polyoma virus-positive and negative Merkel cell carcinoma. Int J Cancer. 2010;126(3):796–8.
72. Sastre-Garau X, Peter M, Avril MF, Laude H, Couturier J, Rozenberg F, et al. Merkel cell carcinoma of the skin: pathological and molecular evidence for a causative role of MCV in oncogenesis. J Pathol. 2009;218(1):48–56.
73. Wetzels CT, Hoefnagel JG, Bakkers JM, Dijkman HB, Blokx WA, Melchers WJ. Ultrastructural proof of polyomavirus in Merkel cell carcinoma tumour cells and its absence in small cell carcinoma of the lung. PLoS One. 2009;4(3):e4958.
74. Varga E, Kiss M, Szabo K, Kemeny L. Detection of Merkel cell polyomavirus DNA in Merkel cell carcinomas. Br J Dermatol. 2009;161(4):930–2.
75. Mangana J, Dziunycz P, Kerl K, Dummer R, Cozzio A. Prevalence of Merkel cell polyomavirus among Swiss Merkel cell carcinoma patients. Dermatology. 2010;221(2):184–8.
76. Paolini F, Donati P, Amantea A, Bucher S, Migliano E, Venuti A. Merkel cell polyomavirus in Merkel cell carcinoma of Italian patients. Virol J. 2011;8:103.
77. Nakajima H, Takaishi M, Yamamoto M, Kamijima R, Kodama H, Tarutani M, et al. Screening of the specific polyoma virus as diagnostic and prognostic tools for Merkel cell carcinoma. J Dermatol Sci. 2009;56(3):211–3.
78. Katano H, Ito H, Suzuki Y, Nakamura T, Sato Y, Tsuji T, et al. Detection of Merkel cell polyomavirus in Merkel cell carcinoma and Kaposi's sarcoma. J Med Virol. 2009;81(11):1951–8.
79. Woo KJ, Choi YL, Jung HS, Jung G, Shin YK, Jang KT, et al. Merkel cell carcinoma: our experience with seven patients in Korea and a literature review. J Plast Reconstr Aesthet Surg. 2010;63(12):2064–70.
80. Jung HS, Choi YL, Choi JS, Roh JH, Pyon JK, Woo KJ, et al. Detection of Merkel cell polyomavirus in Merkel cell carcinomas and small cell carcinomas by PCR and immunohistochemistry. Histol Histopathol. 2011;26(10):1231–41.