DNA from Human Polyomaviruses, MWPyV, HPyV6, HPyV7, HPyV9 and HPyV12 in Cutaneous T-cell Lymphomas

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Abstract. *Background/Aim:* The etiopathogenesis of mycosis fungoides and Sézary syndrome remains obscure. Different viruses have been proposed to have a role in the etiopathogenesis of cutaneous T-cell lymphomas (CTCL). In the present study, the presence of five recently discovered human polyomaviruses 6 (HPyV6), human polyomaviruses 7 (HPyV7), human polyomaviruses 9 (HPyV9), human polyomaviruses 12 (HPyV12), and Malawi polyomavirus (MWPyV), have been analyzed in 55 CTCL in order to confirm the skin tropism and the possible pathological association of these new polyomaviruses. *Materials and Methods:* Human polyomaviruses DNA were amplified from skin lesions were recovered from a total of 55 patients (32 males and 23 females, average age 63±15 years) affected by CTCL. *Results:* When assayed for the presence of 5 different HPyVs, (HPyV6, HPyV7, HPyV9, MWPyV, and HPyV12) HPyV9, HPyV10 and HPyV12 DNA sequences were not found in any skin specimens. HPyV6 and 7 DNA was detected in 1/55 (1.8%) of skin specimens. *Conclusion:* The low-level presence of HPyV6 and HPyV7 DNA, and lack of detection of polyomaviruses HPyV9, MWPyV and HPyV12 in our series do not support a significant role of these HPyVs subtypes in the etiopathogenesis of skin cancers.

Cutaneous T-cell lymphoma (CTCL) is a group of malignancies derived from skin-homing T cells. Mycosis fungoides (MF) and Sézary syndrome (SS) are the most common CTCL variants. Mycosis fungoides (MF) is

characterized by longstanding, scaly patch lesions preferentially involving the buttocks and body areas infrequently exposed to sunlight. Disease progress is slow over years, from patches to plaques and eventually tumors or erythroderma. Lymph node and visceral involvement, as well as large cell transformation, usually occur in the late stages of the disease (1). Sézary syndrome (SS) is an erythrodermic cutaneous T-cell lymphoma with leukemic involvement, with an aggressive clinical behavior and poor prognosis (2, 3).

The etiopathogenesis of MF and SS remains obscure. Persistent antigen stimulation could lead to a continuous proliferation of T-cells and chronic inflammation and, ultimately, to the development of a malignant T-cell clone (4). Another hypothesis suggests that specific viral agents may serve as triggering factors (5). Different viruses have been suggested to have a role in the etiopathogenesis of CTCL, mainly the human T-cell leukemia virus and the Epstein-Barr virus (6, 7). Contradictory results have arisen from studies investigating the role of the Epstein-Barr virus in CTCL (8). Our group demonstrated in previous reports that the Epstein-Barr virus, parvovirus variants (B19, LaL1/K71, V9), human herpesvirus 7 (HHV-7), and human polyomaviruses (HPyVs) HPyV6, HPyV7 and TSPyV were not involved in CTCL pathogenesis (9-11).

More specifically, the first HPyVs, polyomavirus BK (BKPyV) and polyomavirus JC (JCPyV), were discovered in 1971, but it was not until 2007, that two more HPyVs, polyomavirus KI (KIPyV) and polyomavirus (WUPyV), were discovered in nasal aspirates (12, 13) followed by Merkel Cell polyomavirus (MCPyV), isolated from Merkel Cell Carcinomas (MCC) in 2008 (14). Since then, seven new HPyVs have been characterized from samples derived from the skin (Human polyomavirus 6 (HPyV6), human polyomavirus 7 (HPyV7) and Trichodysplasia Spinulosa polyomavirus (TSPyV)) (15-17), blood (human polyomavirus 9 (HPyV9)) (18, 19), feces (Malawi polyomavirus (MWPyV) and STL polyomavirus (STLPyV)) (20, 21), and from the gastrointestinal tract (human

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Key Words: Human polyomavirus, cutaneous T-cell lymphomas, mycosis fungoides, Sézary syndrome.

Table I. Primer and probe sequences used for the amplification of human polyomaviruses (HPyVs).

Target	Orientation	Sequences	Location
HPyV6	Sense	5'-AAGTGGGAAGTGCTGGATATATAAGAG-3'	KX379631.1
	Antisense	5'-TCCACAGGCCCAAAAGTACAT-3'	1624-1650
	Probe	FAM-CCCCTGCTGGTGTAGAAGGTTCCCA-TAMRA	1719-1699
HPyV7	Sense	5'-TGCTGCAGTGCAAGAAGTTACA-3'	1673-1697
	Antisense	5'-CCAAGGCCTCCCTCAACA-3'	KX771235.1
	Probe	FAM-CAAATGCAGCCTGCTACTATCCCTCCAA-TAMRA	2241-2262
HPyV9	Sense	5'-CTAGGGAACAATTTGAATATCAGGAA-3'	2315-2298
	Antisense	5'-ATAGTGTCCAGATCTAGGCTCTGAAC-3'	2269-2296
	Probe	FAM-AAGTTAGGCTGAGGCGGGAGATAGGG-TAMRA	HQ696595.1
MWPYV	Sense	5'-CATTGATGGACAGCCAATGG-3'	1227-1252
	Antisense	5'-TCCTGGAAGAGGTTCTGTTCCCTT-3'	1306-1281
	Probe	FAM-TGGGACTGATAATCAAGTACAGGATGTAAGTGTGT-TAMRA	1254-1279
HPyV12	Sense	5'-GTGGGAAGCTGTCAAGTGTGA-3'	JX262162.1
	Antisense	5'-CCACCTACTGCAAACATGTG-3'	2387-2406
	Probe	FAM-ACTACAGGATGGCCTACCCCATTTGTCAGTC-TAMRA	2468-2446

polyomavirus 12 (HPyV12)) (22). Like most HPyVs, they are also present in a large part of the general population, and their seroprevalence ranges from 20% to >90%, with HPyV9 being the less common among them (23-25). Moreover, some HPyVs, *i.e.*, BKPyV, JCPyV, MCPyV and TSPyV, have clearly been associated with specific diseases and cancer (26-27). In immunocompromised individuals, reactivation of BKPyV is associated with haemorrhagic cystitis, BKV nephropathy, and ureteral stenosis, while JCPyV is associated with progressive multifocal leukoencephalopathy and TSPyV associated with Trichodysplasia spinulosa, a rare skin disease (27). As noted above, MCPyV is a frequent cause of the skin malignancy Merkel cell carcinoma (MCC), mainly in immunosuppressed or older individuals (14). However, despite that only MCPyV has been associated with cancer, most other HPyVs express proteins that may potentially contribute to cancer development; all, except HPyV12, have putative binding sites for the retinoblastoma protein and many also for p53 (16,17, 19, 22).

Herein, the presence of five recently discovered HPyVs, HPyV6, HPyV7, HPyV9, HPyV12 and MWPYV, was analyzed in 55 CTCL in order to confirm the possible skin tropism and the possible pathological association of these new polyomaviruses with the development of CTCL.

Materials and Methods

Patients and samples. The present study was performed in compliance with the principles of good clinical practice and according to the principles of the Declaration of Helsinki. All the patients were included after providing their written informed

consent, and the study was approved by the Ethics Committee of the 'A.O.U. Città della Salute e della Scienza di Torino'. Cryostatic sections OCT-embedded (Tissue-TeK O.C.T. Compound, Sakura Eu, The Netherlands) from skin lesions were recovered from a total of 55 patients (32 males and 23 females, average age 63±15 years) affected by CTCL. All patients had been referred to the Section of Dermatology, at the Department of Medical Sciences of the University of Turin. Diagnosis was histologically confirmed; 43/55 patients had MF, 8/55 had SS; and 4/55 had primary cutaneous T-cell lymphoma (CTCL) non-MF/SS.

DNA extraction. OCT-sections were incubated overnight at room temperature with 500 µl of lysis buffer (100 mM NaCl, 10 mM Tris HCl pH8, 1 mM EDTA pH8, 1% SDS, 2% Triton X-100). The tubes were incubated at +100°C for 10 min. Equal volumes of phenol-chloroform were added to the sample and the tubes were centrifuged at 12,000 rpm for 10 min. Upper aqueous phase was transferred into fresh tubes, followed by adding 1 volume of ice cold ethanol, and incubated at -20°C for 1h and centrifuged at 12,000 rpm for 10 min at +4°C. The DNA pellets were separated from supernatants and washed twice with 70% ethanol; then, re-centrifuged at 12,000 rpm for 10 min and left to air-dry at room temperature. The pellets were dissolved in 20 µl of distilled water. The DNA samples were stored at -20°C until assayed.

Real-Time PCR. Primers and probes sets for HPyV detection are shown in Table I. Sets of primers and a probe targeting Small tumor antigen (STa), a part of early expressed genome of polyomaviruses, were designed using Primer Express Software Version 3.0 (Thermo Fisher, Paisley UK). The primers/probes concentration was 900 nM/250 nM for each target. Real-Time PCR assays were performed using the GoTaq qPCR MasterMix (Promega, Milano IT), on the 7500 Real-Time PCR System (Life Technologies Ltd)

Figure 1. Amplification plot of real-time Taqman PCR. Fluorescent signal was detected only for GAPDH gene.

instrument. The amplifications were run in a 96-well plate at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Amplification was set up in a final volume of 20 µl, including 5 µl of extracted clinical specimen, negative control (sterile double distilled water) or positive control (synthetic fragment containing the target region of amplification constructed by TwinElix (Rho, Milan, Italy)). Housekeeping gene GAPDH was used as internal control. Positive or negative polyomaviruses results were considered only in case of amplifiable GAPDH. Primer, probe and condition of GAPDH amplification were previously published (28).

Results

In total, 55 CTCL samples were analyzed using a Taqman Real time PCR on a 7500 ABI instrument. All of these shown internal control amplification (Figure 1).

In total, the presence of DNA corresponding to 5 different HPyVs (HPyV6, 7, 9, MWPyV, and HPyV12) was examined. HPyV9, HPyV10 and HPyV12 DNA sequences were not detected in any skin specimen.

HPyV6 and HPyV7 DNA sequences were found in 1/55 (1.8%) skin specimen. The patient positive for HPyV6 and HPyV7 had MF.

Discussion

The field of HPyV is emerging, and today there are 13 different HPyV types described, with the majority disclosed in the last 10 years. In the present study 55 CTCL samples, collected between 1999-2013, were analyzed for the presence of five different recently-discovered HPyVs: HPyV6, HPyV7, HPyV9, MWPyV and HPyV12.

HPyV6, HPyV7, HPyV9, and MWPyV are all in general common in the population and sequence data suggests they all are potentially oncogenic, by binding to Rb and p53. Thus, the possibility that one or more of these viruses are causative for a subset of tumors is a plausible hypothesis (29).

The tropism of these viruses is not well understood. HPyV6 and HPyV7 are, similarly to MCPyV, common on skin (30). HPyV9 is mainly, although rarely, found in the blood, but has also been isolated from skin (17) and in a serum sample from a kidney transplant patient (18). MWPyV, isolated from feces, has been detected in fecal samples from different regions with prevalence rates of 2-14% (19, 21) while isolated MWPyV was first described in the skin from a patient with warts, hypogammaglobulinaemia, infections and myelokathexis (WHIM) syndrome (31). Later studies have described the presence of MWPyV in forehead swabs of both human immunodeficiency virus (HIV)-infected and HIV-negative men, confirming skin tropism for this virus (32). HPyV12 was found in organs of the digestive tract, in particular the liver, but also in colon, rectum and stool (22). Many studies have identified HPyV DNA by PCR, which is a very sensitive method. Moreover, the presence of viral DNA provides no information on the activity of the virus. In spite of the high serological prevalence of the newly discovered HPyVs, the prevalence with regard to detection of viral DNA in most sample types is low (11).

In summary, the low-level presence of HPyV6 and HPyV7, and the lack of detection of HPyV9, MWPyV and HPyV12 polyomavirus in our series does not support a significant role of the studied HPyV subtypes in the etiopathogenesis of CTCL.

References

- 1 Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, Ralfkiaer E, Chimenti S, Diaz-Perez JL, Duncan LM, Grange F, Harris NL, Kempf W, Kerl H, Kurrer M, Knobler R, Pimpinelli N, Sander C, Santucci M, Sterry W, Vermeer MH, Wechsler J, Whittaker S and Meijer CJ: WHO/EORTC classification for cutaneous lymphomas. *Blood* 105: 3768-3785, 2005.
- 2 Olsen E, Vonderheid E, Pimpinelli N, Willemze R, Kim Y, Knobler R, Zackheim H, Duvic M, Estrach T, Lamberg S, Wood G, Dummer R, Ranki A, Burg G, Heald P, Pittelkow M, Bernengo MG, Sterry W, Laroche L, Trautinger F and Whittaker S: ISCL/EORTC: Revisions to the staging and classification of mycosis fungoides and Sézary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). *Blood* 110: 1713-1722, 2007.
- 3 Quaglino P, Pimpinelli N, Berti E, Calzavara-Pinton P, Alfonso Lombardo G, Rupoli S, Alaibac M, Bottoni U, Carbone A, Fava P, Fimiani M, Mamusa AM, Titli S, Zinzani PL and Bernengo MG: Gruppo Italiano Linfomi Cutanei: Time course, clinical pathways, and long-term hazards risk trends of disease progression in patients with classic mycosis fungoides: a multicenter, retrospective follow-up study from the Italian Group of Cutaneous Lymphomas. *Cancer* 118: 5830-5839, 2012.

- 4 Zucker-Franklin D and Pancake BA: The role of human T-cell lymphotropic viruses (HTLV-I and II) in cutaneous T-cell lymphomas. *Semin Dermatol* 13: 160-165, 1994.
- 5 Wilcox RA: Cutaneous T-cell lymphoma: 2017 update on diagnosis, risk-stratification, and management. *Am J Hematol*. 92: 1085-1102, 2017.
- 6 Novelli M, Merlino C, Ponti R, Bergallo M, Quaglino P, Cambieri I, Comessatti A, Sidoti F, Costa C, Corino D, Cavallo R, Ponzi AN, Fierro MT and Bernengo MG: Epstein-Barr virus in cutaneous T-cell lymphomas: evaluation of the viral presence and significance in skin and peripheral blood. *J Invest Dermatol* 129: 1556-1561, 2009.
- 7 Willemze R: Mycosis fungoides variants-clinicopathologic features, differential diagnosis, and treatment. *Semin Cutan Med Surg* 37: 11-17, 2018.
- 8 Gru AA and Jaffe ES: Cutaneous EBV-related lymphoproliferative disorders. *Semin Diagn Pathol* 34: 60-65, 2017.
- 9 Sidoti F, Fierro MT, Costa C, Ponti R, Bergallo M, Comessatti A, Fumagalli M, Novelli M, Merlino C, Cavallo R and Bernengo MG: Prevalence and significance of human parvovirus variants in skin from primary cutaneous T cell lymphomas, inflammatory dermatoses and healthy subjects. *Arch Dermatol Res* 301: 647-652, 2009.
- 10 Ponti R, Bergallo M, Costa C, Quaglino P, Fierro MT, Comessatti A, Stroppiana E, Sidoti F, Merlino C, Novelli M, Alotto D, Cavallo R and Bernengo MG: Human herpes virus 7 detection by quantitative real time polymerase chain reaction in primary cutaneous T-cell lymphomas and healthy subjects: lack of a pathogenic role. *Br J Dermatol* 159: 1131-1137, 2008.
- 11 Fava P, Merlino C, Novelli M, Ponti R, Galliano I, Montanari P, Tovo PA, Fierro MT and Bergallo M: HPyV6, HPyV7 and TSPyV DNA sequences detection in skin disease patients and healthy subjects. *J Eur Acad Dermatol Venereol* 30: 624-627, 2016.
- 12 Allander T, Andreasson K, Gupta S, Bjerkner A, Bogdanovic G, Persson MA, Dalianis T, Ramqvist T and Andersson B: Identification of a third human polyomavirus. *J Virol* 81: 4130-4136, 2007.
- 13 Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, Wu G, Brennan DC, Storch GA, Sloots TP and Wang D: Identification of a novel polyomavirus from patients with acute respiratory tract infections. *PLoS Pathog* 3: e64, 2007.
- 14 Feng H, Shuda M, Chang Y and Moore PS: Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 319: 1096-1100, 2008.
- 15 Schowalter RM, Pastrana DV, Pumphrey KA, Moyer AL and Buck CB: Merkel cell polyomavirus and two previously unknown polyomaviruses are chronically shed from human skin. *Cell Host Microbe* 7: 509-515, 2010.
- 16 van der Meijden E, Janssens RW, Lauber C, Bouwes Bavinck JN, Gorbelenya AE and Feltkamp MC: Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an immunocompromized patient. *PLoS Pathog* 6: e1001024, 2010.
- 17 Scuda N, Hofmann J, Calvignac-Spencer S, Ruprecht K, Liman P, Kuhn J, Hengel H and Ehlers B: A novel human polyomavirus closely related to the african green monkey-derived lymphotropic polyomavirus. *J Virol* 85: 4586-4590, 2011.
- 18 Sauvage V, Foulongne V, Cheval J, Ar Gouilh M, Pariente K, Dereure O, Manuguerra JC, Richardson J, Lecuit M, Burguiere A, Caro V and Eloit M: Human polyomavirus related to African green monkey lymphotropic polyomavirus. *Emerg Infect Dis* 17: 1364-1370, 2011.
- 19 Siebrasse EA, Reyes A, Lim ES, Zhao G, Mkakosya RS, Manary MJ, Gordon JI and Wang D: Identification of MW polyomavirus, a novel polyomavirus in human stool. *J Virol* 86: 10321-10326, 2012.
- 20 Lim ES, Reyes A, Antonio M, Saha D, Ikumapayi UN, Adeyemi M, Stine OC, Skelton R, Brennan DC, Mkakosya RS, Manary MJ, Gordon JI and Wang D: Discovery of STL polyomavirus, a polyomavirus of ancestral recombinant origin that encodes a unique T antigen by alternative splicing. *Virology* 436: 295-303, 2013.
- 21 Yu G, Greninger AL, Isa P, Phan TG, Martinez MA, de la Luz Sanchez M, Contreras JF, Santos-Preciado JI, Parsonnet J, Miller S, DeRisi JL, Delwart E, Arias CF and Chiu CY: Discovery of a novel polyomavirus in acute diarrheal samples from children. *PLoS ONE* 7: e49449, 2012.
- 22 Korup S, Rietscher J, Calvignac-Spencer S, Trusch F, Hofmann J, Moens U, Sauer I, Voigt S, Schmuck R and Ehlers B: Identification of a novel human polyomavirus in organs of the gastrointestinal tract. *PLoS ONE* 8: e58021, 2013.
- 23 Nicol JT, Leblond V, Arnold F, Guerra G, Mazzoni E, Tognon M, Coursaget P and Touze A: Seroprevalence of human Malawi Polyomavirus. *J Clin Microbiol* 52: 321-323, 2013.
- 24 Nicol JT, Robinot R, Carpentier A, Carandina G, Mazzoni E, Tognon M, Touze A and Coursaget P: Age-specific seroprevalences of merkel cell polyomavirus, human polyomaviruses 6, 7, and 9, and trichodysplasia spinulosa-associated polyomavirus. *Clin Vaccine Immunol* 20: 363-368, 2013.
- 25 van der Meijden E, Kazem S, Burgers MM, Janssens R, Bouwes Bavinck JN, de Melker H and Feltkamp MC: Seroprevalence of trichodysplasia spinulosa-associated polyomavirus. *Emerg Infect Dis* 17: 1355-1363, 2011.
- 26 Dalianis T and Hirsch HH: Human polyomaviruses in disease and cancer. *Virology* 437: 63-72, 2013.
- 27 Imperiale MJ and Jiang M: Polyomavirus Persistence. *Annu Rev Virol* 29: 517-532, 2016.
- 28 Bergallo M, Galliano I, Montanari P, Cambieri I, Fumagalli M, Casarin S, Ferravante A, Alotto D, Stella M and Castagnoli C: Absolute quantification of residual DNA in a new extracellular matrix derived from human reticular dermis (HADM) using real-time TaqMan® MGB-PCR. *Biomed Mater Eng* 29: 43-52, 2018.
- 29 Ramqvist T, Nordfors C, Dalianis T and Ragnarsson-Olding B: DNA from human polyomaviruses, TSPyV, MWPyV, HPyV6, 7 and 9 was not detected in primary mucosal melanomas. *Anticancer Res* 34: 639-643, 2014.
- 30 Ehlers B and Wieland U: The novel human polyomaviruses HPyV6, 7, 9 and beyond. *APMIS* 121: 783-795, 2013.
- 31 Buck CB, Phan GQ, Raiji MT, Murphy PM, McDermott DH and McBride AA: Complete genome sequence of a tenth human polyomavirus. *J Virol* 86: 10887, 2012.
- 32 Wieland U, Silling S, Hellmich M, Potthoff A, Pfister H and Kreuter A: Human polyomaviruses 6, 7, 9, 10 and Trichodysplasia spinulosa-associated polyomavirus in HIV-infected men. *J Gen Virol* 95: 928-932, 2014.

Received May 7, 2018

Revised May 18, 2018

Accepted May 23, 2018