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SHORT REPORT

HPyV6, HPyV7 and TSPyV DNA sequences detection in skin disease patients and healthy subjects

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Abstract

Background The discovery, from 2007, of eight new human polyomaviruses (HPyVs) has revived interest in thePolyomaviridae family and their association with human diseases and cancer. In particular, HPyV6 and HPyV7 were discovered in skin swabs of healthy donors and TSPyV was discovered in a heart transplant recipient affected by virusassociated Trichodysplasia Spinulosa (TS), a rare skin disease, exclusively found in immunocompromised patients.

Objective The presence of HPyV6, HPyV7 and TSPyV DNA in skin biopsies from patients affected by different skin diseases (cancers and inflammatory disorders) has been evaluated to confirm their skin tropism and the possible pathological association.

Methods DNA extracted was amplified with HPyV6, HPyV7 and TSPyV specific PCR real time on Taqman platform with standard profile.

Results HPyV7 and TSPyV sequences were not found in any skin specimen analysed. HPyV6, on the other hand, was detected in 30% of samples from healthy subjects vs. 14.3% of skin cancer patients and 2.9% of inflammatory disorders. HPyV6 sequences have been detected in primary cutaneous T-cell lymphoma (CTCL) patients (in 18.6% out of Mycosis Fungoides (MF) patients and in 16.7% out of CTCL not MF/SS(S□ezary syndrome) but have not been detected in primary cutaneous B-cell lymphoma (CBCL) patients.

Conclusion Our preliminary data suggest that these three novel human polyomaviruses seem not to play a significant role neither in the pathogenesis of cutaneous malignancies nor in that of inflammatory disorders but, according to literature, can inhabit the skin. On the basis of our data regarding the HPyV6 DNA presence with decreasing percentages in healthy subjects, skin cancer and inflammatory disorders patients, it could be an intriguing matter to study if the activated innate immune response in inflammatory disorders can suppress the virus. Further investigations are needed to better understand their relationship with the human host and its innate immune system.

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Introduction

The discovery, from 2007, of eight new human polyomaviruses (HpyVs) has revived interest in the Polyomaviridae family and their association with human diseases and cancer. As known,oncogenic transformation is mediated by the PyV early gene proteins called T (tumour)-antigen.1

In the mid of 2010, three new polyomaviruses have been discovered using random primers Rolling Circle Amplification (RCA) technology and named HPyV6, HPyV7 and TSPyV (Trichodysplasia Spinulosa Polyomavirus).2,3 In particular, HPyV6 and HPyV7 were discovered in skin swabs of healthy donors during a large-scale screening to explore the possibility that Merkel Cell Polyomavirus (MCPyV) (a novel polyomavirus found in about 80% of Merkel cell skin carcinomas) and other polyomaviruses commonly inhabit healthy human.2 TSPyV was discovered in a heart transplant recipient affected by virus-associated Trichodysplasia Spinulosa (TS), a rare skin disease, exclusively found in immunocompromised patients, characterized by the development of follicular papules and keratin spines known as spicules localized most striking in the face but in other parts of the body, as well. 3 The improvement of the clinical signs after antiviral treatment suggested a close causal relationship between TSPyV and disease. The presence of TSPyV also in clinical unaffected individuals indicated frequent virus transmission causing subclinical, probably latent infections.3 On the other hand, Ramqvist et al. 4 assaying biopsies from mucosal melanomas for the presence of 10 different human PyVs (BKV, JCV, KIPYv, WUPyV, TSPyV, HPyV6, 7, 9, MWPyV) and two simian PyVs (SV40 and LPyV), found that none of the samples were positive for any of these viruses.

In this study, the presence of HPyV6, HPyV7 and TSPyV DNA in skin biopsies from patients affected by different skin diseases (cancers and inflammatory disorders) has been evaluated and compared to that of healthy subjects to confirm the skin tropism and the possible pathological association of these new polyomaviruses.

Materials and methods

Cryostatic sections OCT-embedded (Tissue-TeK O.C.T. Compound, Sakura Eu, The Netherlands) from skin lesions were recovered from a total of 126 patients (57 males and 69 females, average age 55 ± 16 years) affected by neoplastic or inflammatory skin diseases. All patients referred to the Section of Dermatology of the Department of Medical Sciences of the University of Turin. Diagnosis was histologically confirmed.

Of the 126 (72.2%) patients, 95 presented neoplastic diseases: 59/91 mycosis fungoides (MF); 4/91 Sézary syndrome (SS), 12/91 primary cutaneous T-cell lymphoma (CTCL) non-MF/SS; 16/91 primary cutaneous B-cell lymphoma (CBCL). Thirty-five out of 126 (27,8%) was affected by various inflammatory skin disease including: systemic sclerosis (4/35); psoriasis (8/35); pityriasis lichenoides (PLEVA) (4/35); systemic lupus erythematosus (2/35); lichen planus (2/35); lichen simplex chronicus (13/35); lichen sclerosus (2/35). In inflammatory dermatosis, biopsy was carried out to exclude a CTCL.

Sections of OCT-embedded (Sakura Eu, The Netherlands) tissue from normal skin samples (n = 30) from healthy skin donors volunteers correlated by age and sex with the patients studied, kindly provided by the Department of Plastic Surgery, Burn Unit and Skin Bank, CTO Hospital of Turin, were used as control group.

Samples were homogenized using the rotor-stator homogenizer TissueRuptor (Qiagen, Germantown, MD, USA); subsequently incubated twice at 100°C for 10 min and centrifuged for 2 min at 16000 g to harvest the supernatant. Automated extraction of DNA was performed with the NucliSens easyMAG platform (bioMerieux, Marcy l'Etoile, France), according to the manufacturer's instructions. Primers and probes sets for HPyV6 and HPyV7 detection are shown in Table 1. Set of primers and probe targeting Small T region were designed using Primer Express Software Version 3.0 (Life Technologies Ltd, Paisley, UK). Primers and probe for TSPYV, targeting Large T region, were obtained from literature.3 The primers/probes concentration was 900 nM/250 nM for each target. Real-time PCR assays were performed using the Platinum qPCR SuperMix-UDG containing ROX as a passive reference (Life Technologies Ltd). Realtime PCR assays were performed using the 7500 real-time PCR System (Life Technologies Ltd) instrument with Lifetech standard thermal profile. Amplification was set up in a final volume of 20 lL, including 5 lL 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) from HPyV6, HPyV7 and TSPYV genome sequences (GenBank, accession no. NC_014406.1, NC_014407.1, NC_014361.1 respectively). The analytical sensitivity of each assay was 10 copies of HPyV6, 7 and TSPyV standard-plasmid DNA. Cross-reactivity was excluded by the addition of 10 000 and 1 000 000 copies of plasmid DNA of BKPyV, JCPyV, KIPyV, MCPyV, TSPyV, HPyV6 and 7 respectively. Positive results obtained by real-time PCR for HPyV6 were confirmed by sequencing procedure using BigDye® Terminator v1.1 Cycle Sequencing Kit (Life Technologies Ltd) and the same primers listed in Table 1. The sequencing

mix was made according to the manufacturers' instruction as follows: 8 lL of Termi-nator Ready reaction mix, 2 lL of PCR product, 3.2 pmol of primer and deionized water until 20 lL final volume. The cycle sequencing was carried out on 9800 Fast Thermal Cycler (Life Technologies Ltd) with an initial denaturation step at 96°C for 1 min, followed by 25 cycles of 96°C for 10 sec, 50°C for 5 sec and 60°C for 4 min. The Cycle Sequencing product was purified by Ethanol/EDTA/Sodium Acetate precipitation and loaded onto the ABI PRISM 31 Genetic Analyzer (Life Technologies Ltd). Forward and reverse sequences were aligned with the ClustalX software and then compared to HPyV6 sequences of the isolate 601a (GenBank, accession no. NC_014406.1).

Statistical analysis was performed using the Fisher's exact test with GraphPad Prism 5 Software. A p-value <0.05 was considered statistically significant.

Results

HPyV7 and TSPyV sequences were not found in any skin specimen from patients and healthy subjects analysed. HPyV6 sequences were found in 13 of 91 (14.3%) of skin cancer tissue samples. In particular, HPyV6 sequences were detected only in the primary cutaneous T-cell lymphoma (CTCL) group: 11 of 59 (18.6%) Mycosis fungoides (MF) and 2 of 12 (16. 7%) CTCL non-MF/SS. Regarding inflammatory skin conditions, HPyV6 sequences were found in only one of 35 (2.9%) skin sample. In particular, HPyV6 sequences were detected in one of 13 (7.7%) lichen simplex chronicus patient. By contrast, skin samples obtained from volunteers showed HPyV6 sequences in nine of 30 (30%) (Table 2).

Statistical analysis showed a significant negative association between HPyV6 DNA presence and inflammatory skin condition ($P = 0.0040$) and a negative association between HPyV6 DNA presence and skin cancer ($P = 0.0615$; n.s.) (Fig. 1).

Discussion and conclusion

Among of the six human polyomaviruses discovered from 2008,HPVy6, HPVy7 and TSPyV have been characterized from samples derived from the skin.2,3 Like most of HPyVs they are also present in a large part of general population and their seroprevalence range from 20% to >90%.5–7 In spite of the high serological prevalence, the prevalence with regard to detection of viral DNA in most samples types is low: HPVy6, HPVy7 and TSPyV DNA was found in a minority of respiratory and faecal samples from immunocompromised patients and healthy subjects (<1.3%).8The tropism of the new PyVs is not well understood, but HPVy6, HPVy7 and TSPyV, similar to MCPyV, are common on skin.3,9,10

So far, only MCPyV and TSPyV are known to be skin disease associated in immunocompromised patients and none of the polyomaviruses HPVy6, 7, 9 and TSPyV have been found to be associated with cancer development.1–3 Moreover, Ramqvist et al. 4 did not detect DNA from 10 human and two simian polyomaviruses, including HPVy6, 7, 9 and TSPyV, in primary mucosal melanoma patients.

In our study, HPyV 7 and TSPyV sequences were detected in skin samples neither from patients nor healthy subjects.

HPyV6, on the other hand, was detected in 30% of samples from healthy subjects vs. 14.3% of skin cancer patients and 2.9% of inflammatory disorders (Table 2). In particular, HPyV6 sequences have been detected in CTCL patients (in18.6% out of mycosis fungoides patients and in 16.7% out of CTCL not MF/SS) but have not been detected in CBCL patients (Table 2). Since the absence of HPyV 7 and TSPyV DNA sequences in all 126 samples tested and the higher prevalence of HPyV6 DNA sequences in healthy subjects vs. both skin cancer ($P = 0.0615$, n.s.) and inflammatory disorders ($P = 0.0040$) patients, our preliminary data suggest that these three novel human polyomaviruses seem to play a significant role neither in the pathogenesis of cutaneous malignancies nor in that of inflammatory disorders but, according to the literature, can inhabit the skin.

The innate immune system forms the first line of defence against infection. This defence system consists of pattern-recognition receptors, of which the Toll-like receptors (TLR) are best studied. TLR9, which senses viral infection, is a target of several DNA tumour viruses and human polyomaviruses have been shown to efficiently down-regulate TLR9 expression, like other oncogenic viruses such as Epstein–Barr virus, human papillomaviruses and Hepatitis B virus. Inhibition of TLR9 may facilitate the virus in establishing persistent infection.11 Given the increasing number of HPyVs and their known oncogenic and pathogenic potential,

further investigations are needed to better understand their relationship with the human host and its innate immune system.

On the basis of our data regarding the HPyV6 DNA presence (see above) with decreasing percentages in healthy subjects, skin cancer and inflammatory disorders patients, it could be an intriguing matter to study if the activated innate immune response in inflammatory disorders can eliminate the virus. To this aim, we intend to evaluate the TLR expression pattern related to the presence of HPyVs in a higher number of skin cancer and inflammatory disorders patients.

References

1 Dalianis T, Hirsch HH. Human polyomaviruses in disease and cancer. Virology 2013; 437: 63–72.

2 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: 509–515.

3 Van der Meijden E, Janssens RW, Lauber C, Bouwes Bavinck JN, Gorbalenya AE, Feltkamp MC. Discovery of a new human polyomavirus associated with trichodysplasia spinulosa in an immunocompromised patient. PLoS Pathog 2010; 6: e1001024.

4 Ramqvist T, Nordfors C, Dalianis T, Ragnarsson-Olding B. DNA from human polyomaviruses, TSPyV, MWPyV, HPyV6, 7 and 9 was not detected in primary mucosal melanomas. Anticancer Res 2014; 34: 639–644.

5 Van der Meijden E, Kazem S, Burgers MM et al. Seroprevalence of trichodysplasia spinulosa-associated polyomavirus. Emerg Infect Dis 2011; 17: 1355–1363.

6 Van der Meijden E, Bialasiewicz S, Rockett RJ, Tozer SJ, Sloots TP, Feltkamp MC. Different serologic behavior of MCPyV, TSPyV, HPyV6, HPyV7 and HPyV9 polyomaviruses found on the skin. PLoS ONE 2013; 8: e81078.

7 Nicol JT, Robinot R, Carpentier A et al. Age-specific seroprevalences of merkel cell polyomavirus, human polyomaviruses 6, 7 and 9, and trichodysplasia spinulosa-associated polyomavirus. Clin Vaccine Immunol 2013; 20: 363–368.

8 Rockett RJ, Slotts TP, Bowes S et al. Detection of novel polyomaviruses, TSPyV, HPyV6, HPyV7, HPyV9 and MWPyV in feces, urine, blood, respiratory swabs and cerebrospinal fluid. PLoS ONE 2013; 8: e62764.

9 Ehlers B, Wieland U. The novel human polyomaviruses HPyV6, 7, 9 and beyond. APMIS 2013; 121: 783–795.

10 Wieland U, Silling S, Hellmich M, Potthoff A, Pfister H, Kreuter A. Human polyomaviruses 6, 7, 9, 10 and Trichodysplasia spinulosa-associated polyomavirus in HIV-infected. J Gen Virol 2014; 95: 928–932.

11 Moens U, Van Ghelue M, Ehlers B. Are human polyomaviruses co-factors for cancers induced by other oncoviruses? Rev Med Virol 2014; 24: 343–360.

Table 1 Primers and probe used for the amplification of HPyV6 and HPyV7

Target	Primers/Probes	Orientation	Sequences	Location
HPyV6	P6FST	Sense	5′-ACCAGAGACAGTAGCACTTGTAGCA-3′	4457–4482*
	P6RST	Antisense	5′-GCAAGTGGAGATTAATTTAGGAAAGC-3′	4531–4505*
	P6probeST		5′-6FAM-AGAGGCCCCCGTTGG-MGB-3′	4483–4496*
HPyV7	P7FST	Sense	5′-CCAGCATTTGCCCCATGA-3′	4496–4514†
	P7RST	Antisense	5′-AAGAAGGCCAAAGAGTAGC-3′	4556–4536†
	P7probeST		5′-6FAM-AGAGGCCCCCGTTGG-MGB-3′	4515–4530†

*The location of the primers and probe refers to the nucleotide sequence Polyomavirus HPyV6 isolate 601a.

†The location of the primers and probe refers to the nucleotide sequence Polyomavirus HPyV7 isolate 707b.

Table 2 Prevalence of HPyV6, HPyV7 and TSPyV DNA in skin disease patients and healthy subjects

	No. of cases	HPyV6 DNA positive cases	HPyV7 DNA positive cases	TSPyV DNA positive cases
SKIN CANCERS (Total)	91	13/91 (14.3%)	ND	ND
Mycosis fungoides	59	11/59 (18.6%)	ND	ND
S. of Sézary	4	ND	ND	ND
Cutaneous T-cell lymphomas (CTCL) non-MF/SS	12	2/12 (16.7%)	ND	ND
Cutaneous B-cell lymphomas (CBCL)	16	ND	ND	ND
Inflammatory Skin Diseases (Total)	35	1/35 (2.9%)	ND	ND
Systemic sclerosis	4	ND	ND	ND
Psoriasis	8	ND	ND	ND
Systemic Lupus Erythematosus	2	ND	ND	ND
Pityriasis Lichenoides (PLEVA)	4	ND	ND	ND
Lichen planus	2	ND	ND	ND
Lichen simplex chronicus	13	1/13 (7.7%)	ND	ND
Lichen sclerosus	2	ND	ND	ND
Healthy Controls	30	9/30 (30%)	ND	ND

ND, Not Detected.

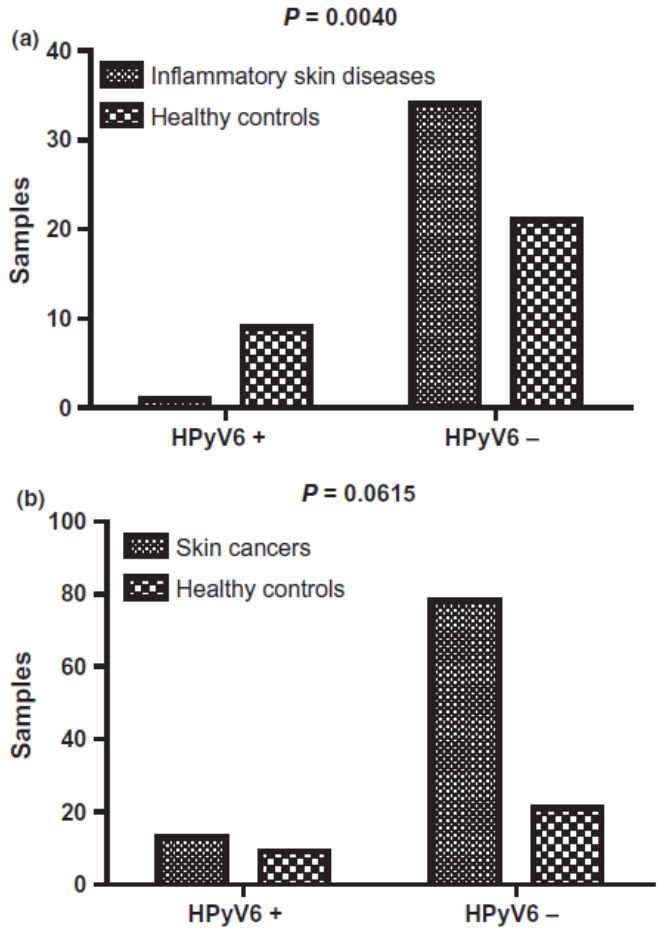

Figure 1 HPyV6 DNA presence in skin biopsies: comparison (Fisher's exact test) between healthy control patients vs. inflammation disease (a) and oncologic disease (b) patients.