DERMATOPATHOLOGY

Human polyomavirus 6 and 7 are associated with pruritic and dyskeratotic dermatoses

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Background: Human polyomavirus (HPyV)6 and HPyV7 are shed chronically from human skin. HPyV7, but not HPyV6, has been linked to a pruritic skin eruption of immunosuppression.

Objective: We determined whether biopsy specimens showing a characteristic pattern of dyskeratosis and parakeratosis might be associated with polyomavirus infection.

Methods: We screened biopsy specimens showing "peacock plumage" histology by polymerase chain reaction for HPyVs. Cases positive for HPyV6 or HPyV7 were then analyzed by immunohistochemistry, electron microscopy, immunofluorescence, quantitative polymerase chain reaction, and complete sequencing, including unbiased, next-generation sequencing.

Results: We identified 3 additional cases of HPyV6 or HPyV7 skin infections. Expression of T antigen and viral capsid was abundant in lesional skin. Dual immunofluorescence staining experiments confirmed that HPyV7 primarily infects keratinocytes. High viral loads in lesional skin compared with normal-appearing skin and the identification of intact virions by both electron microscopy and next-generation sequencing support a role for active viral infections in these skin diseases.

Limitation: This was a small case series of archived materials.

Conclusion: We have found that HPyV6 and HPyV7 are associated with rare, pruritic skin eruptions with a distinctive histologic pattern and describe this entity as "HPyV6- and HPyV7-associated pruritic and dyskeratotic dermatoses." (J Am Acad Dermatol http://dx.doi.org/10.1016/j.jaad.2016.11.035.)

Key words: dyskeratosis; HIV/AIDS; human polyomavirus 6; human polyomavirus 7; immunosuppression; organ transplantation; parakeratosis; polyomavirus; pruritus.

Human polyomaviruses (HPyVs) were first described in 1971, when JC polyomavirus and BK polyomavirus were identified in immunosuppressed individuals with ureteral obstruction and progressive multifocal leukoencephalopathy.[1,2] In the past decade, an additional 11 HPyVs were described.[3] Of these, several appear to reside chronically in human skin: Merkel cell polyomavirus (MCV), trichodysplasia spinulosa polyomavirus, HPyV6, and HPyV7. MCV was discovered within, and was strongly linked to the pathogenesis of, a rare but deadly skin malignancy, Merkel cell

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carcinoma.[4] Trichodysplasia spinulosa polyomavirus was linked to a folliculocentric eruption first described in an immunosuppressed individual.[5,6]

HPyV6 and HPyV7 are closely related polyomavirus species first identified through rolling circle amplification of DNA isolated from swabs of healthy human skin.[7] They are thought to infect the skin in a latent or subclinical manner in the majority of people. In healthy individuals with clinically normal-appearing skin, previous studies detected HPyV6 and HPyV7 sequences from skin swabs in 14% to 28% and 11% to 13% of samples, respectively.[7,8] Less is known about skin diseases associated with HPyV6 and HPyV7. Recent studies revealed that HPyV7 could infect and actively replicate in biopsy specimens taken from immunosuppressed lung transplant recipients. In these patients, the skin infection presented as pruritic, scaly, brown plaques. Biopsy specimens from lesional skin showed a characteristic pattern of parakeratosis described as "peacock plumage."[9] In contrast to HPyV7, HPyV6 has not yet been linked with specific skin disease. Low levels of HPyV6 DNA were detected in several types of epithelial neoplasms and a contribution of HPyV6 to these neoplasms has not been excluded.[10,11]

CAPSULE SUMMARY

- The contribution of human polyomaviruses 6 and 7 to skin diseases remains unclear.
- Novel strains of human polyomaviruses 6 and 7 are associated with pruritic dermatoses showing dyskeratosis and irregular columns of parakeratosis on histology.
- Human polyomavirus 6- and 7-associated pruritic and dyskeratotic dermatoses should be considered in immunosuppressed patients. Their identification could facilitate the characterization and treatment of these diseases.

We identify biopsy specimens showing a characteristic pattern of dyskeratosis and parakeratosis, previously described as "peacock plumage," for HPyV7 skin infections and investigate whether polyomavirus infections might be associated with these eruptions. We identify HPyV6 and HPyV7 infection in 3 additional patients with pruritic dermatoses and provide evidence for the involvement of these viruses in the pathogenesis of the eruptions.[12,13] Our work expands the spectrum of skin diseases associated with HPyV6 and HPyV7 and yields novel insights into the biology of these ubiquitous skin polyomaviruses.

METHODS

This was a retrospective case series of archived skin biopsy specimens. Histologically normal-appearing skin and archived biopsy samples were obtained through an institutional review board–exempt protocol. For patient B, written, informed consent was obtained for collecting skin swabs for diagnostic and research purposes.

HPyV polymerase chain reaction

Formalin-fixed, paraffin-embedded (FFPE) sections were deparaffinized with xylene (Sigma, St Louis, MO) and DNA was extracted using the QIAamp DNA FFPE tissue kit (Qiagen, Hilden, Germany). Typically, polymerase chain reaction (PCR) was performed on 20 ng of genomic DNA with polyomavirus screening primers (Supplemental Table I[7,9,14-17]; available at http://www.jaad.org). Quantitative PCR (qPCR) was used to determine the copy number of HPyV6, HPyV7, and MCV using SYBR green (Bio-Rad, Hercules, CA) and primers targeting the small T-antigen region. Long interspersed nuclear elements (LINE-1) primers were used as a normalization reference. Anonymized skin biopsy specimens from 8 patients with histologically normal-appearing skin were assessed by qPCR as a control.

Histology, immunohistochemistry, and immunofluorescence studies

FFPE sections (5 μm) underwent xylene deparaffinization, rehydration, antigen retrieval, and blocking. These slides were stained overnight at 4°C with 6V32 antibody (1:100, Buck Lab, Bethesda, MD) to detect HPyV6 and HPyV7 viral capsid protein, 2t10t (1:100, Buck Lab) for HPyV7 small T antigen, or 1t1 (1:200, Buck Lab) for HPyV6 small T antigen. Slides were then stained with appropriate secondary antibody conjugated to horseradish peroxidase (Santa Cruz Biotechnology, Dallas, TX). Lastly, the slides were developed with the Vector VIP peroxidase substrate kit (Vector Laboratories, Burlingame, CA). For costaining experiments, slides were incubated with HPyV7 antibodies and rabbit anti-cytokeratin (CK)10 (clone EP1607IHCY, 1:10,000, Abcam, Cambridge, UK), rabbit anti-CK14 (catalog no. PA5-28002, 1:5,000, Thermo Fisher Scientific, Waltham, MA),