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Isolation and characterization of a novel putative human polyomavirus

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ABSTRACT

The small double-stranded DNA polyomaviruses (PyVs) form a family of 73 species, whose natural hosts are primarily mammals and birds. So far, 13 PyVs have been isolated in humans, and some of them have clearly been associated with several diseases, including cancer. In this study, we describe the isolation of a novel PyV in human skin using a sensitive degenerate PCR protocol combined with next-generation sequencing. The new virus, named Lyon IARC PyV (LIPyV), has a circular genome of 5269 nucleotides. Phylogenetic analyses showed that LIPyV is related to the raccoon PyV identified in neuroglial tumours in free-ranging raccoons.

Analysis of human specimens from cancer-free individuals showed that 9 skin swabs (9/445; 2.0%), 3 oral gargles (3/140; 2.1%), and one eyebrow hair sample (1/439; 0.2%) tested positive for LIPyV.

Future biological and epidemiological studies are needed to confirm the human tropism and provide insights into its biological properties.

1. Introduction

Members of the polyomaviruses (PyVs) are non-enveloped double-stranded DNA viruses with a genome of approximately 5000 nucleotides. The organization of the viral genome is highly conserved throughout the PyV family and comprises early and late coding regions and the viral non-coding control region (NCCR) of approximately 500 bp. The early region encodes for two regulatory proteins: small T-antigen (ST-Ag) and large T antigen (LT-Ag). The late region encodes for three viral proteins that are necessary for formation of the capsid: the major capsid protein VP1 and two minor capsid proteins, VP2 and VP3. The NCCR contains the origin of DNA replication, regulatory elements, and transcription promoters (Moens et al., 2008).

With the development of high-performance molecular biology tools, many of the PyVs have been isolated during the past decade, mainly from mammals, birds, and fish (Johne et al., 2011; Peretti et al., 2015). Based on the observed distance between LT-Ag coding sequences, the International Committee on Taxonomy of Viruses (ICTV) Polyomaviridae Study Group has classified the different species of PyVs into four genera: alpha-, beta-, gamma- and delta-PyV) (Calvignac-Spencer et al., 2016).

To date, a total of 13 PyVs have been isolated from humans: BKPyV (Gardner et al., 1971), JCPyV (Padgett et al., 1971), KIPyV (Allander et al., 2007), WUPyV (Gaynor et al., 2007), Merkel cell PyV (MCPyV) (Feng et al., 2008), human PyV 6 (HPyV6) (Schowalter et al., 2010), human PyV 7 (HPyV7) (Schowalter et al., 2010), trichodysplasia spinulosa-associated PyV (TSPyV) (van der Meijden et al., 2010), human PyV 9 (HPyV9) (Scuda et al., 2011), Malawi PyV (MWPyV) (Siebrasse et al., 2012), Saint Louis PyV (STLPyV) (Lim et al., 2013), human PyV 12 (HPyV12) (Korup et al., 2013) and New Jersey PyV (NJPyV) (Mishra et al., 2014). PyVs are widely spread in the human population. Many PyV infections occur early in life, and in most cases it remains asymptomatic (Nickeleit et al., 2015). Serological studies have shown that up to 90% of the human population has been exposed to HPyV, with several HPyV infections occurring during childhood (Egli et al., 2009; Kean et al., 2009; Sroller et al., 2016).

Four HPyVs have been clearly associated with human diseases, many occurring more frequently in immunocompromised individuals. JCPyV has been associated with progressive multifocal leukoencephalopathy, a fatal brain disease, in immunocompromised individuals (Jiang et al., 2009; Koralnik, 2006), and BKPyV has been associated

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with nephropathy and hemorrhagic cystitis (Azzi et al., 1994; Coleman et al., 1978), particularly among kidney transplant patients; MCPyV has been isolated from Merkel cell carcinomas of the skin (Feng et al., 2008), a cancer with higher incidence in immunocompromised individuals, and TSVPyV has been associated with a rare cutaneous condition, trichodysplasia spinulosa, in an immunocompromised patient (van der Meijden et al., 2010). A possible association between HPyV7 and non-neoplastic diseases in immunosuppressed individuals has also been reported recently (Ho et al., 2015; Toptan et al., 2016). In addition, HPyV7 has been found in human thymic epithelial tumours, but a causal association has not been established (Rennspiess et al., 2015). The remaining PyVs that have been isolated from humans, KIPyV, WUPyV, HPyV6, HPyV9, HPyV12, MWPyV, STLPyV and NJPyV, have not so far been associated with any human diseases.

The oncogenic potential of HPyVs has been extensively studied in experimental animal models, where these viruses induce a wide range of tumours. The inoculation of JCPyV in a small rodent model and in non-human primates leads to the development of brain tumours (Miller et al., 1984; Varakis et al., 1978; Walker et al., 1973; Zu Rhein et al., 1979). Transgenic mice expressing the early region of HPyVs have been used to investigate the carcinogenesis induced by MCPyV (Shuda et al., 2015; Verhaegen et al., 2015), JCPyV (Shollar et al., 2004), and BKPyV (Dalrymple et al., 1990). In addition, simian virus 40 (SV40), BKPyV, and JCPyV have been shown to display transforming activity in *in vitro* experimental models (Moens et al., 2008). It is still unclear whether other HPyVs exist. Here, we report the characterization of a new PyV isolated from human skin swabs. We found it to be phylogenetically related to the raccoon PyV (RacPyV) associated with brain tumours in free-ranging raccoons, and gave it the provisional name of Lyon IARC PyV (LIPyV).

2. Materials and methods

2.1. Human specimens

Skin swabs, eyebrow hairs and oral gargles from three different ongoing studies aiming to determine the prevalence of human papillomaviruses (HPVs) and HPyVs were used in the present analysis (Franceschi et al., 2015; Hampras et al., 2015, 2014; Nunes et al., 2016; Pierce Campbell et al., 2016, 2013). Skin swabs and eyebrow hairs were collected at baseline from 448 subjects participating in the VIRUSCAN study, an ongoing five-year (2014–2019) prospective cohort study being conducted at Moffitt Cancer Center and the University of South Florida (R01CA177586-01; “Prospective study of cutaneous viral infections and non-melanoma skin cancer”). In addition, 25 cutaneous skin swabs were randomly selected from the HPV Infection in Men (HIM) study, a large, multi-national prospective cohort study of the natural history of HPV infection in men. The 25 skin swabs were collected from men in Tampa, Florida, USA. The HIM study methods have been described in detail previously and are similar to those used in the VIRUSCAN Study (Giuliano et al., 2011). An area of approximately 5×5 cm of the top of the sun-exposed forearm was sprayed with 0.9% saline solution. A cotton-tipped Dacron swab (Digene, Gaithersburg, MD, USA) was then rubbed back and forth a few times to collect exfoliated skin cells. Individual swabs were placed in a separate vial and preserved in Digene Standard Transport Medium (STM). Three or four eyebrow hairs were plucked from each eyebrow using disposable tweezers. The eyebrow hairs with attached follicles were snap-frozen in liquid nitrogen and stored at −80 °C until further use.

We used 140 oral gargles that were collected for the Study of Natural History of HPV Infection and Precancerous Lesions in the Tonsils (SPLIT), which is an ongoing study on the detection of HPV infection and precancerous lesions in age-stratified immunocompetent individuals who underwent tonsillectomy for benign diseases in selected university hospitals across France (Combes et al., In press; Franceschi et al., 2015).

After DNA extraction, all samples were analysed at the International Agency for Research on Cancer (Lyon, France) for HPVs and all known HPyVs.

2.2. Design of degenerate primers and PCR conditions for PyV screening

Complete HPyV sequences were obtained from GenBank and were used for alignment of the early region genes. A pair of degenerate primers was developed based on the more conserved parts of LT-Ag of several PyV genomes. The accession numbers of the GenBank sequences that were used as references, with the corresponding HPyV types given in parentheses, are EU37584 (MCPyV), NC_001538 (BKPyV), NC_001669 (SV40), EF520287 (KIPyV), NC_009539 (WUPyV), and NC_001699 (JCPyV). Two oligonucleotides (forward primer, 5′-CAW GCT GTR TIT AGT AAT A-3′ and reverse primer, 5′-RWT TAT TMA CHC CIT TAC-3′), allowing the amplification of a region of approximately 240 bp, were synthesized by MWG Biotech (Ebersberg, Germany). The polymerase chain reaction (PCR) mix contained 1x PCR buffer, 200 µmol/L of each dNTP, 0.2 µmol/L of each primer, and 0.625 U of HotStarTaq DNA polymerase in a final volume of 25 µL (Qiagen). Forty-five amplification cycles were run in the GeneAmp PCR System 2400 with a 94 °C denaturation step (1 min), a 48 °C annealing step (1 min), and a 72 °C extension step (1 min), including an initial denaturation step of 15 min and a final extension step of 10 min, resulting in a 240-bp product.

2.3. Next-generation sequencing

The libraries were prepared using 50 ng of the PCR products with DNA NEBNext Fast DNA Library Prep Set for Ion Torrent (New England Biolabs, Ipswich, MA, USA) following the manufacturer’s protocol, and sequenced with the Ion Torrent PGM sequencer (Life Technologies) at 100x coverage using the Ion OneTouch 200 Template Kit v2 DL and the Ion PGM Sequencing 200 Kit v2 with the 314 or 316 chip kits (all produced by Life Technologies), following the manufacturer’s instructions. The data analysis was conducted using Geneious version 6.0.1 (http://www.geneious.com) (Kearse et al., 2012).

2.4. Luminex assay for high throughput screening of LIPyV

As described previously, LIPyV DNA from eyebrow hairs, and skin swabs was detected using a highly sensitive and specific assay which combines multiplex PCR and bead-based Luminex technology (Schmitt et al., 2006, 2010). The following PCR primers and Luminex probe were used: forward primer, 5′-CAA GCC TTG CTG CAG CAT TCC TAG-3′ and reverse primer, 5′-ATC TTT GTT TTG TCC TCT AGA ACC CT-3′; and probe, 5′-ATC TAT CTT GGG GGC AAT-3′. Briefly, PCR products were denatured and hybridized to the beads coupled with specific probes for LIPyV. Results were expressed as the median fluorescence intensity (MFI) of at least 100 beads per bead set. For each probe, MFI values with no respective PCR product added to the hybridization mixture were considered background values. The cut-off was computed by adding 5 MFI to 1.1x the median background value.

2.5. Rolling circle amplification

DNA was extracted and purified from skin swabs as described previously (Schowalter et al., 2010). The DNA was amplified by multiply primed rolling circle amplification (RCA) using the Illustra TempliPhi 100 Amplification Kit according to the manufacturer’s recommendations (GE Healthcare, Piscataway, NJ), with supplementation of 450 µM dNTPs as described by Rector et al. (2004).

2.6. Long-range PCR

Long-range PCR was performed for amplification of the entire