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Genetic Analysis of Human Adenovirus Type 54 Detected in Osaka, Japan

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Human adenoviruses (HAdVs) are double-stranded DNA viruses that belong to the genus Mastadenovirus of the family Adenoviridae and cause a range of diseases such as respiratory infection, gastroenteritis, and keratoconjunctivitis. HAdVs are grouped into 7 species (HAdV-A to HAdV-G) with 56 types (1–8). Several HAdVs are known to cause adenoviral keratoconjunctivitis, including HAdV-3 (HAdV-B), HAdV-4 (HAdV-E), HAdVs-8, -19, and -37 (HAdV-D), and the recently identified types HAdV-53, -54, and -56 (HAdV-D) (4–8). HAdV-54 is genetically similar to HAdV-8 and is associated with inflammation of the cornea and conjunctiva (8). HAdV-54 was first detected in 2000 in Hyogo, a prefecture neighboring Osaka. Since then, it has been reported in nearly all regions of Japan (8–12); however, the epidemiology of these viruses, particularly in Osaka, remains unclear.

Although HAdV typing has classically been done by neutralization test, new HAdV types are difficult to classify serologically because type-specific antiserum is not available. Therefore, we used the newer and more efficient method of analyzing nucleotide sequences, which is especially valuable for the detection of new HAdVs (13–17). HAdV type classification is based on the sequences of the main type-specific epitopes within loops 1 and 2 of the hexon protein, which contain 7 hypervariable regions, and the minor type-specific epitopes of the fiber protein (17–19). The hexon gene is divided into 4 conserved (C1–C4) and 3 variable (V1–V3) regions (20). Since the C4 region is conserved across different types (20), we used this region to detect HAdVs, as reported previously (16), and then determined the nucleotide sequences of the hexon and fiber genes to identify the type of the HAdV strains.

A conjunctival swab was obtained from each of the 12 patients with keratoconjunctivitis at a hospital in Osaka from September 2010 to November 2010. Then 200 μL of the swabbed solution were inoculated into both A549 and HEp-2 cell cultures in 24-well microplates for virus isolation. If cytopathic effects (CPE) were not observed within 1 week of inoculation, 200 μL of the culture medium was passaged once a week and observed until day 28 after inoculation. Viral DNA was then extracted from 150 μL of the fluid from the CPE-positive cell cultures using an E.Z.N.A.™ Total RNA Kit (OmegaBio-Tek, Madison, Wiss., USA). DNA was also extracted from 3 preserved HAdV strains that had been isolated from conjunctivitis patients in 2000, 2002, and 2003 and serologically typed as HAdV-
Polymerase chain reaction (PCR) was performed in a reaction volume of 25 μL with puRe Taq Ready-To-Go PCR Beads (GE Healthcare, Little Chalfont, UK). 1 μL of template DNA and 2 μM of primer using previously described methods (14–17). Briefly, a partial sequence of the C4 region was amplified for HAdV detection and typing, and then the sequences of the loops in the hexon and fiber genes were amplified. Then, the PCR products were purified, the nucleotide sequences were analyzed with an Applied Biosystems 3130 genetic analyzer (Applied Biosystems, Foster City, Calif., USA), and the sequence data were aligned using CLUSTAL W in the MEGA 5 software package (Molecular Evolutionary Genetics Analysis software) (21).

In total, 7 HAdV strains were isolated from the A549 cells: 220309/Osaka/2010, 220325/Osaka/2010, 220327/Osaka/2010, 220328/Osaka/2010, 220334/Osaka/2010, and 220335/Osaka/2010. CPE emerged on the second or third subculture in 6 strains, while the remaining 1 strain showed such effects after the third blind passage. No virus was isolated using HEp-2 cells.

Nucleotide sequence analysis of 350 bp of the C4 region (nucleotide position 19608–19957 in GenBank accession no. AB333801), loops 1 and 2 in hexon (nucleotide positions 18098–18626 and 18787–19067, respectively), and the fiber gene (nucleotide position, 30714–31799) indicated that 220325/Osaka/2010, 220327/Osaka/2010, 220328/Osaka/2010, and 220335/Osaka/2010 had sequences identical to 220309/Osaka/2010. These results indicate that antiserum specific for HAdV-8 shared 100% identity with 220309/Osaka/2010. These results indicate that antiserum specific for HAdV-8 cross-reacts with HAdV-8 antisera (8), we also analyzed 3 HAdV strains that had been previously isolated and serologically typed as HAdV-8. A similar analysis of the hexon and fiber genes revealed that 151013/Osaka/2003 (GenBank accession nos. AB665056 to AB665059) and shared 100% identity with HAdV-54 (GenBank accession no. AB333801), which was isolated in 2000 in Kobe, Japan (8). Because HAdV-54 is antigenically similar to HAdV-8 and reacts with HAdV-8 antisera (8), we also analyzed 3 HAdV strains that had been previously isolated and serologically typed as HAdV-8.

A similar analysis of the hexon and fiber genes revealed that 151013/Osaka/2003 (GenBank accession nos. AB665060 to AB665063) was actually HAdV-54 and shared 100% identity with 220309/Osaka/2010. These results indicate that antiserum specific for HAdV-8 cross-reacts with HAdV-54, leading to identification of the virus as HAdV-8 in a neutralization test. In contrast, genetic analysis proved to be a more powerful means to distinguish virus types, and showed that HAdV-54 has been in Osaka since at least 2003.

In 2010, HAdV-54 caused adenoviral keratoconjunctivitis cases in Osaka, and we found evidence of its spread in nearly all regions of Japan (8,9). Our results indicate that HAdV-54 emerged in Osaka as early as 2003, and the nucleotide sequence of the virus was conserved during the intervening years from 2003 to 2010. Furthermore, we found that isolation of HAdV-54 in A549 cell culture monolayers required at least 28 days, supporting previous results, which showed that isolation of HAdV-54 requires more time than that needed to isolate HAdV-3, HAdV-4, HAdV-19, or HAdV-37 (22).

According to the National Surveillance data on HAdVs in Japan (http://idsc.nih.go.jp/iasr/prompt/s2graph-ke.html), HAdV-8 was detected from epidemic keratoconjunctivitis cases every year during the period from 2007 to 2010, although the data for HAdV-54 have not been determined. However, because HAdV-54 has been occasionally identified as HAdV-8, and only a few prefectoral and municipal public health institutes have reported detection of HAdV-54 (10–12), surveillance for new HAdV types was insufficient to elucidate its epidemiology throughout Japan. HAdV-54 has only been identified in Japan, and re-typing of HAdV strains isolated between 1990 and 2009 revealed that, since 1995, the number of HAdV-8 cases has decreased while the number of HAdV-54 cases has increased (22). Although we have a stock HAdV-8 strain isolated in 1983, most of the HAdV-D strains preserved in our laboratory were isolated after 2000, and neither HAdV-8 nor HAdV-54 were isolated between 1984 and 2009 except the HAdV-54 strain in 2003. Therefore, precise determination of when HAdV-54 emerged in Osaka has been difficult. HAdV-54 strains that were isolated between 2000 and 2005 from 8 prefectures, including Hyogo Prefecture, had nucleotide sequences that were identical to each other (8) and to the strains reported in this study, indicating that genetic variations within the hexon and fiber genes of HAdV-54 have yet to be found. Although viral genome mutations are difficult to predict, variations in the hexon and fiber genes from HAdV-8 and HAdV-37 were found during epidemics of adenoviral keratoconjunctivitis (23,24). Likewise, if HAdV-54 causes a large keratoconjunctivitis epidemic, it might be due to appearance of new mutant variants.

In the present study, we confirmed the spread of HAdV-54 in Osaka, and verified that for the classification of new HAdV types, genetic analysis is more effective than serological analysis, and is required for the precise typing of isolated adenoviruses. Continuous surveillance will be necessary to clarify the epidemiology of adenoviral keratoconjunctivitis, and identification of the alterations in HAdVs over time will require both previously isolated strains as well as those isolated in the future.

Conflict of interest None to declare.

REFERENCES