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Full genome sequence analysis of a novel adenovirus of rhesus macaque origin indicates a new simian adenovirus type and species

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ABSTRACT

Multiple novel simian adenoviruses have been isolated over the past years and their potential to cross the species barrier and infect the human population is an ever present threat. Here we describe the isolation and full genome sequencing of a novel simian adenovirus (SAdV) isolated from the urine of two independent, never co-housed, late stage simian immunodeficiency virus (SIV)-infected rhesus macaques. The viral genome sequences revealed a novel type with a unique genome length, GC content, E3 region and DNA polymerase amino acid sequence that is sufficiently distinct from all currently known human- or simian adenovirus species to warrant classifying these isolates as a novel species of simian adenovirus. This new species, termed Simian mastadenovirus D (SAdV-D), displays the standard genome organization for the genus *Mastadenovirus* containing only one copy of the fiber gene which sets it apart from the old world monkey adenovirus species HAdV-G, SAdV-B and SAdV-C.

1. Introduction

The family Adenoviridae comprises a large family of non-enveloped double stranded DNA viruses. It can be subdivided into five genera which contain a plethora of viruses infecting a wide range of hosts including

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mammals, birds, fish, and amphibians (Wold and Horwitz, 2007). The genus *Mastadenovirus* includes 68 human types identified to date which divide into 7 known species (A–G) (Harrach et al., 2011). These types differ greatly in their tropism and pathogenicity, but they are generally known to infect the human respiratory-, urinary- or gastrointestinal tract or the conjunctiva causing a range of medical conditions including pneumonia, croup, bronchitis, keratoconjunctivitis, gastroenteritis and cystitis (Jones et al., 2007; Lewis et al., 2009; Louie et al., 2008; Saad et al., 1997; Treacy et al., 2010; Walsh et al., 2009; Wold and Horwitz, 2007; Wood, 1988). Adenoviral infections are usually innocuous, but can be serious in immunocompromised patients such as AIDS patients (Echavarria, 2008). Adenovirus types isolated from great apes are most closely related to human types and cluster together with them within the human adenovirus (HAdV) species A–F (Roy et al., 2009). In contrast, simian adenoviruses (SAdVs) of old-world monkey (OWM) origin form separate species (A–C). Although adenoviruses are generally considered to be species specific, zoonotic infections have been reported (Gillespie et al., 2008; Wolfe et al., 2007). The recently described human type 52 (HAdV-52), the founding and so far only human type of the Human mastadenovirus species G (HAdV-G), was isolated from the stool of five different patients during an outbreak of gastroenteritis in Los Angeles county (Jones et al., 2007). Interestingly, although considered a human species, HAdV-G also includes multiple types isolated from OWM and no further types of human or great ape origin (Chiu et al., 2013; Roy et al., 2012; Wevers et al., 2011). HAdV-52 is thus likely of monkey origin and could therefore represent a documented case of an OWM simian adenovirus crossing the species barrier to cause disease in humans.

Besides being important human and animal pathogens, adenoviruses are also widely used as vectors in vaccine development and gene therapy. Human adenovirus 5 (HAdV-5) is by far the most widely used vector but it showed poor efficacy in HIV-1 clinical trials (Buchbinder et al., 2008; McElrath et al., 2008) presumably due to the fact that this type is widespread in the human population and preexisting humoral immune responses against HAdV-5 impair the immunogenicity of HAdV-5-vectored vaccines (Casimiro et al., 2003; Catanzaro et al., 2006; McElrath et al., 2008; Priddy et al., 2008). To bypass this problem animal adenoviruses are being developed as alternative to human vaccine vectors. Although simian adenoviruses of great ape origin are preferred because they belong to the same species as their human counterpart without showing significant seroprevalence in the human population (Dicks et al., 2012) adenoviruses of OWM origin could also conceivably be considered since they have shown the capability to naturally cross the species barrier and infect human individuals.

Here we describe the isolation and full genome analysis of a novel simian adenovirus type of rhesus macaque origin. Full genome analysis of our isolate using NextGen sequencing revealed a genome sequence substantially different from all previously described human and simian types and species. Therefore, we classify our novel simian adenovirus type as a novel species of simian adenoviruses termed Simian mastadenovirus D (SAdV-D).

2. Results

2.1. Isolation of simian adenoviruses 26296 and 23336

Rhesus macaques (RM) suffering from AIDS like symptoms demonstrate an SIV-associated expansion of the enteric virome including the increased appearance of multiple adenoviruses in the gastrointestinal tract (Handley et al., 2012). To determine whether SIV-infected RM secreted novel viruses in their urine we collected urine from several late stage SIV_{mac239}-infected RM and spinoculated primary rhesus fibroblasts with the fresh samples. The cells were monitored in regular intervals and most samples developed owl eyed plaques within 1–2 weeks of infection indicative of cytomegalovirus (CMV) (data no shown). This observation was consistent with close to 100% of the animals in our cohort being naturally CMV^{+}. However, one viral isolate showed a different phenotype of infection (Fig. 1A) with infected cells resembling human foreskin fibroblasts infected with HAdV-5 (Rothmann et al., 1998). In a second experiment, samples were frozen prior to spinoculation, a process that should reduce live CMV contamination, and a second viral isolate with similar phenotype was obtained (Fig. 1B).

To examine whether these newly isolated viruses were adenoviruses, we isolated DNA from primary rhesus fibroblasts inoculated with viral isolates from the urine of late stage-SIV infected rhesus macaques (19262, 19936, 23336, 24514 and 26296) or RhCMV strains 68-1 and UCD59 as positive RhCMV controls and performed diagnostic PCRs with primers for RhCMV gO, for the adenovirus hexon protein (Roy et al.,