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Short Communication

Evolutionary relationships of the hexon and penton base genes of novel squirrel adenovirus

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ABSTRACT

Squirrel adenovirus (SqAdV) was reported previously. However, only partial sequences of its hexon and polymerase genes have been revealed. For the first time, we report the full-length genome of SqAdV including the complete hexon and penton base genes. From internal body organs of 59 red squirrels archived in Korea Bank for Pathogenic Viruses, the hexon, penton base, and full-length genome of SqAdV were determined by a PCR method. Of the internal body organs examined, the spleen showed the highest detection rate (25.42%) for SqAdV whereas the kidney and lung exhibited 18.64% and 3.39% rates, respectively. Based on the phylogenetic relationships of the hexon and penton base genes, SqAdV appears to belong to the genus *Mastadenovirus*, and, at least in our study, the hexon of SqAdV exhibits the closest relationship to that of an alpaca AdV. Compared with the hexon, the penton base of SqAdV appears to be genetically more divergent from that of other mastadenoviruses. It was also revealed that the full-length SqAdV genome retained AT nucleotide content similar level to AT-rich atadenoviruses, which is unusual for mastadenoviruses. Our results emphasize that SqAdV is classified into the genus *Mastadenovirus* and demonstrate the AT-biased nucleotide constitution of SqAdV.

1. Introduction

Adenoviruses (AdVs) of the family *Adenoviridae* causes respiratory, gastrointestinal, and eye diseases to humans (Wold and Ison, 2013). Since their first identification in human adenoids in 1953 (Rowe et al., 1953), it has been shown that AdVs are widely distributed in a variety of animal hosts including humans (Davison et al., 2003).

AdVs have linear double-stranded DNA genome, ranging in nucleotide size between 26 and 45 kb (Davison et al., 2003), that is encapsidated in an icosahedral protein structure (Dulbecco and Ginsberg, 1988). There are more than 100 distinct types of AdVs that can be classified into the five genera: *Aviadenovirus*, *Atadenovirus*, *Ichtadenovirus*, *Mastadenovirus*, and *Siadenovirus* (ICTV, 2016). The genera *Aviadenovirus* and *Mastadenovirus* have been identified in birds and mammals, respectively (Benko et al., 2005; Davison et al., 2003). The genus *Atadenovirus* exhibits characteristic high contents of adenine and thymine nucleotides in the genome and has been found in various animal hosts, such as birds, ruminants, snakes, and lizards (Benko et al., 2005; Papp et al., 2009; Thomson et al., 2002; Wellehan et al., 2004). The *Siadenovirus* has been reported in birds, frogs, and turtles whereas the fifth genus *Ichtadenovirus* was only identified from a white sturgeon (Kovacs et al., 2003; Rivera et al., 2009). AdVs of the five different genera have genus-common genes located in the central region of their genome but differ from each other by retaining genus-specific genes mostly found near either terminus of the genome (Davison et al., 2003). From the genus-common genes, which are considered to be inherited from common evolutionary ancestors, hexon and penton base protein genes are well-characterized in terms of their functions and viral structures (Athappilly et al., 1994; Stewart et al., 1993). Additionally, immunological features of hexon and penton base proteins are also well-documented (Rosen, 1960; Toogood et al., 1992).

AdV in red squirrels (*Sciurus vulgaris*) was first noticed in diarrheal animals during the investigation of the decline of red squirrels in UK (Sainsbury et al., 2001). Since then, AdV-mediated enteropathy has been implicated in the epizootic spreads of AdV infection not only in naturally living red squirrels but also in confined red squirrels of breeding purpose (Everest et al., 2014). AdV infection was also detected in other sympatric woodland rodents, such as grey squirrels *Sciurus carolinensis* and wood mice *Apodemus sylvaticus* (Romeo et al., 2014). However, as resources of the complete genomic information of squirrel AdV (SqAdV) are limited, which makes it difficult to trace molecular evolutionary dynamics of AdVs in red squirrels and other sympatric

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animals (Everest et al., 2013; Peters et al., 2011; Romeo et al., 2014), the history of interspecies spread of these AdV infections cannot be reconstructed in detail. Moreover, absence of clinical signs or subclinical infection with AdVs in red squirrels challenges epidemiological efforts of AdV surveillance in red squirrels (Everest et al., 2014). In this regard, the full-length genomic information of AdVs from zoological specimens may have great importance for the molecular epidemiological analysis of AdV distribution not only in red squirrels but also in overall animal hosts.

In this study, we investigated the prevalence of AdVs in red squirrels in Korea using captured specimens and decoded the complete sequence of SqAdV for the first time. Furthermore, we analyzed the phylogenetic relationships based on the hexon and penton base genes and discussed the molecular evolutionary dynamics of SqAdV.

2. Materials and methods

2.1. Specimens

Through a surveillance project of small rodents living in the Republic of Korea during 1995–1996, a total of 59 red squirrels were captured in Gyeonggi-do (Gapyeong, n = 7, in 1995 and Namhansanseong, n = 17, in 1996) and Chungcheongnam-do (Gongju, n = 35, in 1996) and archived in Korea Bank for Pathogenic Viruses, Korea University College of Medicine. Kidney, lung, and spleen specimens of the red squirrels were then used for sequencing the hexon and penton base genes as well as the full-length genome of SqAdV.

2.2. Sequencing

Kidney, lung, and spleen of the red squirrels were homogenized and used for the extraction of viral genomic DNA using Exgene™ Tissue SV mini kit (GeneAll Biotechnology, Seoul, Republic of Korea) following a manufacturer's protocol. As presented in Table 1, degenerate PCR primers (AdV_Hex_F and AdV_Hex_R) detecting conserved regions of AdV hexon gene (Kiss et al., 1996) were initially used to detect AdV traces from the red squirrel specimens. Two different sets of primers (AdV_CSq1 and AdV_CSq3) targeting the hexon gene were used to additionally confirm AdV infection. Since SqAdV is known as one of the rodent-borne AdV member of the genus *Mastadenovirus* (Peters et al., 2011), we designed PCR primers for the hexon (SqHexon_1st and SqHexon_2nd) and penton base (SqPenton base) genes by comparing the reference sequences of bovine AdV A (Bovine_1, GenBank accession number AC_000191), bovine AdV B (Bovine_3, NC_001876), canine AdV A (Canine_1, AC_000003), equine AdV A (Equine_1, JN418926), human AdV type 2 (Human_2, J01917), murine AdV A (Murine_1, NC_000942), murine AdV C (Murine_3, EU835513), porcine AdV C (Porcine_5, AF289262), and tree shrew AdV A (Tree Shrew_1, AC_000190). Based on the sequences of the complete hexon and penton base genes of SqAdV, multiple sets of primers were designed for the extension of sequence readout and for the complete sequencing of the full-length SqAdV genome (Data S1). Primer sequences are available upon request.

2.3. Phylogeny and genetic divergence analysis

The best-fit models of nucleotide substitution of the hexon and penton base genes were determined as GTR + I + G and TrN + I + G, respectively, using jModelTest (v2.1.5) (Darriba et al., 2012). Based on these best-fit nucleotide substitution models, phylogenetic relationships of the hexon and penton base genes were reconstructed using maximum likelihood (ML) method of MEGA7 (Kumar et al., 2016). The reliability of phylogenetic trees were tested using a bootstrap method (500 replicates), and a subtree-pruning-regrafting approach was selected for ML heuristic search. The inferred phylogenetic trees of the hexon and penton base genes were visualized using FigTree (v1.4.3, http://tree.bio.ed.ac.uk/software/figtree/). Genetic divergence of the hexon and penton base genes was also analyzed using MEGA7 by estimating the number of base substitutions per site via the Maximum Composite Likelihood model (Tamura et al., 2004). Standard error estimates were obtained by a bootstrap procedure (500 replicates).

3. Results

3.1. Detection rates of AdV from red squirrel specimens

By implementing the designed primer sets (AdV_Hex, AdV_CSq1, and AdV_CSq3) (Table 1), red squirrel specimens were screened for AdV infection by a conventional PCR method. Of a total of 59 red squirrels, 19 exhibited AdV genome traces (32.20% positive rate) (Table 2). The red squirrels of Gapyeong, Gongju, and Namhansanseong showed 28.57%, 28.57%, and 41.18% positive rates of AdV infection, respectively. It is notable that, regardless of the different geographical origins of the red squirrels examined, the spleen exhibited the highest detection rate (25.42%) of AdV infection, and the kidney and lung were followed with 18.64% and 3.39% positive rates, respectively (Table 2). No AdV genome traces were observed in the liver samples. Of the 19 AdV-positive animals, only the two red squirrels, 96_28 and 96_29, exhibited

Table 1
Primers used for the detection and sequencing of SqAdV.

Primer set	Primer	Nucleotide sequence (5′ → 3′)	Polarity	Product size, bp (starting position, nt)[†]
AdV_Hex[§]	AdV_Hex_F	GCCGCARTGGTCYTACATGCACATC	(+)	301[‡]
	AdV_Hex_R	CAGCRYRCCGCGGATGTCAAART	(−)	
AdV_CSq1[§]	SqHexon_124F	GGCAACAAATTCAGAAACCCAAC	(+)	160 (15,000)
	SqHexon_284R	TCCAATACCCTATTATCCCCAAC	(−)	
AdV_CSq3[§]	SqHexon_1278F	CAGAGTACTTTGGGGAATGACCTCAG	(+)	774 (16,515)
	SqHexon_2052R	GGATAAGGCCAGTTAGATGGATATG	(−)	
SqHexon_1st[¶]	Sq_pVI_437F	TTCCATCAAAATCCGCCCCATCT	(+)	3363 (14,610)
	Sq_23k_361R	ATGCCGCACTATGAGGTCCTTGAACAC	(−)	
SqHexon_2nd[¶]	Sq_pVI_613F	ATTGTTGGTTTAGGTGTCAGAAGC	(+)	3006 (14,786)
	Sq_23k_180R	ATTCCAGCCGAATGCAATCCAATG	(−)	
SqPenton base[¶]	Sq_pIIIa_1297F	AGATGGAAAACTTACAGACAGGA	(+)	1674 (11,156)
	Sq_pVII_72R	CCACCCTGTATTATTTGACGGGGA	(−)	

[§] Primers used to detect an adenovirus in squirrel tissue samples.
[¶] Primers used to sequence hexon and penton base genes.
[†] Product size (base pairs, bp) and starting position (nucleotide, nt) of a given primer set at the full-length sequence of SqAdV genome (Data S1).
[‡] Adopted from the study of Kiss et al. (1996).