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ANNOTATED SEQUENCE RECORD

Complete genome sequence of a novel bat mastadenovirus C strain isolated from *Rhinolophus cornutus* in Japan

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Abstract

Here, we report a novel bat adenovirus strain isolated from apparently healthy bats of the species *Rhinolophus cornutus* in Japan. The genome of the isolate was 36,506 bp in length and encoded at least 33 proteins. Phylogenetic analysis of the DNA polymerase amino acid sequence, which provides one demarcation criterion for adenoviral species, indicated that the isolate belongs to the species *Bat mastadenovirus C* in the genus *Mastadenovirus*. Most of the encoded proteins shared high sequence similarity with those of known bat adenovirus C strains detected in different species of *Rhinolophus*, whereas the fiber protein and some E3- and E4-related proteins shared moderate similarity, and only the large E3 protein, which contains several host immune-suppression-related motifs, showed considerably lower similarity.

Bats are an important reservoir of many zoonotic viruses [1–4] including adenoviruses [5, 6]. Adenoviruses are non-enveloped icosahedral viruses with a double-stranded DNA genome that infect a wide range of vertebrate hosts from amphibians and fish to mammals. The family *Adenoviridae* is divided into six genera (*Atadenovirus, Aviadenovirus, Ichtadenovirus, Mastadenovirus, Siadenovirus,* and *Testadenovirus*). Mastadenoviruses infect a variety of mammalian hosts, including bats. Bat mastadenoviruses (BtAdVs) have been classified as members of the species *Bat mastadenovirus A-J* by the International Committee on Taxonomy of Viruses (ICTV) [7]. BtAdVs are divided into three groups depending on host family classification: group 1, comprised of members of species A, B, G, and J, isolated from Vespertilionidae bats and genetically closely related to canine adenoviruses; group 2, comprised of members of species C from Rhinolophidae bats; and group 3, comprised of members of species D, E, F, H, and I from Miniopteridae and Pteropodidae bats [8].

To investigate bat viruses, we collected fecal samples from apparently healthy insectivorous *Rhinolophus cornutus* bats with permission from the local government in Shizuoka Prefecture of Japan in September 2020. We then inoculated antibiotic-treated fecal suspensions onto African green monkey Vero cells expressing a transmembrane protease, serine 2 (Vero/TMPRSS2), which may support the replication of certain unknown viruses [9]. A clear cytopathic effect was observed in the cells at 7 days post-inoculation. The cell supernatant was passed through a 0.22-μm filter and inoculated onto fresh Vero/TMPRSS2 cells, producing an extensive cytopathic effect (Supplementary Fig. S1). This indicated that the virus had been isolated successfully. To identify the genome type of the isolate, we analyzed its growth in the presence of ribavirin or 5-iodo-2'-deoxyuridine (IUDR), which inhibit the growth of RNA and DNA viruses, respectively. Growth was inhibited with IUDR but not with ribavirin, suggesting that the isolate was a DNA virus. Then, to determine the complete genome sequence of the viral isolate, we grew it in cells for 4 days and purified it from the culture medium by ultracentrifugation with a 20% sucrose cushion. Viral DNA was extracted from the purified virus using a NucleoSpin Tissue Kit (Macherey-Nagel, Duren, Germany). A DNA library was prepared using a TruSeq

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Nano DNA Low Throughput Library Prep Kit (Illumina, San Diego, CA, USA) and subjected to next-generation sequencing (NovaSeq 6000, Illumina). The sequence dataset was assembled *de novo* using CLC Genomics Workbench software (v.8, CLC bio, Aarhus, Denmark), and sequence comparisons indicated that the isolate was a BtAdV strain, which was named Rc-kw20. The complete genome of Rc-kw20 is 36,506 nucleotides long with 54% G+C content. The complete nucleotide sequence has been deposited in the DDBJ/GenBank database under the accession number MZ683934.

The full genomic nucleotide sequence was analyzed phylogenetically (Fig. 1a). The phylogenetic tree

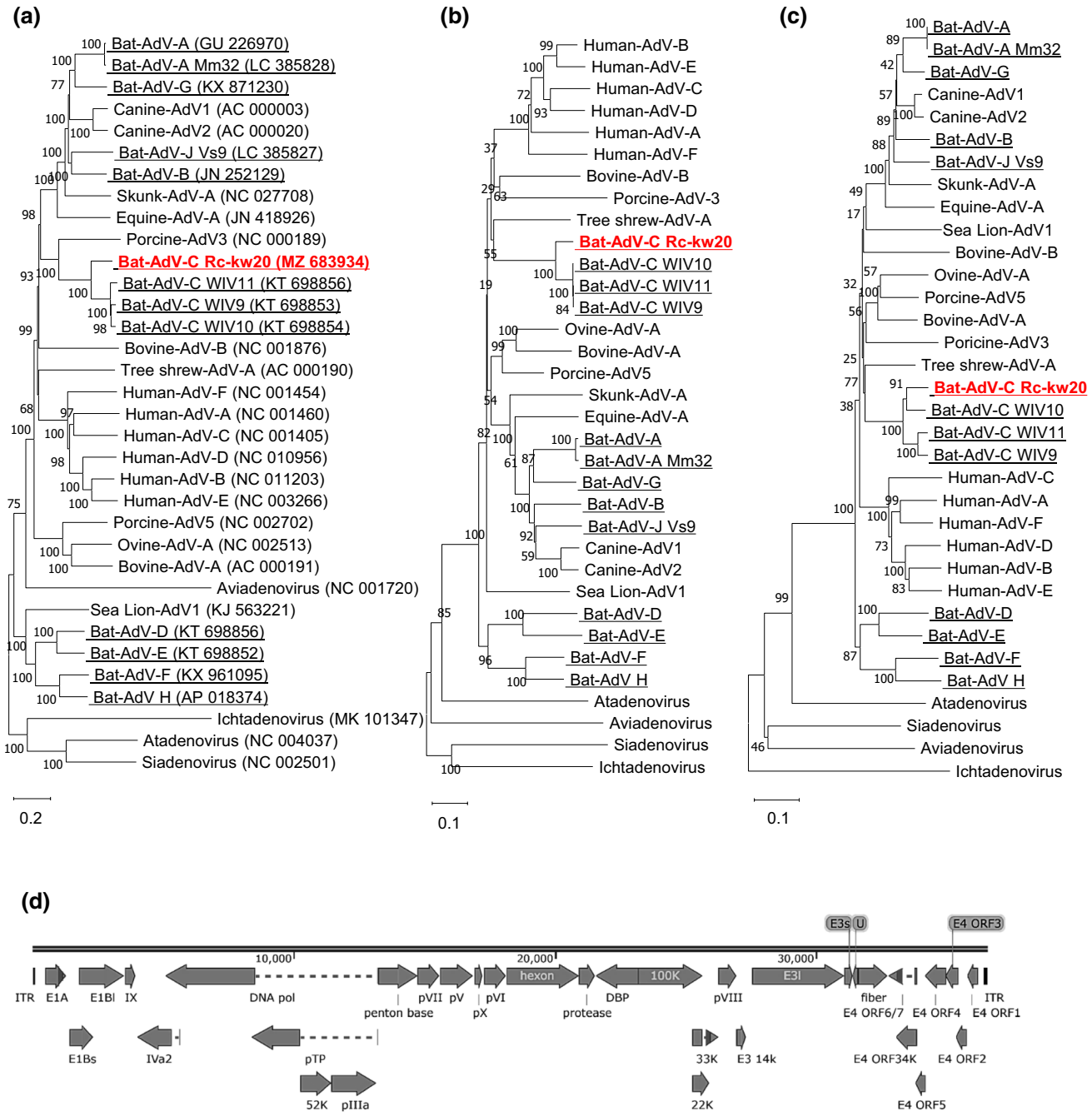

Fig. 1 Genetic analysis of bat mastadenoviruses. Phylogenetic trees based on (a) full-length nucleotide sequences, (b) DNA polymerase amino acid sequences, and (c) hexon amino acid sequences were constructed by the neighbor-joining method with 1,000 bootstrap replicates using MEGA X software. Bootstrap values are indicated as percentages at the nodes. Bars indicate evolutionary distance in units of substitutions per site. All bat mastadenoviruses are underlined, and the novel isolate identified in this study is shown in bold red. (d) Gene map of BatAdV-Rc-kw20 in which genes are shown as grey arrows and ITR sequences are shown as black rectangles

Table 1 Amino acid sequence identity of Rc-kw20 proteins to those of other BtAdV-C strains

Gene product	Genomic position (orientation*)	Identity (%)		
		WIV9	WIV10	WIV11
E1A	529-1024, 1115-1310 (R)	72.3	72.3	72.8
E1Bs	1457-2368 (R)	69.4	68.2	68.2
E1Bl	1822-3528 (R)	68.0	68.1	68.3
pIX	3578-3964 (R)	80.3	80.3	80.3
IVa2	4032-5362, 5641-5653 (L)	94.2	94.0	94.2
pol	5123-8575, 13227-13235 (L)	89.8	89.8	89.8
pTP	8422-10284, 13227-13235 (L)	90.6	90.4	90.4
52k	10277-11455 (R)	92.8	92.8	92.8
pIIIa	11484-13184 (R)	90.9	90.7	90.9
Penton base	13265-14767 (R)	89.0	89.0	89.2
pVII	14773-15588 (R)	91.2	91.2	91.2
pV	15648-16880 (R)	85.2	85.4	85.2
pX	16957-17238 (R)	95.7	95.7	95.7
pVI	17236-18129 (R)	82.4	82.4	82.5
Hexon	18178-20928 (R)	88.8	90.9	89.2
Protease	20943-21563 (R)	90.5	91.5	91.0
DBP	21592-23205 (L)	78.8	78.7	78.7
100k	23219-25687 (R)	85.2	85.1	85.3
33k	25302-25699, 25935-26289 (R)	79.8	79.5	80.1
22k	25302-25922 (R)	75.9	75.5	76.2
pVIII	26292-26984 (R)	96.1	96.1	96.5
E3 14k	26971-27351 (R)	69.4	68.5	68.5
E3l	27579-31118 (R)	34.6	33.6	31.8
E3s	31120-31419 (R)	57.6	57.6	57.6
U exon	31434-31610 (L)	52.6	52.6	52.6
Fiber	31632-32753 (R)	60.1	50.3	48.0
E4 34k	33085-33882 (L)	75.7	74.5	75.3
E4 ORF6/7	32782-33059, 33791-33882 (L)	62.4	62.4	62.4
E4 ORF5	33827-34192 (L)	83.6	79.8	80.7
E4 ORF4	34194-34970 (L)	51.6	50.4	51.6
E4 ORF3	34997-35464 (L)	56.2	56.2	55.4
E4 ORF2	35391-35768 (L)	67.2	67.2	66.4
E4 ORF1	35799-36212 (L)	50.7	50.7	53.7

*Strand orientation; L, leftward; R, rightward

indicated that strain Rc-kw20 was positioned in the same cluster with previous BtAdV-C strains, all of which had been detected in *Rhinolophus sinicus* bats in China, suggesting that Rc-Kw20 belongs to the species *Bat mastadenovirus C* in the genus *Mastadenovirus*. The full genomic nucleotide sequence of Rc-kw20 was 83.8%, 83.9%, and 83.7% identical to BtAdV-C strains, WIV9, WIV10, and WIV11, respectively (GenBank accession numbers KT698853-5).

Open reading frames (ORFs) were predicted using SnapGene Viewer software (SnapGene v.5; GSL Biotech, San Diego, CA, USA) (Table 1), and 33 putative ORFs were identified. Comparisons of the DNA polymerase amino acid sequences of Rc-kw20 and other BtAdV-C strains, which provide one demarcation criterion for adenoviral species [10], showed that they were nearly 90% identical (Table 1). Phylogenetic analysis confirmed that the Rc-kw20 strain was positioned in the BtAdV-C cluster (Fig. 1b), and this was supported by analysis using hexon protein amino acid sequences (Fig. 1c), confirming that Rc-Kw20 is a BtAdV-C strain and that *Rhinolophus cornutus* should be included as a susceptible host for group 2 BtAdV.

We also generated a genomic map, which included the 54-bp-long inverted terminal repeat (ITR) sequences (Fig. 1d). Twenty of the 33 proteins encoded by Rc-kw20 shared high sequence similarity (more than 80% identity) with those of previously characterized BtAdV-C strains, whereas the fiber protein and several E3- and E4-related proteins shared moderate similarity (ranging from 48.0% to 67.2% sequence identity), and only the large E3 (E3l) protein, which contains host immune-suppression-related motifs [11], showed considerably lower similarity (31.8% to 34.6% sequence identity) (Table 1). Possible functional differences involving proteins with lower sequence similarity might determine the host specificity of BtAdV-C strains.

Recent studies have indicated that *Rhinolophus* spp. bats may represent an ancestral host for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes COVID-19. Indeed, related bat sarbecoviruses have been detected in *Rhinolophus affinis, R. sinicus, R. pusillus, R. malayanus*, *R. acuminatus*, and *R. shameli* in Asian countries [12–17]. In addition, we detected a bat sarbecovirus in *R. cornutus* in Japan [18]. Further studies are needed to assess the zoonotic potential of bat viruses inhabiting these *Rhinolophus* species, including BtAdV-C.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00705-021-05357-8.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This study did not involve experiments with human participants performed by any of the authors. Experiments with bat samples were approved by the Animal Experiment Committee of the Graduate School of Agricultural and Life Sciences at the University of Tokyo (approval number P21-058).

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