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Genome sequence of a pathogenic isolate of monkey B virus (species *Macacine herpesvirus 1*)

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Abstract

The only genome sequence for monkey B virus (BV; species *Macacine herpesvirus 1*) is that of an attenuated vaccine strain originally isolated from a rhesus monkey (BVrh). Here we report the genome sequence of a virulent BV strain isolated from a cynomolgus macaque (BVcy). The overall genome organization is the same, although sequence differences exist. The greatest sequence divergence is located in non-coding areas of the long and short repeat regions. Like BVrh, BVcy has duplicated Ori elements and lacks an ORF corresponding to the μ34.5 gene of herpes simplex virus. Nine of ten miRNAs and the majority of ORFs are conserved between BVrh and BVcy. The most divergent genes are several membrane-associated proteins and those encoding immediate early proteins.

Monkey B virus (BV; species *Macacine herpesvirus 1*) is a macaque α-herpesvirus related to the human herpes simplex viruses [6]. Primary BV infections are usually asymptomatic, although oral or genital lesions can occur [10]. When BV infects humans,it rapidly invades and replicates within the central nervous system (CNS), resulting in serious and usually fatal disease [5, 8]. One strain of BV (E2490) has been attenuated for use as a vaccine [9] and serves as the reference BV strain. The sequence of the BV E2490 genome has been determined and is arranged much like the genome of HSV and other nonhuman primate α-herpesvirus genomes [11, 13, 14, 16, 20-22].

Since lethal human BV cases have historically been associated with exposure to rhesus macaques, the possibility exists that rhesus BV isolates (BVrh) are more lethal than BV from other macaque species. Restriction analysis suggested that BV isolates from different macaque species could be distinguished from one another, and restriction mapping of a cynomologous macaque (*M. fascicularis*) BV isolate (BVcy) suggested that the genome is organized differently from that of BVrh E2490 [7, 24]. Comparative sequencing of ~1 kbp of BV isolates from different macaque species identified sequence differences that correlate with the host macaque species, resulting in identification of BV genotypes (BVrh from

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rhesus monkeys, BVcy from cynomologous macaques, etc.) [12, 18, 19]. However, the full extent to which BV genotypes differ is unknown. Here, we report the genome sequence of a virulent BVcy strain and its comparison to that of the attenuated BVrh reference strain.

BVcy strain E90-136 was isolated from a young cynomologous macaque that succumbed to a generalized infection [17]. BVrh strains 24105G and 32425G were isolated from genital swabs of rhesus macaques [15]. All work with infectious BV was performed in accordance with the Oklahoma State University Select Agent Program as approved by the US Centers for Disease Control and Prevention. Viral DNA used for sequencing and PCR reactions was purified from infected Vero cells as described [3]. Initial genomic sequencing utilized cloned restriction fragments and Sanger dideoxy sequencing performed by the Oklahoma Medical Research Foundation DNA Sequencing Facility (Oklahoma City, OK) and at Nagasaki University. The BVcy E90-136 genome sequence was subsequently determined using Illumina sequencing technology performed by the Oklahoma University Health Sciences Center Laboratory for Molecular Biology and Cytometry, Oklahoma City, OK) as described [4]. Sequence data were assembled and analyzed using the Vector NTI version 9.1.0 and CLC Main Workbench version 6.7.1 software packages. The BVcy genome sequence has been deposited in the GenBank database under accession no. KJ566591.

Despite previous reported differences in the U_S region [7], the BVrh E2490 and BVcy E90-136 genomes are completely orthologous. Predicted transcriptional grouping of BVcy genes was consistent with that previously reported for BVrh as well as simian herpesviruses HVP2 and SA8 [11, 13, 14, 21, 22]. The BVcy ‘a’ repeat has two copies of the DR1 element as in HSV [23], while BVrh E2490 has only one [14]. The BVcy and BVrh genomes differ in size by ~2.9 kbp, the BVcy genome being 153,891 bp, as compared to 156,789 bp for BVrh. A substantial part of the size difference is due to the absence in BVcy of reiterations within non-coding sequences of the long and short repeat regions (RL and RS, respectively). When the BVcy and BVrh genome sequences are aligned, ~50% of gaps are located within the RL region, 25% in the RS region, 15% in the unique long (U_L) region and 10% in the U_S region. The greatest nucleotide sequence variation also lies within the noncoding regions of RL and RS.

The level of variation in coding sequences between the two BV strains reflected the level of sequence variation between homologous genes of other primate α-herpesviruses; genes strongly conserved among other primate viruses were most strongly conserved between BVrh and BVcy, and genes that varied most among the other primate viruses showed the greatest sequence variation between the two BV strains (see Supplemental Data, Table 1). Most BVcy proteins have a very high level of DNA and amino acid (AA) sequence identity to the homologous BVrh ORFs/proteins (range 79-99% AA sequence identity; average, 95%). The most highly conserved ORFs included those encoding several capsid proteins (UL18, UL19, UL25 and UL38), DNA packaging proteins (UL15, UL28 and UL33), UL48 (VP16/αTIF), ssDNA binding protein (UL29), glycoprotein gK (UL53) and a subunit of the helicase/primase (UL5). The most divergent genes encoded glycoproteins (gL [UL1], gG [US4] and gJ [US5]), immediate early regulatory proteins (ICP0 [RL2], ICP22 [US1] and ICP47 [US12]), the uracil DNA glycosylase (UL2), a nuclear protein (UL4), and a membrane-associated protein (UL56).

Ten miRNAs have been identified in BVrh [1, 2]. BVcy has clear homologues of all eight pre-miRNAs located within the repeat regions and one (Pre-hbv-miR-B2) located between OriL and UL30. Of the miRNAs located in the repeat regions, seven were strongly conserved, with sequence identities of 95.9 – 98.3%. A homologue of Pre-hbv-miR-B3RC (located, between ORFs UL45 and UL46) was not evident in BVcy. Over the 190-bp region where BVrh Pre-hbv-miR-B3RC is located in BVrh, there is only 70% sequence identity between BVrh and BVcy (20% substitutions and 10% insertions/deletions), the two polyA signals (for UL44/45 and UL46/47) being the only clearly conserved sequence in this area.

Based on splice junctions reported for BVrh E2490 RL2/ICP0 [14], the BVcy RL2 (ICP0) ORF has an in-frame stop codon in exon 3, resulting in a truncated ICP0 protein. However, a different reading frame encodes AA sequences homologous to the C-terminal region of E2490 ICP0, suggesting that the reported splice junction between exons 2 and 3 is incorrect. To determine splice junctions for the BV ICP0 gene experimentally, Vero cells were infected (MOI = 5 PFU/cell) with virus (BVrh E2490, BVcy E90-136, two low-passage BVrh field isolates) and harvested at 3 h PI, and total RNA was isolated (RNAeasy; QIAGEN, Valencia, CA). cDNA was prepared (GoScript; Promega, Madison, WI), and PCR primers located in exons 1 and 3 were used to amplify a region of ICP0 cDNA spanning the two reported splice junctions. PCR products were sequenced and aligned with genome sequences to identify splice junctions. The splice junction between exons 1 and 2 was identical to that reported previously [14], but the splice junction between exons 2 and 3 was not (Fig. 1). The experimentally identified splice junction between exons 2 and 3 was consistent in all four BV strains examined, resulting in ICP0 proteins with nearly identical AA sequences.

Despite there being sequence differences that clearly delineate the two BV genotypes, the overall similarity of the BVrh and BVcy coding sequences confirms that these two strains of BV are not distinct viruses but rather different strains of BV. While the greatest sequence variation was in the noncoding regions of RL and RS, the significance of this sequence variation is not clear since there are no known functions assigned to these sequences.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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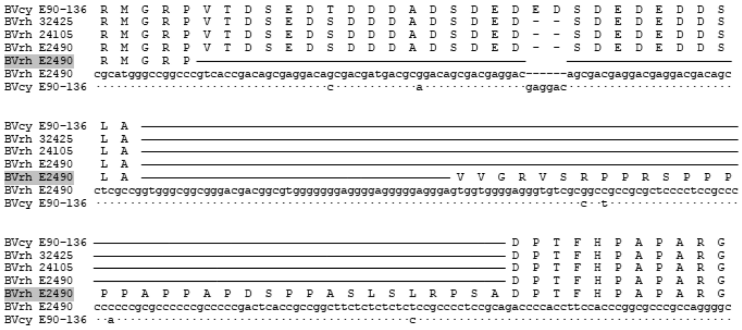

Fig. 1.

Location of the RL2 (ICP0) exon 2/3 splice junction. Predicted AA sequences are shown in the top 5 lines, with the shaded BVrh E2490 AA sequence being that originally reported [14]. DNA sequences for BVrh E2490 and BVcy E90-136 are shown in the two lower lines. PCR/sequencing of RL2 mRNA from the three BVrh strains and BVcy resulted in predicted splice junctions producing ICP0 AA sequences that are identical in all four BV strains.