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Isolation, characterization and prevalence of a novel Gammaherpesvirus in *Eptesicus fuscus*, the North American big brown bat

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ABSTRACT

Little is known about the relationship of Gammaherpesviruses with their bat hosts. Gammaherpesviruses are of interest because of their long–term infection of lymphoid cells and their potential to cause cancer. Here, we report the characterization of a novel bat herpesvirus isolated from a big brown bat (*Eptesicus fuscus*) in Canada. The genome of the virus, tentatively named *Eptesicus fuscus* herpesvirus (*Ef*HV), is 166,748 base pairs. Phylogenetically *Ef*HV is a member of Gammaherpesvirinae, in which it belongs to the Genus *Rhadinovirus* and is closely related to other bat Gammaherpesviruses. In contrast to other known Gammaherpesviruses, the *Ef*HV genome contains coding sequences similar to those of class I and II host major histocompatibility antigens. The virus is capable of infecting and replicating in human, monkey, cat and pig cell lines. Although we detected *Ef*HV in 20 of 28 big brown bats tested, these bats lacked neutralizing antibodies against the virus.

1. Introduction

In recent years several bat viruses have been discovered, most appear to have little deleterious effect on their hosts (Calisher et al., 2006; Wynne and Wang, 2013). These unique and benign virus-host relationships are likely a byproduct of evolutionary adaptations to flight and long-term associations between the bats and their viruses (Schountz, 2014). In addition to simply satisfying scientific curiosity, a better understanding of these unique relationships may provide clues to mitigating the much more serious pathologic virus-host interactions in other species.

Gammaherpesviruses are a sub-family of Herpesviridae with a primary tropism for cells of lymphoid lineage (David M. Knipe, 2013). Gammaherpesviruses establish latent infections in long-lived lymphoid cells and in some cases cause neoplasias, such as Burkitt's lymphoma and Kaposi sarcoma in humans (Jha et al., 2016). One of the challenges in studying the reactivation and pathogenesis of these oncogenic viruses is the lack of a suitable animal model (Grinde, 2013). Murine Gammaherpesvirus-68 in mice, the model used most extensively, is not ideal as the virus is not readily transmitted between laboratory mice (Aligo et al., 2015). Previous studies have shown that bats and primates harbor a large diversity of Gammaherpesviruses that might have led to higher chances of cross-species transmission from these taxa to other mammals (Ehlers et al., 2008; Escalera-Zamudio et al., 2016; Zheng et al., 2016). Therefore, a naturally occurring Gammaherpesvirus in a readily accessible bat species may provide us with vital information on evolution of these viruses and serve as a model for studying pathogenesis.

Even though there are a few reports of detection of Gammaherpesvirus genomes (Molnar et al., 2008; Paige Brock et al., 2013; Watanabe et al., 2009), and a single report of isolation of a Gammaherpesvirus from bats (Shabman et al., 2016), knowledge about bat Gammaherpesviruses is limited. As bats do not appear to normally display viral pathology, studying a Gammaherpesvirus in its natural bat host may provide information about novel co-evolutionary adaptations that lead to balanced and benign host-virus relationships.

Here, we report the discovery, isolation and characterization of a novel bat Gammaherpesvirus from the lungs of a North American big brown bat (*Eptesicus fuscus)*. We provisionally name the virus, *E. fuscus* Herpesvirus (*Ef*HV). The morphology and protein composition of *Ef*HV are similar to those of other herpesviruses. Furthermore, we obtained the sequence of its genome, determined its ability to infect cells from several mammalian species and characterized its growth characteristics. A comparison of the sequence of *Ef*HV with those of other

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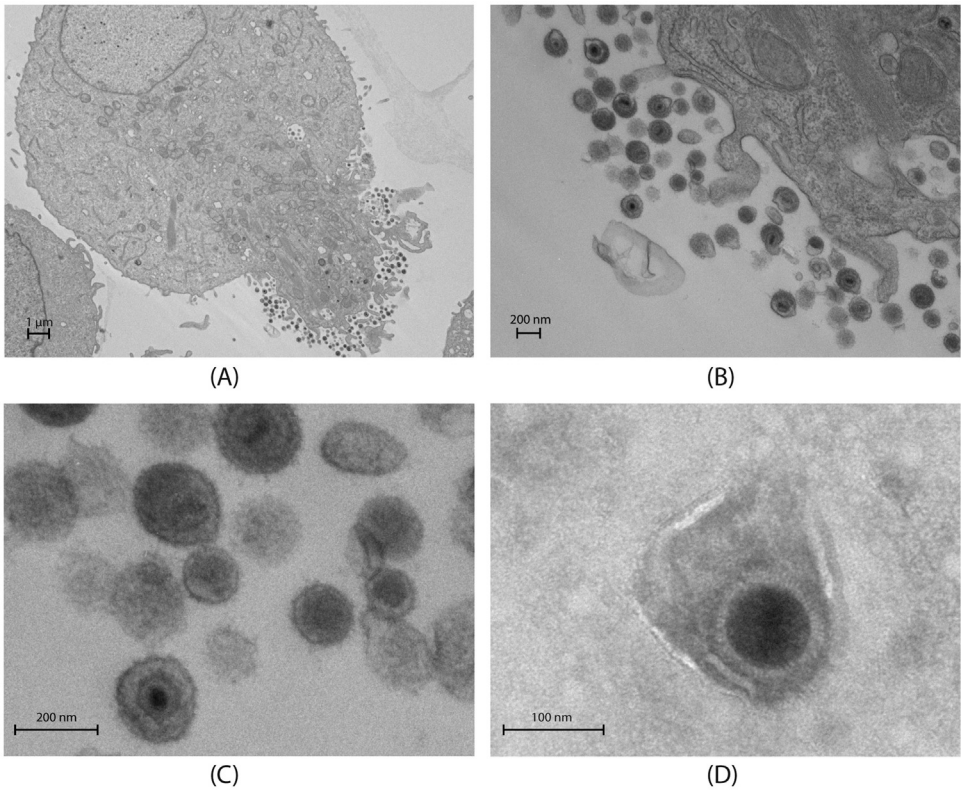

Fig. 1. Electron micrographs of *Ef*HV. *Ef*K cells inoculated with homogenate of lungs from a big brown bat showing enveloped virions (approximately 200 nm in size) budding out from the cells (A), (B) and (C). Negative staining of purified virions showing a magnified image of the virus (D). The capsid is approximately 100 nm in size.

herpesviruses indicates that the virus is closely related to other bat Gammaherpesviruses. In addition to transducing host cytokine genes like interleukin 10 (IL-10), the *Ef*HV genome is unique among Gammaherpesviruses in that it contains the coding sequences for four putative host MHC-I antigens and one MHC-II antigen.

2. Results

2.1. Isolation of a novel herpesvirus from lungs of a big brown bat (E. fuscus)

As part of ongoing surveillance of viruses in local North American bats, we observed cytopathic effects in *Ef*K cells, a big brown bat kidney cell line (Banerjee et al., 2016), inoculated with a homogenate of lungs from a big brown bat submitted to the Canadian Wildlife Heath Cooperative (CWHC). Using pan-herpesvirus primers in a PCR (Ehlers et al., 1999) we established the presence of a herpesvirus. To further confirm this, we performed electron microscopy on the cells and observed virus particles having herpesvirus-like morphology (Fig. 1-A, B and C). In addition to microscopy of the cells, we observed herpesvirus particles in negative stained preparations of purified virus (Fig. 1-D).

2.2. Genome sequencing of EfHV

To determine the nucleotide sequence of the *Ef*HV genome, we used a combination of paired-end (Illumina MiSeq) and long read (Oxford Nanopore) sequencing. We obtained 1458,704 paired-end reads from an Illunima Mi-seq run, and de-novo assembly on these reads (using Geneious Assembler (Kearse et al., 2012)) led to the assembly of contigs of a maximum of ~100 kb. As we were unable to join all the contigs, we resorted to performing a long read sequencing i.e. Nanopore sequencing, from which 27,002 long reads were obtained. Finally, we used Geneious assembler to combine the long reads and the paired end reads in order to get the complete genome of the herpesvirus which is 166,748 bp with a GC content of 59.6% (NCBI Accession number MF385016) (Kearse et al., 2012). The arrangement of the sequence contigs was verified by restriction enzyme mapping (Supplementary Figure 1). We annotated the genome based on results of BLASTp comparisons of predicted open codon reading frames (ORFs) (Rastogi, 2000) from the assembled sequence to all sequences in the NCBI database, which resulted in the successful identification of 75 genes (Table 1, Fig. 2). Repeats in the genome were identified using Geneious R10 (Kearse et al., 2012).

The genome length of *Ef*HV was well within the range of known herpesviruses, i.e. 124–295 kb (David M. Knipe, 2013). Based on BLASTp percent similarity, most of the genes matched with orthologues in other Gammaherpesviruses. Within the first 8 kb of the 5' end of the genome, we detected four putative major histocompatibility complex (MHC) class I genes and one MHC class II gene (Fig. 2). In addition to MHC antigens, we detected an interleukin-10 homolog in the genome. We verified expression of all the MHC-I, MHC-II and IL-10 homolog genes in bat cells infected with *Ef*HV by analyzing transcripts from virus-infected cells - we used quantitative real time PCR (qRT-PCR) followed by analysis of the products by agarose gel electrophoresis (Supplementary Figure 2) and determined their nucleotide sequence. *Ef*HV also encoded proteins similar to the latency associated nuclear antigen (LANA1) and replication and transcription activator (Rta), proteins that are key regulators of Kaposi sarcoma virus latency and lytic cycles (Groves et al., 2001; Sun et al., 1998).

2.3. EfHV belongs to the Genus Rhadinovirus in Gammaherpesvirinae

We initially compared the amino acid sequence of the entire DNA polymerase protein of *Ef*HV with that of homologues in representative alpha, beta and gammaherpesviruses (Supplementary Figure 3). *Ef*HV clustered with Gammaherpesviruses, and most closely aligned with the *Rhadinoviruses* Kaposi's sarcoma herpesvirus and *Saimirine herpesvirus* 2. We next aligned the amino acid sequences of the DNA polymerase and glycoprotein B gene of representative viruses from Gammaherpesvirinae based on sequences reported by Escalera-Zamudio et al. (2016)