GENOMIC ANNOTATION OF OVINE HERPESVIRUS-2

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The 131 611 bp complete genome sequence of OvHV-2 has a GC content of 52 % (L-DNA) and 69% (H-DNA) at the terminal repeat region. Comparison of the whole genome sequence analysis between OvHV-2 and closely related gammaherpesvirus Alcelaphine herpesviruses 1 (AlHV-1) showed that most of the homologous regions were within the sequence where the ORFs were predicted in both sequences. Phylogenetic analysis of the whole genome with other herpesvirus complete genomic sequences showed the close relationship with AlHV-1 and confirmed as a member of the genus Rhadinovirus of the family Gammaherpesvirus.

1. INTRODUCTION

Malignant catarrhal fever (MCF) is a severe, usually fatal, lymphoproliferative and inflammatory syndrome primarily of ruminant species caused by either of the two closely related gammaherpesviruses Alcelaphine herpesvirus-1 (AlHV-1) and Ovine herpesvirus-2 (OvHV-2 [1]. AlHV-1 and OvHV-2 are members of the rhadinovirus subfamily of gammaherpesviruses, which includes Human herpesvirus-8 (Kaposi's sarcoma associated herpesvirus, KSHV), Epstein Barr Virus (EBV) and murine gammaherpesvirus (MHV 68).

The similarity of disease, cross-reactivity of antisera and limited information from sequencing of gene fragments, all suggested that OvHV-2 was most closely related to AlHV-1 [2]. The complete genomic sequence available for OvHV-2 will enable to deduce the different complex area including other molecular aspects of pathogenesis of this virus in details.

2. METHODS

There are certain computational genome annotation tools available some of which including Artemis, a free DNA sequence viewer and annotation tool has mostly been used in this study [3]. Potential protein coding ORFs were identified by the following criteria: ORF size larger than 60 aa, presence of potential transcriptional start and stop sites, a high GeneMark score and homology to other known herpesvirus or cellular ORFs. The Blast module Blastall, which supports all five Blast programs (blastp, blastn, blastx, tblastn and tblastx) was used frequently for the present study.

MPsrch utilizes a rigorous exhaustive algorithm compared to Blast and Fasta searches and the tool was used from the MRI Linux server. The OvHV-2 sequence was submitted to polyADQ, eukaryotic (human polyadenylation poly (A) signal search engine: (Cold Spring Harbor Laboratory [4]. polyADQ decides whether a given AATAAA or ATTAAA hexamer is a true polyA signal by comparing with the set cut off values. The putative location of possible polyA site is 20 bp downstream of the polyA signal. The software CpGIE was downloaded via the website: http://bioinfo.hku.hk/cpgieintro.html [5]. The following cutoff values were used to determine the CpG island in a given genomic sequence. >=200 nt , G+C content 50%, and an observed : expected CpG ratio 0.6.

The Artemis Comparison Tool (ACT), written by Kim Rutherford, was used for the pair wise comparisons between OvHV-2 and AlHV-1 genomes. The Lasergene software from DNASTAR provides a number of modules for sequence analysis. Some of the modules were used in this study for multiple sequence and Phylogenetic analysis.

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3. RESULTS

3.1 Coding potential

The complete version of the OvHV-2 genome used in this project consists of 131 611 bp. About 129kbp has GC content of 52% (L-DNA) flanked by repeat sequences with GC content of about 69% (H-DNA).

The L-DNA of OvHV-2 was found to encode 84 potential open reading frames. The genes were evenly distributed except for two regions of non-repetitive sequence which contained no ORF of significant size and is similar to AlHV-1 in this respect.(between ORF11 and 17; ORF 69 and O8.5/ORF73).

Among the ORFs identified seven of them were found to be unique to OvHV-2 and 13 ORFs were found to have homology with the AlHV-1 unique genes. Most of the ORFs were homologous to other herpesviruses.

There are certain limitations of similarity search for example, they can not identify new genes especially those divergent from the known homologs; some genes may be missed which are unique to the genome; gene identification based on sequence homology is tentative until independently verified. Thus approach was taken to predict regulatory region for validation of annotation.

3.2 Regulatory Regions

Most of the ORFs of OvHV-2 genome were within the CpG islands and a few CpG islands were found within the noncoding region between ORF11 and ORF18.

Not all the ORFs had polyA signals in their downstream region. In the 3' end of ORF O2.5e polyA signal was available whereas it was absent in the other spliced sites of O2.5. On the other hand, several signals available within the spliced ORFs O6a, O6b and O6c which might indicate the alternative splicing of these spliced reading frames

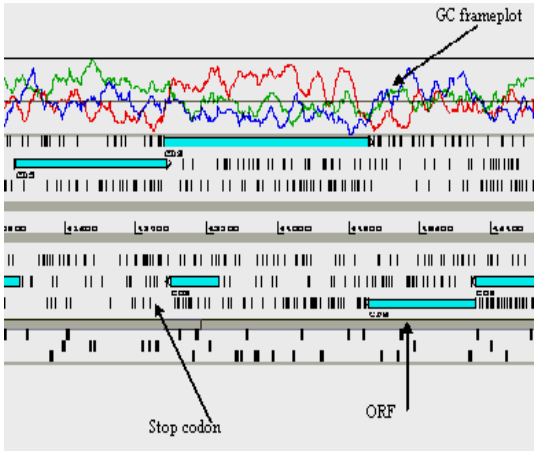

Figure 1: Artemis overview of Ovine Herpesvirus-2 whole genome

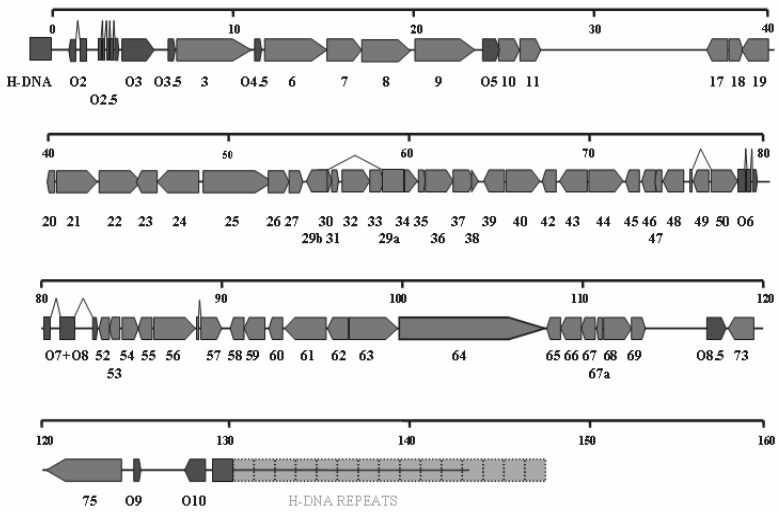

Figure 2: Organization of the OvHV-2 genome. ORFs are shown by the arrow which showing the direction of transcription/ translation, and non-coding DNA as a solid line. The ORFs are drawn to scale with the relative co-ordinates shown below in kbp. Splice sites are shown as lines above connecting exons. Major repetitive elements are shown as shaded rectangles (Source: George Russell, Moredun Research Institute, Edinburgh, UK)

3.3 Comparison between OvHV-2 and AlHV-1 whole genome

Most of the ORFs in both the genomes were in homologous positions and the conserved regions were mostly within the positions where the ORFs were available. Most of the nonconserved regions were in positions where no ORFs were available. Interestingly most ORFs predicted in case of OvHV-2were within the CpG islands, whereas AlHV-1 genome is known as CpG suppressed. A few predicted promoters of OvHV-2 were also found conserved in the corresponding upstream of ORFs in AlHV-1 indicating the possible regulation of genes in similar fashion such as ORFs including : 24, 26, 31, 32, 29a, 29b, 38, 39, 43, 54, 58, 59, 62 etc.

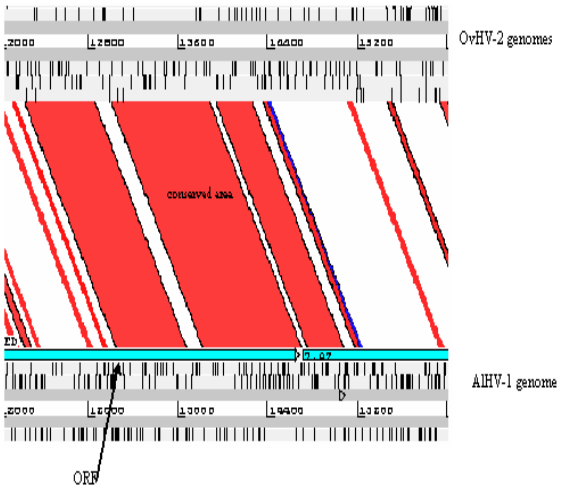

Figure 3: ACT overview of genome comparison between OvHV-2 and AlHV-1. Dark bars indicate the conservation between two genomes

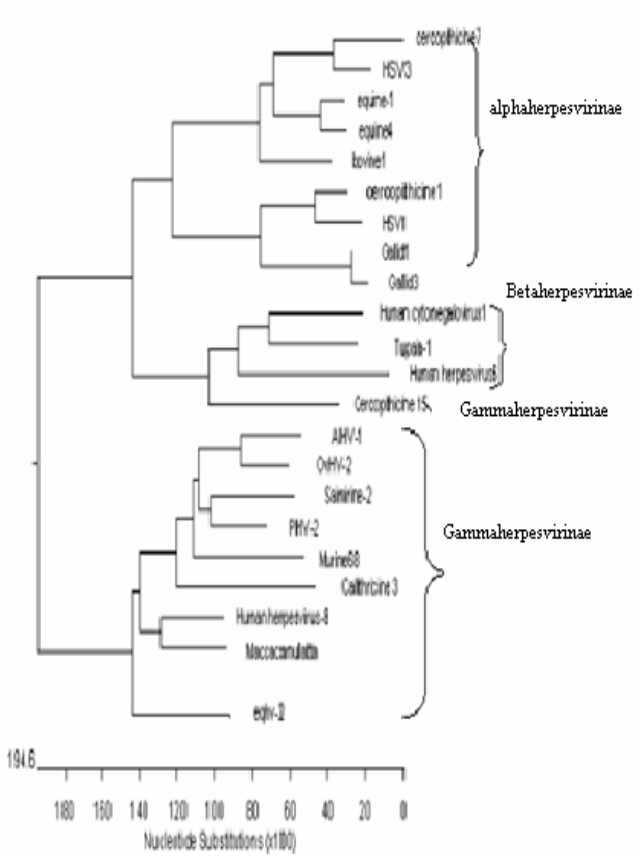

Figure 4: Phylogenetic analysis of Herpesvirus family

4. Discussion

It has been proposed that the γHVs associated with MCF be placed in their own genus, Macavirus, based on evolutionary relatedness of conserved ORF sequences [6]. The sequence presented here confirms this new grouping, showing that OvHV-2 is highly similar to AlHV-1 and PLHV-1, not only in the nucleotide similarity of conserved ORFs, but also in terms of ORFs that are present only in this group of viruses. These macavirus-specific ORFs are likely to be involved in host-specific pathogenesis and the development of MCF. Thus, comparative genetic analysis of OvHV-2 and related viruses enabled by the completion of this sequence will be core to the understanding of the mechanisms underlying MCF

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