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Short Communication

DRAFT GENOME SEQUENCE OF HUMAN HERPES VIRUS-4 VRF_EBV_01, AN EPSTEIN BARR VIRUS OBTAINED FROM A PEDIATRIC POST TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD) PATIENT

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ABSTRACT

Objectives: To decode the sequence of Epstein-barr virus (EBV) genome isolated from a pediatric patient with post transplant lymphoproliferative disorder (PTLD).

Methods: EBV culture harvested from the blood sample of a 4-year-old patient with post liver transplant lymphoproliferative disorder prior to treatment was subjected to whole genome sequencing using Illumina platform. Generated data were subjected to various quality analysis and the filtered sequences were submitted to NCBI and published under accession number KM269735-KM269744.

Results: Annotation results of VRF_ EBV01 genome using Prokka tool and manual blast search infers 48 hypothetical proteins, each 3 genes coding for Epstein-Barr nuclear antigen 3A and 3B and each one gene coding for Epstein-Barr nuclear antigens 1, 4, 4B and DNA primase UL70 protein. Single copy of genes coding for each variant of Tegument protein BSRF1, BLRF2, BGLF2 mir-BART 1,2,3,5,7,12,15,17,20 and two copies of gene coding for primary envelopment factor BFRF1 gene was also found.

Conclusion: To date, only **e**ight EBV genome sequences have been reported worldwide and there is no genome sequence reported from India. This study is the first of its kind to report on EBV genome from a post–transplant lymphoproliferative disorder to the scientific community for the welfare of research against EBV diagnostic markers and drug discovery.

Keywords: Herpes virus, Genome sequence, Epstein - Barr virus, lymphoproliferative disorder

Epstein-Barr virus (EBV) is a ubiquitous human gamma-herpes virus that infects more than 90% of the population worldwide. It has been implicated in the development of malignancies of lymphoid or epithelial origin, including B-cell lymphoma, T-cell lymphoma and B-cell lymphoproliferations in immunocompromised patients [1], endemic Burkitt's lymphoma [2], Hodgkin's disease [3], undifferentiated nasopharyngeal carcinoma (NPC) [4], gastric carcinoma [5], Oral hairy leukoplakia in HIV patients [6].

To date there are eight EBV whole-genome sequences has been reported worldwide from various studies. A complete 171 kb wild-type EBV reference genome, WT-EBV, (NCBI accession no: NC_007605.1/AJ507799.2) was later constructed using B95-8 [7] as a backbone while an 12 kb missing fragment in the EBV genome was provided by the EBV sequence found in Raji cells [8, 9]. Akata (KC207813) and Mutu (KC207814) genomes were reported recently from cases of Burkitt's lymphomas from Japan and Kenya, respectively [10]. But there were no reports from India about the EBV genome, due to the complexity of the viral genome and insertion into the host genome making sequencing the data a difficult task [11-13]. This study provides the first draft genome analysis of EBV strain isolated from post transplant lymphoproliferative disorder (PTLD)-derived EBV strain VRF_EBV01. In the current study, Blood sample was collected from a 4-year-old liver transplant patient with PTLD prior to treatment at the Global hospitals, Chennai, India. The patient's consent as well as approval from the Institute Research Ethics Committee was obtained. The serological titrations of immunoglobulin M antibodies against the EBV viral capsid antigen showed the presence of circulating antibody titre and real time PCR revealed 1,23,714 copies/ml of EBV.

Human Umbilical cord blood mononuclear cells were isolated on Ficoll-Hypaque density gradients, further 10^6 mononuclear cells were infected for 2 h with EBV in 200 microlitres (µl) of the Buffy separated from the collected patient's blood. After infection, the cells were washed with phosphate-buffered saline and then cultured in RPMI 1640 (plus 10% FBS and 0.5% pen/strep). The cultures were fed weekly with fresh medium. Small colonies began to appear within 35 to 50 days. Individual colonies were then expanded and maintained for DNA extraction.

Purified genomic DNA extracted by QIAamp DNA Mini Kit (Hilden, Germany) was then used to prepare a sequencing library using a Nextera DNA sample preparation kit (Illumina, San Diego, CA). Genome sequencing was performed at Scigenome, Bangalore, India using Illumina MiSeq version 2 systems. Generated Fastq files were validated, and filtered data was subjected to de novo assembly using CLC Genomics Workbench version 6.5[14]. Obtained result was subjected to Blast in NCBI and found to carrying viral genome with the host genome. Hence, viral genome was separated by mapping with reference genome EBV-B95-8 in CLC Genomics Workbench version 6.5 and progressive alignments made using Mauve v2.3.1 with reference genome EBV-B95-8 resulted into 10 contigs [15]. Obtained viral genome was annotated using Prokka tool [16] and manual blast search with CLC Genomics Workbench version 6.5 and NCBI server.

Annotation of VRF_EBV01 genome resulted in 48 hypothetical proteins in the total genome. The genome was comprised of 3 set of genes encoding for Epstein-Barr nuclear antigen 3A and 3B and each single gene sequences encoding for Epstein-Barr nuclear antigen 1, 4, 4B and DNA primase UL70 protein. Single copy of genes encoding for each variant of Tegument protein BSRF1, BLRF2, BGLF2 and mir-BART 1,2,3,5,7,12,15,17,20 and two copies of gene coding for primary envelopment factor BFRF1 revealed from the annotation study.

Since, the available EBV genome sequences are scanty with different origins from all over the world comprised of host genome sequence. Report from this study will fetch into knowledge on EBV genome sequence from Indian origin, which may lead to better understanding of viral genome adaptation and lineation to its environment.

Nucleotide sequence accession number

The draft genome sequence of the Epstein Barr virus strain VRF_EBV01 is available in NCBI under the accession number KM269735-KM269744.

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CONFLICT OF INTERESTS

Declared None

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