Establishment of a Novel Equine Cell Line for Isolation and Propagation of Equine Herpesviruses

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ABSTRACT. In the present study, an equine-derived cell line was established by transfecting primary fetal horse kidney (FHK) cells with expression plasmid encoding simian virus 40 (SV40) large T antigen and then cloning them by limiting dilution. The cloned cell line, named FHK-Tcl3, grew well and could be propagated over 30 times by splitting them 1:3. Equine herpesvirus (EHV)-1 and EHV-4 replicated well in FHK-Tcl3. EHV-2 and EHV-4 were isolated from samples collected from horses in the field using FHK-Tcl3, and EHV-3 also propagated in FHK-Tcl3. These results indicated that this novel cell line, FHK-Tcl3, can be used for isolation and propagation of equine herpesviruses.

KEY WORDS: cell line, equine, equine herpesvirus.

Equine herpesvirus (EHV)-1 can propagate well in many cell lines, including rabbit- and bovine-derived cell lines (RK13 and MDBK cells, respectively). However, it has been reported that EHV-1 propagated in non-equine cells possesses a mutation in the glycoprotein C gene and becomes more susceptible to heparin [5]. In addition, EHV-4 only replicates well in equine-derived cells. Therefore, cells derived from horses should be used for isolation and research of EHV.

Primary cells from the fetal horse kidney (FHK) cells seem to be the most useful type of cell for isolation and propagation of EHV. However, obtaining constant supply of freshly prepared FHK cells is difficult, because a pregnant mare is needed as the source of the fetal horse kidney. On the other hand, only one cell line, E. Derm (NBL-6) cell (ATCC number: CCL-57), is available from American Type Culture Collection (ATCC). However, E. Derm cells grow slowly and have a limited propagation ability (less than 40 passages). In addition, only E. Derm cells from passage No. 31 were available at the point of this study from Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). Therefore, we attempted to establish a novel cell line for isolation and research of EHV.

Primary FHK cells were maintained in Dulbecco’s modified Eagle’s minimal essential medium (DMEM) supplemented with 10% of fetal calf serum and streptomycin and 100 µg/ml of penicillin. For transformation, an expression plasmid DNA encoding the large T antigen of replication origin-defective simian virus 40 (SV40) was transfected to the FHK cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, U.S.A.) and the transfected cells were selected by addition of 200 µg/ml of G418. The cells were maintained in selective medium and then cloned by limiting dilution. Eight different clones were obtained; one of these clones, FHK-Tcl3, was used for further experiments.

For comparison of cell growth, approximately 2.5 × 10^5 FHK-Tcl3 cells (passage No. 24) and E.Derm cells (NBL-6, passage No. 33) were cultured in 35 mm dishes. The number of cells was counted each day, and the results showed that the FHK-Tcl3 cells grew faster than E.Derm cells and became almost confluent in the 35 mm dishes on days 4 (Fig. 1). In addition, the EHK-Tcl3 cell line grew well over 30 passages, and then growth gradually slowed.

To examine viral growth in FHK-Tcl3, EHV-Tcl3 cells infected with EHV-1 89c25p [4] and EHV-4 TH20p [2] at a multiplicity of infection of 0.1, and the virus titer in the culture supernatant was then determined. The result showed that both viruses can grow well in FHK-Tcl3 cells and that the peak of virus growth was 2–3 days after inoculation (Fig. 2). Figure 3 shows the clear cytopathic effect (CPE) of EHV-1 and EHV-4 on FHK-Tcl3. Although the viral growth in FHK-Tcl3 was not compared with that in primary FHK cells, the viral growth and CPE seemed to be similar.

Next, we attempted to isolate herpesviruses from horses in the field using FHK-Tcl3 cells. Peripheral blood cells were collected from a healthy riding horse and co-cultured with FHK-Tcl3. CPE was observed after one blind passage. The genomic DNA was extracted from the virus-infected cells by treatment with 100 µg/ml of proteinase K and 0.1% sodium dodecyl sulfate, purified with phenol/chloroform,
and then precipitated with ethanol. PCR was carried out using the following EHV-2-specific primers: EHV-2-P1 5’-GGCAGTGAACCC ATACTG-3’ and EHV-2-P2 5’-AAAACCATCCTGCTCAAC-3’ [1]. Only one band of approximately 1.3 kilobase pairs was detected (data not shown). Next, nasal swab samples from 3 Thoroughbred yearlings with respiratory symptoms were tested. The viruses isolated from all 3 yearlings were confirmed to be the EHV-4 strain by EHV-4-specific PCR as described previously [3] (data not shown). These results indicated that this FHK-Tcl3 cell can be used for virus isolation.

Furthermore, FHK-Tcl3 cells were also infected with the EHV-3 ECE-V-T2 strain, kindly provided by the National Institute of Animal Health of Japan. The virus titer for EHV-3 at 5 days post-infection was $2.5 \times 10^5$ PFU/ml. However, the virus titer in the FHK-Tcl3 cells was less than that in the parental FHK cell ($1 \times 10^7$ PFU/ml).

In conclusion, the cell line established in the present study, FHK-Tcl3, can be used for isolation and propagation of equine herpesviruses. In particular, since EHV-4 grows poorly in non-equine cells, the FHK-Tcl3 cell line would be useful for diagnosis and research of EHV-4. Although propagation of the FHK-Tcl3 cells was less than 40 times as
effective as propagation of E. Derm (NBL-6) cells, the cell
growth of the FHK-Tcl3 cells was better than that of the E.
Derm (NBL-6) cells. In addition, FHK-Tcl3 cells should be
useful for research of other equine viruses.

ACKNOWLEDGEMENTS. This study was supported by a
Grand-in-aid from the Ministry of Education, Culture,
Sports, Science and Technology of Japan. We would like to
thank the National Institute of Animal Health of Japan for
providing EHV-3.

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