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RESEARCH PAPER

Construction of Recombinant Retroviral Vector Carrying *Lab* Gene of Foot-and-mouth Disease Virus and Its Expression in Bovine Kidney (MDBK) Cells

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Abstract: In this study, foot-and-mouth disease virus (FMDV) strain OA/58 RNAs were used as templates for RT-PCR. By the molecular cloning, the *Lab* gene encoding leader protease called L^{pro} were cloned in retroviral vector pBPSTR1 to obtain reconstruction retroviral vector termed pBPSTR1-Lab. At different concentrations of puromycin and tetracycline respectively in the cell culture mediums, the growth of bovine kidney cells (MDBK) showed that the optimal puromycin resistant selection concentration was 3 μg/mL and tetracycline regulatory concentration was 1 μg/mL. Pseudotyped retroviral virus particles were produced by transiently co-transfecting GP2-293 cells with a retroviral vector DNA and VSV-G plasmid. Then MDBK cells were infected by pseudotyped retroviral virus and were continually seeded in the medium at the optimal tetracycline regulatory concentration and puromycin selection concentration for 12 days to obtain puromycin resistant colonies whose genomes contained the *Lab* gene. After tetracycline removal, synthesis of L^{pro} induced severe morphological changes in the puromycin resistant MDBK cells. PCR and Western blotting proved that a stable MDBK cell line inducibly expressing the *Lab* gene under the control of tetracycline was obtained. The experiment might provide a basis for studying that L^{pro} of FMDV plays an important role in MDBK cell pathogenesis.

Keywords: foot-and-mouth disease virus; leader protease; puromycin; tetracycline; pBPSTR1

Introduction

Foot-and-mouth disease virus (FMDV) belongs to the *Aphthovirus* genus of the family *Picornaviridae*. The virus contains a positive-sense RNA genome, which has some functions like an mRNA and encodes a viral polyprotein. The FMDV genome is about 8500 nt in length. When the viruses infect host cell, the genome which contains a single long open reading frame (ORF), encodes all its proteins in the form of a polyprotein. The encoded polyprotein can be processed into several nonstructural proteins and four kinds of structural proteins by three viral proteinases (L^{pro}, 2A, and $3C^{pro}$). The L^{pro} is coded for by the 5′-end of the ORF, and cleaves itself from the polyprotein to turn into an active proteinase[1]. Within the L^{pro} coding region of all serotypes of FMDV are two in-frame AUG codons that encode proteins called Lab and Lb. Although both Lab and Lb have been detected *in vitro* as well as in infected cells, the latter is the major protein synthesized *in vivo*. During the course of identifying the nature of L^{pro} is a thiol-type proteinase related to the papain family of proteinase[2]. When the L^{pro} releases itself from the nascent polyprotein, it can act as a *trans*-proteinase in the

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form of a dimmer, which cleaves the host-cell translation factor eIf4G by Cys and His, resulting in the shut-off of host cap-dependent mRNA translation[3–5].

Nowadays, the technology of infectious cDNA clone plays a major role in studying the L^{pro} functions. The Plum Island group deleted L from the infectious copy of FMDV serotype A12 and suggested that L^{pro} is an important determinant of virulence[6]. In addition, nonstructural protein gene for *3ABC* of FMDV has expressed in sf9 cells and its activity has been analyzed by Ma et al[7]. More and more researchers have focused on the functions of nonstructural proteins of FMDV. However, there is no report that a stable bovine kidney (MDBK) cell line, which inducibly expresses FMDV L^{pro}, has been obtained to study the relationship between L^{pro} virulence and host-cell cytopathic.

To further analyze the effects of FMDV L^{pro} on both gene expression and ongoing cellular translation, we decided to use a tetracycline (tet)-dependent expression system[8] and a puromycin-dependent screen system[9]. The Tet-off system can be used to express FMDV Lpro avoids the presence of other viral nonstructural proteins, which characterizes most eukaryotic expression system thus far analyzed. To this end, stably transformed MDBK cell clones that express FMDV L^{pro} under the control of an inducible, tightly regulated promoter were obtained. As a result, a biochemical and morphological analysis of the consequences of FMDV L^{pro} expression in MDBK cells has been carried out.

1 Materials and methods

1.1 Cells and virus

The following cell lines were used: bovine kidney cells (MDBK) and packaging cells GP2-293 (National foot-and-mouth disease reference laboratory collection). MDBK cells were grown in DMEM medium (HyClone, Beijing.) containing 10% newborn calf serum (SiJiQing, Hangzhou.). The packaging cell GP2-293 was in DMEM medium supplemented with 15% fetal bovine serum (HyClone, Southern American).

The pathological tissue containing FMDV OA/58 strain was collected in the virus strains center of national foot-and-mouth disease reference laboratory. Following the RNeasy Mini Kit (Qiagen) instruction, the FMDV OA/58 strain RNAs were extracted from the pathological tissue. Also, consulting for RNA PCR Kit (AMV) Ver.3.0 (TaKaRa Biotechnology Company) instruction, FMDV OA/58 strain RNAs were used as templates for RT-PCR.

1.2 Construction of retroviral plasmid pBPSTR1-Lab

Using the OLIGO software to design up and down primers for the L^{pro} gene of FMDV OA/58 strain, L1 was an up-primer containing a new *Not* I site, and L2 was a down primer adding a new *Bam*H I site. (L1, L2 were sent to TaKaRa Biotechnology Company for making) L1: 5′-ACGCGGCCGCCATGGACACAACTGATTG-3′ (the underlined nucleotides were the *Not* I site). L2: 5′-AAGGATCCTCACTTGAGCCGCTTCTG AAC-3′ (the underline nucleotides were the *Bam*H I site). The coding sequence of L^{pro} was initially PCR generated using the cloned full-length cDNA of FMDV OA/58 strain as template and L1, L2 primers. First, the PCR product was cloned into the pGEM-T Easy Vector (Promega). The resulting plasmid was transformed and amplified in E. *coli* JM109 competent cells (TaKaRa), and subsequently purified by B Type: Mini-Plasmid Rapid Isolation Kit (Bio Dev). The purified plasmid was digested with *Not* I /*Bam*H I restriction enzymes to create the L^{pro} insert, which was subsequently ligated into *Not* I /*Bam*H I cloning site of the pBPSTR1 retroviral vector and transformed to E. *coli* JM109 competent cells (TaKaRa). The resulting plasmid (pBPSTR1-Lab) was purified by B Type: Max-Plasmid Rapid Isolation Kit (Bio Dev).

1.3 Test of selection density and regulation density

When MDBK cells were 80% confluent, they were grown in DMEM medium containing 10% newborn calf serum and different densities of puromycin dihydrochioride (Sigma) including 1 μg/mL, 2 μg/mL, 3 μg/mL, 4 μg/mL, 5 μg/mL, 6 μg/mL, 7 μg/mL, 8 μg/mL, 9 μg/mL, and 10 μg/mL to continually select MDBK cells for 12 days. The optimal selecting mini-density was defined as that MDBK cells were all killed in the cell culture medium with the mini density of puromycin dihydrochloride on the 12^{th} day. Following the lipid-mediated transfection instruction, the MDBK cells were transfected with retroviral plasmid pBPSTR1-Lab and were grown in cell culture medium containing different densities (0.25 μg/mL, 0.5 μg/mL, 0.75 μg/mL, and 1 μg/mL) of tetracycline hydrochloride (Sigma) to determine the optimal regulation density.

1.4 Transfection and generation of reconstruction viruses

Briefly, 10^6 packaging cells GP2-293 were plated in 10 mL of cell culture medium with 5% CO_2 at 37°C. After 24 h, the scale of packaging cells GP2-293 reached 80% confluent. Cells were transfected with 5μg of retroviral plasmid pBPSTR1 and 5 μg of the packaging plasmid pVSV-G, using the lipid-mediated transfection technique. Seven hours post-transfection, the medium was removed and 20 mL of fresh cell culture medium was added. Cells were grown in this medium for 72 h, and the medium contained reconstruction viruses. Finally, the medium was stored at –80°C.

1.5 Generation of stably transformed MDBK cells bearing the FMDV L^{pro} gene

First, 10^6 MDBK cells were seeded in a 100 mL cell culture bottle. After 18 h, the confluent of MDBK cells