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Research Article

Establishment and Evaluation of a Suckling Mouse Integrin $\alpha_v\beta_8$ Transgenic CHO-677 Cell Line with Increased Susceptibility to Type O/BY/CHA/2010 Foot-and-Mouth Disease Virus

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Abstract

Integrin $\alpha_v\beta_8$ may play an important role in the initial stage of FMDV infection and influence the host specificity and tissue tropism. To study the role of integrin $\alpha_v\beta_8$ in FMDV infection, a cell line, which stably expressed the suckling mouse αvβ8 heterodimer, was established and designated as CHO-677-m$\alpha_v\beta_8$. Polymerase chain reaction (PCR) and indirect immunofluorescent assay (IFA) were used to determine the presence of integrin subunits α_v and β_8 in the cell line at the 20th passage, respectively. The results showed that these genes were successfully transduced and expressed in the cell line. Biological characteristics of FMDV were determined in the cell line. After the cell line was infected with FMDV type O/BY/CHA/2010, there was an obvious increase for viral RNA and proteins, compared with those in the parental cells. Our data showed that a stable cell line was successfully established. Additionally, the results of antibody blockade assay show that the cells treated with anti-β_8 serum had a lower virus titre, compared to the mock-treated cells, implying that the β_8 was important for maintaining $\alpha_v\beta_8$ in receptor function required for productive infection by FMDV. The cell line may be used to isolate the FMDV, study the function of integrin $\alpha_v\beta_8$ in infection and entry of FMDV.

Keywords: *$\alpha_v\beta_8$, Cell line, Foot-and-Mouth Disease Virus, Integrin receptor, Mouse*

Tip O/BY/CHA/2010 Şap Hastalığı Virusuna Karşı Artırılmış Duyarlılıkta Süt Emen Fare $\alpha_v\beta_8$ Transgenik CHO-677 Hücre Hattının Oluşturulması ve Değerlendirilmesi

Öz

İntegrin $\alpha_v\beta_8$ Şap hastalığının ilk aşamasında önemli bir rol oynayabilir ve konak spesifikliği ile doku tropizmini etkileyebilir. Şap hastalığında integrin $\alpha_v\beta_8$'ınrolünü araştırmak amacıyla stabil olarak süt emen fare αvβ8 heterodimer eksprese eden bir hücre hattı geliştirildi ve CHO-677-m$\alpha_v\beta_8$ olarak adlandırıldı. Yirminci pasajda hücre hattında α_v ve β_8 integrin subünitlerinin varlığını belirlemek amacıyla sırasıyla polimeraz zincir reaksiyonu ve immunofloresan teknikleri kullanıldı. Sonuçlar bu genlerin hücre hattına başarıyla aktarıldığını ve eksprese edildiğini gösterdi. Şap hastalığı virusunun biyolojik karakterleri hücre hattında belirlendi. Hücre hattı Şap hastalığı virusu tip O/BY/CHA/2010 ile enfekte edildikten sonra hücre hattında viral RNA ve proteinleri parental hücreler ile karşılaştırıldığında belirgin olarak artmıştı. Elde edilen veriler stabil bir hücre hattının başarıyla oluştuğunu gösterdi. Ayrıca, antikor blokajı yönteminin sonuçları, anti-β_8 serumu uygulanan hücrelerin plesibo uygulanan hücrelerle karşılaştırıldığında daha düşük virus titresine sahip olduğunu ve böylece Şap hastalığı virusu ile hastalık oluşturmak için gerekli reseptör fonksiyonunda $\alpha_v\beta_8$'nin oluşturulmasında β_8'in önemli olduğunu gösterdi. Bu hücre hattı Şap hastalığı virusunun izolasyonunda, enfeksiyonda integrin $\alpha_v\beta_8$'in fonksiyonunun ve virusun hücreye girişinin çalışılmasında kullanılabilir.

Anahtar sözcükler: *$\alpha_v\beta_8$, Hücre hattı, Şap hastalığı Virusu, İntegrin reseptörü, Fare*

INTRODUCTION

Foot-and-mouth disease (FMD) is a highly infectious disease of domestic and wild cloven-hoofed animals, including cattle, swine, sheep, goats, and over 70 species of wild animals. The causative agent, foot-and-mouth virus (FMDV), belongs to

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a member of the family *Picornaviridae*, genus *Aphthovirus*, and has at least seven serological types, A, C, O, Asia1, South Africa serotype (SAT)1, 2, and 3, and multiple subtypes [1]. The virus is a non-enveloped particle of icosahedral symmetry containing a single-stranded, positive-sense RNA genome approximately 8.5 kb in length. There is a single open reading frame (ORF) in the viral genome, which is translated into ten nonstructural proteins (NSP) and four structural proteins [2,3].

The recognition of specific receptors on the cell plasma membrane by proteins on the virus surface is necessary for virus attachment and subsequent infection [4,5]. Thus far, four integrins ($\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_6$, and $\alpha_v\beta_8$) have been demonstrated by several laboratories to be FMDV receptors [6-9]. Integrin $\alpha_v\beta_8$ is the fourth integrin receptor identified for FMDV and may play an important role in the initial stage of FMDV infection and influence the host specificity and tissue tropism. While the natural hosts of FMDV include domestic and wild cloven-hoofed animals, the suckling-mouse is one of the most important experimental animals in the laboratory [1]. Thus, in the present study, suckling-mouse integrin α_v and β_8 subunits were cloned, and the Chinese hamster ovary 677 (CHO-677) cell line was transduced to stably express suckling-mouse integrin $\alpha_v\beta_8$ using a highly efficient lentivirus-based gene transfer technology [10]. The CHO-677 cell line expresses neither four integrin receptors nor heparin sulfate (HS) receptor for FMDV and is usually used in the studies of receptor pathway for FMDV [11-13]. IBRS-2 cell doesn't only expresses single integrin $\alpha_v\beta_8$ of four integrin receptors, but also HS receptor. Therefore, it is necessary for establishing the CHO-677-m$\alpha_v\beta_8$ cell line.

MATERIAL and METHODS

Cells, Virus and Antibodies

Human embryonic kidney (HEK) 293T cells and HS-deficient Chinese hamster ovary (CHO-677 or pgsD-677, ATCC, CRL-2244) cells were cultured in Ham'S/F-12 (SH30026.01, HyClone, USA) medium supplemented with 10% fetal bovine serum (FBS, SH30070.03, HyClone, USA), 1% streptomycin (0.2 mg/mL) and penicillin (200 U/mL). Baby hamster kidney (BHK-21, ATCC, CCL-10) cells were maintained in Eagle's Minimal essential Medium (EMEM, Invitrogen, USA) containing 10% FBS. All the cells were incubated at 37°C with 5% CO_2.

Foot-and-mouth virus type O/BY/CHA/2010 (GenBank accession No. JN998085.1) was isolated from a naturally infected pig in the city of Baiyun in Guangdong Province, China, during the 2010 outbreak and was propagated on BHK-21cells.

Guinea pig anti-FMDV serum and rabbit polyclonal anti-serum directed against mouse integrin subunit α_v (anti-α_v serum) and β_8 (anti-β_8 serum) were obtained from the Lanzhou Veterinary Research Institute. Fluorescein isothiocyanate (FITC)-conjugated goat anti-guinea-pig IgG antibody (F6261) and FITC-conjugated goat anti-rabbit IgG antibody (F0382) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cloning and Sequencing The Integrin Subunit α_v and β_8Genes

Genomic RNA was extracted from the tongue or lung tissues of suckling mice using an RNeasy Mini kit (74104, Qiagen, Hilden, Germany), according to the manufacturer's specifications. The use of all animals in this study was approved by the Review Board of Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences. cDNA was synthesized from the extracted RNA with AMV reverse transcriptase (12328-019, Invitrogen, USA) using random primers (20 pmol/mL), and used as the template for the amplification of the α_v and β_8 transcripts with PCR. The PCR primer pairs, α_vF/α_vR and β_8R/β_8F are shown in *Table 1*. The PCR reaction system was: 25 μL Premix LA Taq(RR903A, Takara Bio, Japan), 1 μL primer (10 pmol/L), 2 μL DNA, 21 μL dH_2O. The cycling parameters were: 40 cycles of denaturation at 95°C for 3 min, annealing at 58°C for 30 sec, and the elongation at 72°C for 4 min, followed by a final elongation step at 72°C for 10 min before the reaction was cooled to 16°C for further processing. The amplicon was purified with the MiniBEST Agarose Gel DNA Extraction KitVer.4.0 (9762, Takara Bio, Japan) and cloned separately into the pGEM-T Easy vector (A1360, Promega, Shanghai, China). The positive plasmids were sequenced in both directions by GeneWizInc (Suzhou, China).

Construction of the Recombinant Lentiviral Plasmid

The recombinant plasmid was constructed as previously described [14]. Briefly, pOK_{12} and the internal ribosome entry site (IRES) fragment were amplified from pOK_{12} and the pIRES2-EGFP plasmid (stored in our laboratory), respectively, with the corresponding primer pairs *(Table 1)*, and the IRES fragment was then cloned into pOK_{12} to generate pOK-IRES. The α_v PCR product was digested by the restriction enzymes *Not*(1623) and *Nhe*(1622) (Takara Bio, Japan), and cloned into the pOK-IRES plasmid to generate the recombinant plasmid pOK-α_v-IRES, which was digested to obtain the α_v-IRES fragment by the restriction enzymes *Not*(1623) and *Xba*(1634) (Takara Bio, Japan). This fragment was then cloned into the pLVX-Tight-Puro vector (Clontech, USA) to generate the recombinant plasmid pLVX-α_v-IRES. The amplified β_8 fragment was digested by the restriction enzymes *Xba*(1634) and *Mlu*(1619) (Takara Bio, Japan), and cloned into pLVX-α_v-IRES to generate the recombinant lentiviral plasmid pLVX-α_v-IRES-β_8. All the products were sequenced by GeneWiz Inc.

Establishment of The CHO-677-m$\alpha_v\beta_8$Cell Line

Using the Lenti-X™ Tet-Off® Lentiviral Advanced Induced

Table 1. *Oligonucleotides used in this study*

Name	Nucleotide sequence (5′-3′) [a, b]	Genome Position
α_vF	ATGGCTGCTCCCGGGCGC	1-18
α_vR	TCAGGTTTCAGAGTTTCCT	3117-3135
β_8F	ATGTGCGGCTCGGCCCTGGCT	1-21
β_8R	TTAGAAGTTGCACCTGAAG	2286-2304
IRESF IRESR pL-α_vF	CTAGCTAGCGCCCCTCTCCCTCCCCCCCCCCTAA CGGTCTAGATGTGGCCATATTATCATCGTGT TTTGCGGCCGCGCCACCATGGCTGCTCCCGGGCGC	1-25 562-584 1-18
pL-α_vR	CTAGCTAGCTCAGGTTTCAGAGTTTCCTTCGCCATT	3117-3143
pL-β_8F	CGGTCTAGAGCCACCATGTGCGGCTCGGCCCTGGCT	1-20
pL-β_8R	TCGACGCGTTTAGAAGTTGCACCTGAAG	2343-2364

[a] *Red letters represent restriction enzyme site;* [b] *Blue letters represent Kozak sequence*

Expression System (TakaraBio, Japan), we transduced 293T packaging cells with the lentiviral vector pLVX-α_v-IRES-β_8 to yield the disguised lentivirus, which was then used to infect the CHO-677 target cells according to the Lentiviral Expression System User Manual. To establish a stable CHO-677-m$\alpha_v\beta_8$ cell line, a single clone was cultured under selection with 500 mg/mL G418(E859, Amresco, US) and 2 mg/mL puromycin (P9620, Sigma-Aldrich, St. Louis, MO, USA). After approximately 2 weeks, cell cloning islands were observed. After 20 rounds of continuous cloning, a stable $\alpha_v\beta_8$-transgenic CHO-677 cell line was obtained and designated as "CHO-677-m$\alpha_v\beta_8$". The presence of α_v and β_8 genes in the CHO-677-m$\alpha_v\beta_8$ cells at the twentieth passages were analyzed by PCR using the primers (α_vF, α_vR and β_8F, β_8R) *(Table 1)*. The PCR reaction system was: 12.5 μL Premix LA Taq, 0.5 μL primer (10 pmol/L), 0.5 μL template, 11 μL dH_2O. The cycling parameters were: 30 cycles of denaturation at 95°C for 3 min, annealing at 58°C for 30 sec, and the elongation at 72°C for 4 min, followed by a final elongation step at 72°C for 10 min. Furthermore, the expression of integrin subunits α_v and β_8 in CHO-677-m$\alpha_v\beta_8$ cells were confirmed by an indirect immunofluorescence assay (IFA).

Growth Characterization of FMDV Type O/BY/CHA/2010 in CHO-677-m$\alpha_v\beta_8$Cell Line

In order to examine the functional activity of $\alpha_v\beta_8$ in CHO-677-m$\alpha_v\beta_8$ cell line, FMDV was used to infect the cell line. Plaque assay, growth kinetic, IFA and quantitative real-time PCR (qRT-PCR) of FMDV type O/BY/CHA/2010 were analyzed in the CHO-677-m$\alpha_v\beta_8$ cell line.

Plaque Assay

CHO-677-m$\alpha_v\beta_8$ and the parental CHO-677 cells were seeded in six-well cell culture plates 48 h before infection. Dilutions (10-fold) of the virus were prepared in Ham's F-12 medium. The inoculum volume was 200 mL per well. After One hour adsorption, the culture medium was removed, and then the cells were overlain with 50% gum tragacanth(G1128, Sigma-Aldrich, St. Louis, MO, USA) and 50% 2′ minimal essential medium supplemented with 2% fetal bovine serum. The plates were incubated for 48 h, fixed with acetone and methanol (1:1), and stained with crystal violet (Histochoice, Amresco, Solon, Ohio).

Growth Kinetics of the Virus

Growth kinetic of the virus was analyzed in BHK-21 cells. The parental and m$\alpha_v\beta_8$ expressing cells were infected with FMDV type O/BY/CHA/2010 at the same multiplicity of infection (MOI=1), and the virus was allowed to adsorb for 1 h at 37°C. The medium was removed and then the cells were washed. Fresh medium was added. At 12, 24, 36, 48, 60, and 72 h post-infection (hpi), the plates were freeze/thawed three times and the viral titers in cell culture media were determined by $TCID_{50}$ on BHK-21 cells using the Reed-Muench method [15]. Three independent experiments were performed and the mean value was used to determine the viral growth curve.

qRT-PCR

To determine the level of viral replication, the parental and m$\alpha_v\beta_8$ expressing cells were prepared in triplicate as described above and the viral supernatants were harvested at the indicated times after infection. Total RNA was extracted from the cells with the QIAxtractor kit (Qiagen), according to the manufacturer's instructions. qRT-PCR was performed with the StrataGene Mx3000P® Real-time PCR System (Agilent, Santa Clara, USA) using the SYBR Premix Ex Taq Kit (TaKaRa), as described previously [16].

IFA

Viral proteins were detected with IFA. CHO-677-m$\alpha_v\beta_8$ and CHO-677 cells were seeded on 35 mm diameter plates, grown to approximately 80% confluence, and then

infected with FMDV type O/BY/CHA/2010 at MOI=1. At 24 hpi, the cells were fixed, permeabilized, and blocked as described previously [17]. The cells were incubated with guinea pig anti-FMDV serum (1:500 dilution) at 37°C for 2 h, and then reacted with FITC-conjugated goat anti-guinea-pig IgG antibody (1:500 dilution) for 1.5 h in a dark room. The cell nuclei were stained by the 4′, 6-diamidino-2-phenylindole (DAPI, Invitrogen).The treated cells were viewed by the microscope.

The Effect of Antibody Blockade for β_8 Subunit in FMDV Infection

Antibody blockade assay was performed to examine the role of β_8 subunit in the FMDV infection. CHO-677-m$\alpha_v\beta_8$ cells were seeded in 35-mm-diameter dishes and incubated for 48 h at 37°C with 5% CO_2. Confluent cell monolayers were rinsed with PBS (pH 7.5, 2 mM $CaCl_2$, 1 mM $MgCl_2$). The cells were divided into two groups and there were three dishes in each group. One group was incubated with PBS as control; while the other was incubated with the anti-β_8 serum (1:500 dilution). After 1.5 h, the supernatants were removed, and then both groups were infected with FMDV type O/BY/CHA/2010 (MOI=2) at 37°C. At 24 hpi, the titer of virus in the cell culture media was determined by $TCID_{50}$ on BHK-21 cells.

RESULTS

Cloning and Sequencing The Integrin Subunit α_v and β_8 Genes

To study the integrin $\alpha_v\beta_8$ of suckling mouse, we amplified the integrin subunit α_v and β_8 genes from the tongue or lung tissues, respectively *(Fig. 1)*. The results of DNA sequencing confirmed that the sequences of both genes were consistent with the corresponding one from the GenBank.

Construction of Lentivirus Recombinant Plasmid

In order to confirm the lentiviral recombinant plasmid pLVX-α_v-IRES-β_8 was built successfully, double enzyme digestion experiments were performed. The pLVX-α_v-IRES-β_8 plasmid was digested by *Not*I and *Nhe*I to identify the α_v fragment, and digested by *Xba*I and *Mlu*I to identify the β_8 fragment. As shown in *Fig. 2*, the results of double enzyme digestion were verified by electrophoresis.

Construction of the CHO-677-m$\alpha_v\beta_8$ Cell Line

To detect the presence of the suckling mouse integrin subunits α_v and β_8 at the gene in CHO-677-m$\alpha_v\beta_8$ cell line, α_v and β_8 genes at the twentieth passage were amplified by PCR *(Fig. 3A)*. The sequencing results proved the identity of the PCR product. Furthermore, the expression of integrin $\alpha_v\beta_8$ protein in CHO-677-m$\alpha_v\beta_8$ cell line was confirmed by IFA *(Fig. 3B)*, demonstrating that CHO-677-m$\alpha_v\beta_8$ cell line was successfully established.

Growth Characterization of FMDV Type O/BY/CHA/2010 in CHO-677-m$\alpha_v\beta_8$ Cell Line

To study the functional features of $\alpha_v\beta_8$, the cells were infected with FMDV type O/BY/CHA/2010 to analyze viral

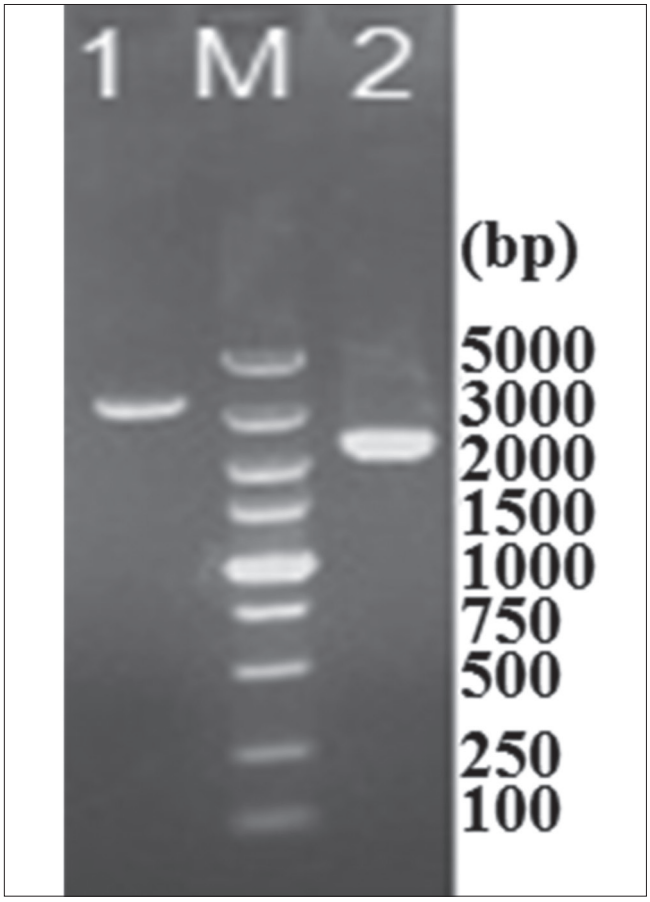

Fig 1. PCR products of both subunit genes of suckling mouse integrin $\alpha_v\beta_8$.Lane1: α_v gene fragment (3135bp.); Lane2: β_8 gene fragment (2304bp.); Lane M: 5000 bp. DNA Ladder

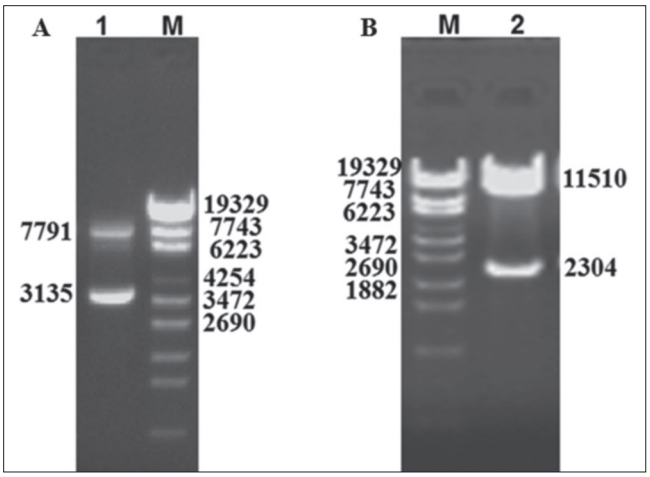

Fig 2. Identification of the recombinant plasmid pLVX-α_v-IRES-β_8 by double enzyme digestion. Lane1: Double enzyme digestion identification about the α_v fragment. Lane 2: Double enzyme digestion identification about the β_8 fragment. Lane M: DNA molecular weight marker λ-EcoT14 I

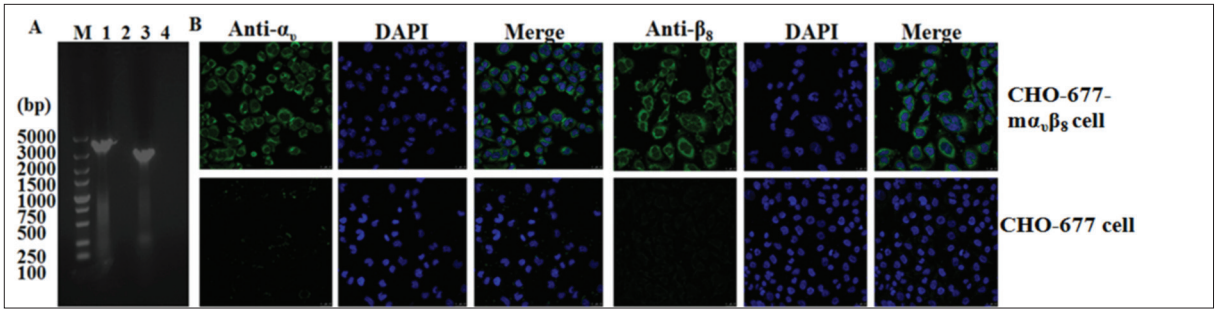

Fig 3. Identification of CHO-677-mα$_v$β$_8$ cell line. A. Detection of integrin subunit α$_v$ and β$_8$ genes in CHO-677-mα$_v$β$_8$ cell line at the twentieth passage. The PCR products of α$_v$ and β$_8$genes (lane 1 and 3, respectively) are shown, relative to the negative controls for the α$_v$ and β$_8$ genes (lanes 2 and 4, respectively). Molecular weight marker in lane M. B. Expression of integrin subunits α$_v$ and β$_8$ were detected in CHO-677-mα$_v$β$_8$ cells at the twentieth passage by IFA. Photographs were taken at a magnification of 40 fold. One representative experiment is shown

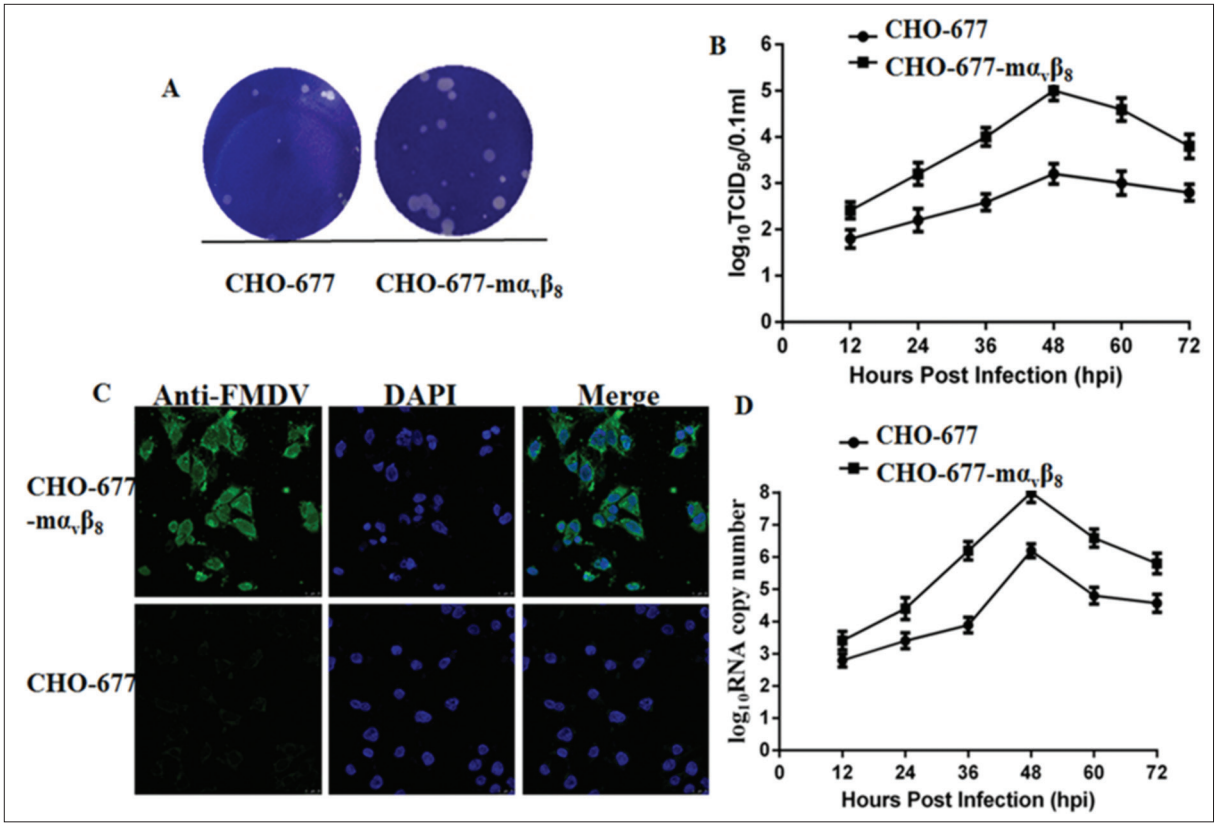

Fig 4. Growth characteristics of FMDV type O/BY/CHA/2010 in CHO-677-mα$_v$β$_8$ and CHO-677 cells. A. Plaque morphology of CHO-677-mα$_v$β$_8$ and CHO-677 cells after inoculation with FMDV type O/BY/CHA/2010. B. Growth kinetics of FMDV type O/BY/CHA/2010 on CHO-677-mα$_v$β$_8$ and CHO-677 cells. C. Immunofluorescence analysis of FMDV type O/BY/CHA/2010 on CHO-677-mα$_v$β$_8$ and CHO-677 cells. The virus proteins were probed with guinea pig anti-FMDV serum and then reacted with FITC-conjugated goat anti-guinea pig IgG antibody. D. The levels of type O/BY/CHA/2010 FMDV RNA copy numbers were determined in infected CHO-677-mα$_v$β$_8$ and CHO-677 cells at different time points by real-time PCR

growth characteristics. Plaque assays were performed to compare the plaque size and quantity produced in CHO-677-mα$_v$β$_8$ and CHO-677 cells, and the plaque phenotypes and FMDV yields were characterized in both cells. As shown in *Fig. 4A*, the virus produced more plaques in the CHO-677-mα$_v$β$_8$ cells than in the CHO-677 cells.

In order to compare the *in vitro* growth characteristics of FMDV type O/BY/CHA/2010 in the CHO-677-mα$_v$β$_8$ cell line and the parental cells, samples were collected at 12h intervals, up to 72 hpi and $TCID_{50}$ was titrated on BHK-21 cells. The peak titers of FMDV type O/BY/CHA/2010 were $10^{5.0}$ $TCID_{50}$/0.1 mL in CHO-677-mα$_v$β$_8$ cells at 48 hpi and $10^{3.2}$ $TCID_{50}$/0.1 mL in the parental cells at 48 hpi *(Fig. 4B)*. We then determined the expression of viral proteins with IFA. Intracellular cytoplasmic fluorescence was detected in the infected CHO-677-mα$_v$β$_8$ cell line, while weak fluorescence was detected in the CHO-677 cells *(Fig. 4C)*, indicating the

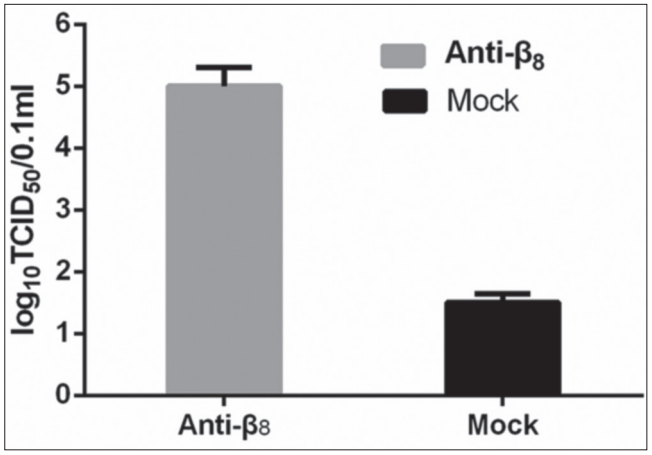

Fig 5. The result of antibody blockade assay. The virus titres were detected by $TCID_{50}$ on BHK cells (mean ± SEM of three independent experiments)

CHO-677- mα$_v$β$_8$ cell line increased the susceptibility to FMDV type O/BY/CHA/2010.

To analyze the replication capacity of the virus, we determined the copy numbers of FMDV type O/BY/CHA/2010 RNAs in both cells with qRT-PCR. As shown in *Fig. 4D*, the parental cells showed clearly lower levels of RNA replication than the CHO-677-mα$_v$β$_8$ cell line. These results indicated that the CHO-677-mα$_v$β$_8$ cells are susceptible to FMDV.

Antibody Blockade Assay

In order to determine whether the integrin subunit β$_8$ plays an important role in FMDV-mediated infection, the antibody of β$_8$ ligand binding domain (LBD) was used to block the integrin subunit β$_8$. Cells treated with anti-β$_8$ serum had a lower virus titer, compared to the mock-treated cells *(Fig. 5)*, indicating that integrin α$_v$β$_8$ increased the susceptibility of CHO-677-mα$_v$β$_8$ cell line to FMDV and that integrin subunit β$_8$ played an important role in FMDV-mediated infection.

DISCUSSION

Many host cells express more than one integrin receptor [18], which may hinder the study of viral infection and invasion mediated by single receptor. Therefore, cells expressing single integrin receptor are necessary for researching individual receptor pathways. PerhapsIBRS-2 cells, which only express integrin α$_v$β$_8$, are commonly used to study FMDV infection mediated by integrin α$_v$β$_8$ [12,19]. However, there is no representative cell from other hosts, which only express integrin α$_v$β$_8$. Suckling mice are widely used as experimental animals for FMDV. For example, serial passages and determining the LD_{50} of FMDV both require suckling mouse as experimental animals. Moreover, the suckling mouse integrin receptor genes have high sequence homology to swine, bovine and camel counterparts. Therefore, we cloned the full-length cDNAs of the integrin subunits α$_v$ and β$_8$ from the suckling mouse, and established a CHO-677-mα$_v$β$_8$ cell line stably expressing suckling mouse integrin α$_v$β$_8$.

Integrin is a family of allosteric, heterodimeric, transmembrane glycoproteins that regulate cell-cell, cell-extracellular matrix, and sometimes cell-pathogen interactions [20,21]. Miller et al.[22] studied the role of the β$_6$ cytoplasmic domain in infection, showing that α$_v$β$_8$ not only contributed to passing the virus onto a next receptor for internalization but also played an important role in the whole infection process. Zhang et al.[23] showed that integrin β$_6$-1 subunit could induce partial protection against FMDV in guinea pigs. Integrin α$_v$β$_8$ was identified as a receptor for FMDV. Zhang et al.[14] established a CHO-677-mα$_v$β$_8$ cell line stably expressing suckling mouse integrin α$_v$β$_8$. Although integrin α$_v$β$_8$ and α$_v$β$_8$ had been studied widely, the study of integrin α$_v$β$_8$ in infection was not well defined. Several reseachers studied the function of integrin by a transient expression system [9,24]. Wang et al. [25]. determined the role of α$_v$β$_8$ and α$_v$β$_8$ in FMDV replication. Although the transient expression system is easy and conveniently manipulated, its repeatability is low. Here, a highly efficient lentivirus-based inducible expression system was used to establish the CHO-677-mα$_v$β$_8$ cell line. As a member of integrin family, α$_v$β$_8$ has many functions, which include the receptor function for FMDV [6]. Therefore, in order to examine the CHO-677-mα$_v$β$_8$ cell line, FMDV was selected to infect the cell line. Our results suggested that the suckling mouse integrin α$_v$β$_8$ increased the susceptibility of CHO-677-mα$_v$β$_8$ cell line to FMDV. In this paper, we used antibody blockade assay to study the role of the β$_8$ subunit in mediated infection, showing that the β$_8$ subunit played a critical role in integrin α$_v$β$_8$-mediated infection. Of course, the CHO-677-mα$_v$β$_8$ cell line should be further determined and evaluated.

In this study, a CHO-677-mα$_v$β$_8$ cell line was established as a cell model for studying the interaction between FMDV and single integrin-expressed cell line. Our results demonstrated that integrin α$_v$β$_8$ expression increased the susceptibility of CHO-677-mα$_v$β$_8$ cell line to FMDV and that the β$_8$ was important for maintaining α$_v$β$_8$ in receptor function required for productive infection by FMDV. The cell line may be used to isolate the FMDV, study the function of integrin α$_v$β$_8$ in infection and entry of FMDV, and so on.

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Competing Interests

The authors declare they have no competing interests.

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