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Establishment of a Murine αυβ1 Transgenic CHO-K1 Cell Line and its Susceptibility to Foot-and-Mouth Disease Virus Type Asia l/HN/2006 in China

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Abstract: Field isolates of Foot-and-Mouth Disease Virus (FMDV) were found to use four αυ integrins (αυβ1, αυβ3, αυβ6 and αυβ8) as cellular receptors. Researchers established a stable Chinese Hamster Ovary clone K1 (CHO-K1) cell line expressing the murine αυβ1 heterodimer (designated as CHO-K1-αυβ1) using a highly efficient lentiviral-based gene transfer technology to deliver murine αυ, Internal Ribosome Entry Site (IRES) and β1 genes into cell chromosomes and the inserted genes were then transcribed from a Cytomegalovirus (CMV) promoter. αυβ1 expression was stringently regulated by Doxycycline (Dox) and was found to be stable. CHO-K1-αυβ1 cells were susceptible to FMDV type Asia l/HN/2006. The plaque assay revealed that the virus produced bigger and more plaques in CHO-K1-αυβ1 cells (1.05×10^{4} PFU mL^{-1}) than in CHO-K1 cells. When sodium heparin (1 and 2 mg mL^{-1}) was used as the inhibitor, the number of plaques in CHO-K1 cells were significantly decreased (4.0×10^{3}-35 and 20 PFU mL^{-1}), supported by time-course of replication and proliferation. The number and size of plaques on CHO-K1-αυβ1 cells showed no obvious change, indicating that the αυβ1 heterodimer expressed on CHO-K1-αυβ1 can be used as an FMDV receptor.

Key words: Foot-and-mouth disease, foot-and-mouth disease virus, integrin, receptor, cellular

INTRODUCTION

Foot-and-Mouth Disease (FMD) is a highly infectious disease caused by the FMD Virus (FMDV) and it affects domestic and wild cloven-hoofed animals, including cattle, swine, sheep and goats. The disease is characterized by the appearance of vesicles on the feet and in and around the mouth (Pega *et al*., 2012). The FMDV belongs to genus *Aphthovirus* in the family Picornaviridae and it exists in seven serotypes (O, A, C, Asia l, SAT1, SAT2 and SAT3) (Rueckert and Wimmer, 1984; Grubman and Baxt, 2004). FMDV particles consist of 60 copies each of four capsid proteins (VP1-VP4) which encapsidate a single, positive-sense RNA genome (Belsham, 1993; Fry *et al*., 2005). Two families of cellular receptors-integrins and Heparan Sulfate Proteoglycans (HSPG) have been found to mediate FMDV infection (Ruiz-Saenz *et al*., 2009). The tripeptide Arg-Gly-Asp (RGD) located in the VP1 GH loop is the signature recognition motif for the integrin receptor used by FMDV for cell attachment (Mason *et al*., 1994). FMDV type Asia l/HN/2006 carries RGD receptor recognition sites from naturally infected pigs. Its characteristics and the underlying molecular mechanism by which it infects pigs are not yet well understood. Evidence suggests that the Asian-type FMDV generally infects cattle and sheep but it rarely infects pigs (Zhang *et al*., 2008). The viral receptor plays a major role in both FDMV host and tissue tropism (Duque *et al*., 2004).

Integrins are α and β heterodimeric adhesion receptors that relay signals bidirectionally across the plasma membrane between the extracellular matrix and cell-surface ligands and cytoskeletal and signaling effectors (Campbell and Humphries, 2011). The physical and chemical signals regulated by integrins are essential for intercellular communication and support all aspects of metazoan existence. To mediate such diverse functions, integrins exhibit structural diversity, flexibility and dynamism. Conformational changes as opposed to surface expression or clustering are central to the regulation of receptor function (Askari *et al*., 2009). Four integrins (αυβ1, αυβ3, αυβ6 and αυβ8) have been identified as FMDV receptors (Ruiz-Saenz *et al*., 2009).

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Integrin $\alpha\upsilon\beta3$ was originally identified as a cellular receptor for FMDV. The roles of $\alpha\upsilon\beta3$ and $\alpha\upsilon\beta6$ as FDMV cellular receptors have been studied in depth and the role of $\alpha\upsilon\beta8$ has been studied to a satisfactory extent. Studies have reported that virulent FMDV utilizes the $\alpha\upsilon\beta3$ integrin as a primary receptor to initiate infection of cultured cells and that adaptation of FMDV O1 to cell culture results in the ability of the virus to utilize heparan sulfate as a receptor with concomitant loss of virulence (Neff *et al*., 2000). Integrin $\alpha\upsilon\beta6$ which is expressed on epithelial cells was subsequently identified in an *in vivo* study as a major candidate for use as an FMDV receptor (Jackson *et al*., 2000). The infection of $\alpha\upsilon\beta6$-expressing cells (transfected with cDNAs encoding $\alpha\upsilon\beta6$) by FMDV occurs in association with the integrin in vesicular structures in a clathrin-mediated endocytosis pathway. It followed by acidification within endosomes that facilities viral replication, causing the breakdown of the viral capsid structure and genome release by a hitherto unidentified mechanism (Berryman *et al*., 2005; O'Donnell *et al*., 2005). Integrin $\alpha\upsilon\beta8$ can also function as a receptor for FMDV (Jackson *et al*., 2004). The involvement of $\alpha\upsilon\beta8$ in infection was confirmed by demonstrating that virus attachment to the transfected cells could be inhibited by function-blocking monoclonal antibodies specific for either $\alpha\upsilon\beta8$ or $\alpha\upsilon$. In contrast, although $\alpha\upsilon\beta1$ has been identified as a receptor for FMDV, very limited data are available for this integrin since its expression appears to be restricted in a cell-specific manner and while several cell types express both subunits of this integrin in excess few cell types express this heterodimer (Vogel *et al*., 1990; Sheppard *et al*., 1992; Jackson *et al*., 2002). Therefore, researchers used a highly efficient lentiviral-based gene transfer technique to deliver murine *αυ*, *IRES* and *β1* genes into cell chromosomes so that they can be transcribed from the CMV promoter. Here, researchers attempted to establish a cell line with which we could obtain stable and controlled expression of $\alpha\upsilon\beta1$ and evaluated the susceptibility of this cell line to FMDV type Asia 1/HN/2006.

MATERIALS AND METHODS

Cells and viruses: Chinese Hamster Ovary clone K1 (CHO-K1) cells were cultured in Ham's F12 (HyClone) medium containing 10% fetal bovine serum (FBS; Gibco), 200 U mL^{-1} penicillin, 0.2 mg mL^{-1} streptomycin and 100 U mL^{-1} mycostatin. FMDV type Asia 1/HN/2006 was isolated from naturally infected pigs in China during the 2006 outbreak and propagated on baby hamster kidney cell line (BHK-21) cells grown in Dulbecco's Modified Eagle's Minimal Medium (DMEM) (invitrogen, USA) supplemented with 2% FBS, 200 U mL^{-1} penicillin, 0.2 mg mL^{-1} streptomycin and 100 U mL^{-1} mycostatin. Cell culture supernatants were harvested, frozen and thawed 3 times and the cell debris was removed by centrifugation at 500×g for 10 min. The supernatants which contained the virus were stored at -70°C for later use.

Construction of a lentivirus recombinant plasmid: To obtain the coding region of murine integrin subunits $\alpha\upsilon$ and $\beta1$, genomic RNA was extracted from tongue or lung tissues with an RNeasy Mini kit (Qiagen, Germany) according to the manufacturer's instructions. The tissues had been previously obtained from suckling mice. The cDNAs of murine *αυ* and *β1* genes were synthesized using AMV reverse transcriptase (20 U mL^{-1}, Takara, Japan) with the oligo-dT18 primer (20 pmol mL^{-1}) and a random primer (20 pmol mL^{-1}) in a 40 μL reaction mixture, according to the manufacturer's instructions (Du *et al*., 2009). These cDNAs were then used as templates for amplification of full lengths of the *αυ* (3135 bp) and *β1* (2397 bp) genes with the following PCR parameters: A pre-denaturation step of 95°C for 5 min; 35 cycles of 95°C for 5 min, 58°C for 30 sec, 72°C for 4 min; followed by the final extension step of 72°C for 10 min. The primers used to amplify the *αυ* and *β1* genes were αυF and αυR, β1F and β1R, respectively. The amplified PCR products were separately cloned into the pGEM-T easy vector (Promega, USA).

As shown in Fig. 1, pOK_{12} was chosen as the transition carrier and the IRES sequence was used as the bridge connecting $\alpha\upsilon$ and $\beta1$ in the construction of the lentivirus recombinant plasmid. First, the pOK_{12} fragment was amplified from the pOK_{12} plasmid with primers POK-F and POK-R bearing four restriction enzyme sites (XbaI, MluI, NheI and NotI) and the IRES fragment, from pIRES2-EGFP with primers IRES-F and IRES-R. The IRES fragment was then cloned into pOK_{12} to generate pOK-IRES which was confirmed by PCR and digestion with restriction enzyme. Second, an $\alpha\upsilon$ fragment containing NheI and NotI restriction sites was generated from recombinant pGEM-T containing the *αυ* coding gene, using primers pL-αυF and pL-αυR. Plasmid pOK-IRES was linearized after digestion with NheI and NotI. The earlier mentioned PCR product was also digested with NheI and NotI and cloned into the pOK-IRES plasmid to generate recombinant plasmid pOK-αυ-IRES which was subsequently confirmed by PCR and digestion with restriction enzyme. Third, the recombinant plasmid pOK-αυ-IRES was digested by NotI and XbaI to obtain the αυ-IRES fragment which was subsequently cloned into pLVX-Tight-Puro vector

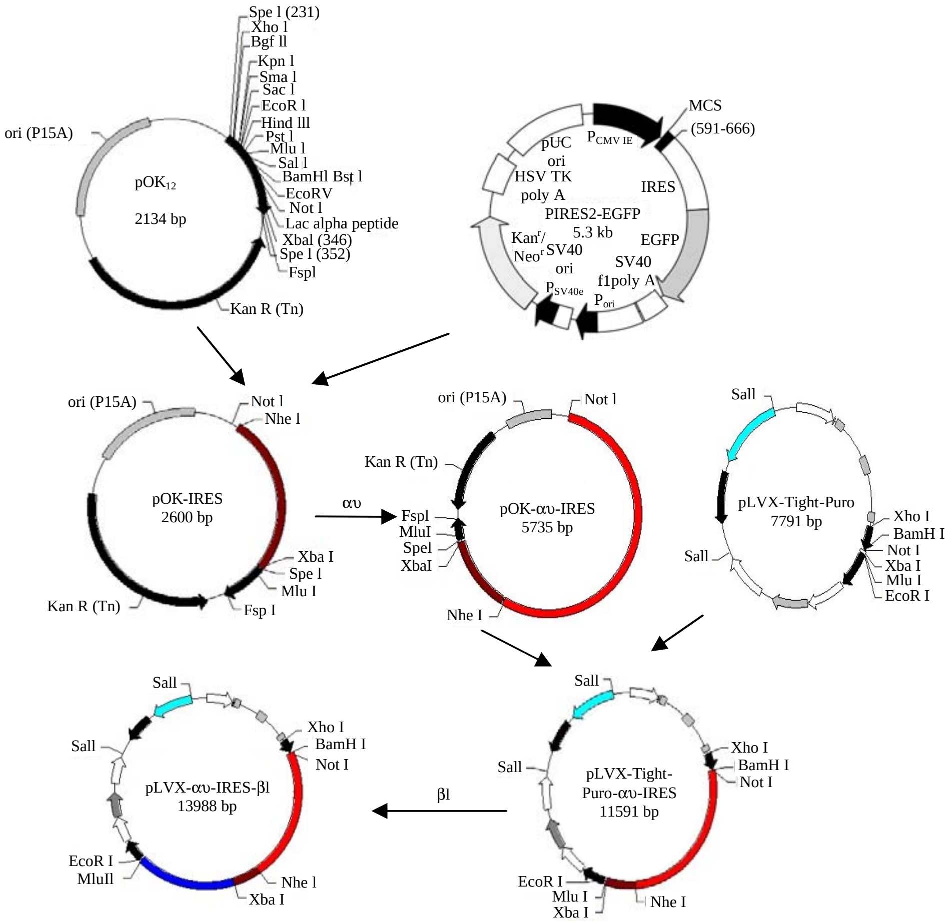

Fig. 1: Schematic diagram of the construction of a lentivirus recombinant plasmid

Table 1: Primer used for RT PCR or PCR amplification

Primer	Sequence (5′-3′)	Restriction enzyme
αυF	ATGGCTGCTCCCGGGCGC	-
αυR	TCAGGTTTCAGAGTTTCCTTCGCCATT	-
β1F	ATGAATTTGCAACTGGTTTTCTGGA	-
β1R	TCATTTTCCCTCATACTTCGGATTG	-
pL-αvF	CATTTTATTTT*GCGGCCGCGCCACC*ATGGCTGCTCCCGGGCGC	NotI
pL-αυR	CGTCTA*GCTAGC*TCAGGTTTCAGAGTTTCCTTCGCCATT	NheI
pL-β1F	CGG*TCTAGAGCCACC*ATGAATTTGCAACTGGTTTTCTGGA	XbaI
pL-β1R	GTTGTCG*ACGCGT*TCATTTTCCCTCATACTTCGGATTG	MluI
IRES-F	CGTCTA*GCTAGC*GCCCCTCTCCCTCCCCCCCCCCTAA	NheI
IRES-R	CGG*TCTAGA*TGTGGCCATATTATCATCGTGTTTTTCAA	XbaI
POK-F	GCTTGGGCCC*TCTAGA*TTGTGCACT*ACGCGTG*AACTAGTGGATCGATCCCCAATTCG	XbaI and MluI
POK-R	TTTTGGGCCC*GCTAGC*TTTGTACAT*GCGGCCGCA*GTGGCGTAATCATGGTCATAGCTGTT	NheI and NotI

*Letter in italics represent restriction enzyme sites and Kozak sequences

(Clontech, USA) resulting in generation of the recombinant plasmid pLVX-αυ-IRES. Finally, the β1 fragment containing the XbaI and MluI sites was generated from recombinant pGEM-T containing the *β1*-coding gene by using primers pL-β1F and pL-β1R. The amplified β1 fragment was digested with XbaI and MluI and cloned into pLVX-αυ-IRES to generate the recombinant plasmid pLVX-αυ-IRES-β1. All the products were sequenced using an ABI-Prism 377 DNA Sequencer (Applied Biosystems, USA). The primers used in this study are listed in Table 1.

Establishing an inducible expression system on CHO-K1 cells: To establish the CHO-K1-αυβ1 cell line, CHO-K1 cells were simultaneously co-transduced with the regulator and response lentivirus vectors (Clontech, USA) for 12 h according to the manufacturer's instructions. The virus-containing medium was then removed and replaced with DMEM supplemented with 10% "Tet System-Approved FBS" (Clontech, USA). The cells were cultured without Dox for 48 h and harvested for analysis. The expression of the αυβ1 heterodimer in co-transduced CHO-K1 cells (designated as LV-CHO-K1) was analyzed by indirect Immunofluorescence Assay (IFA), the cells were incubated with a 1:20 dilution of the polyclonal antibody of mice β1 protein (it was preserved in the laboratory) for 1 h at RT, FITC-monoclonal rat anti-mouse IgG1 (invitrogen) at a 1:100 dilution was then added for 1 h at RT as described previously (Zheng *et al*., 2009).

Establishing stable and inducible αυβ1 expression in CHO-K1 cells: To select a stable αυβ1 transgenic CHO-K1 cell line, a single clone of LV-CHO-K1 cells were cultured under the selection pressure of 500 μg mL^{-1} G418 and 2 μg mL^{-1} puromycin. Cell-cloning islands were observed after approximately 15 days. After continuously cloning for twenty times, stable αυβ1 transgenic CHO-K1 cell line was obtained (designated as CHO-K1-αυβ1). After twenty passages of CHO-K1-αυβ1 cells, the presence of *αυ* and *β1* genes in CHO-K1-αυβ1 cells was analyzed by PCR assay using the primers αυF and αυR and β1F and β1R. Furthermore, the expression of αυβ1 on CHO-K1-αυ β1 cells was confirmed by IFA as mentioned before. The inducibility of αυβ1 expression on CHO-K1-αυβ1 was analyzed by flow cytometry. Briefly, CHO-K1-αυβ1 cells obtained were seeded in six-well plates at a cell density of 1×10^6 cells/well, each of which contained fresh medium with or without 500 ng mL^{-1} Dox. The cells were incubated for 48 h, harvested with Ethylene Diamine Tetraacetic Acid (EDTA) and resuspended at a concentration of 1×10^7 cells mL^{-1} in Tris-buffered saline (pH 7.4) containing 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 2% normal goat serum and 3% bovine serum albumin (buffer A). The cells were incubated with a 1:20 dilution of the polyclonal antibody of β1 protein (it was preserved in the laboratory) on ice for 20 min. FITC-monoclonal rat anti-mouse IgG1 (invitrogen) at a 1:100 dilution was then added for 1 h at RT. Background fluorescence was determined in the absence of the primary antibody. The fluorescence staining was assessed by flow cytometry with a FACSCalibur (Becton Dickinson) by counting 10,000 cells per sample.

Infectivity assays: CHO-K1-αυβ1 and CHO-K1 cells were seeded at 1-2×10^6 cells/well in six-well plates and incubated for 16 h at 37°C with 5% CO_2. The monolayers were washed with phosphate-buffered saline (PBS; pH 7.5) containing 2 mM $CaCl_2$ and 1 mM $MgCl_2$. Subsequently, the FMDV growth curve in CHO-K1-αυβ1 cells was determined and the results were compared to the corresponding growth curve in CHO-K1 cells. Both cells were prepared as described before and inoculated with the FMDV at 1.0×10^5 $TCID_{50}$. After adsorption for 1 h, cell monolayers were washed 3 times with PBS and cultured in DMEM supplemented with 2% FBS (500 μL/well) at 37°C in a 5% CO_2 atmosphere. The viral supernatants were harvested at 1, 6, 12, 24, 36, 48 and 60 h after inoculation and sampling at each time point was carried out in triplicate. Total RNA was extracted from the cells using a QIAxtractor Kit (Qiagen, Germany), according to the manufacturer's instruction. One-step rRT-PCR was performed on each sample according to the procedure described previously (Shaw *et al*., 2007). The results from all samples were analyzed using Stratagene® MxPro™ QPCR software and a CT value was assigned to each reaction as described previously (Reid *et al*., 2002). A standard plaque assay was used to determine the pathogenicity of FMDV on CHO-K1 and CHO-K1-αυβ1 cells (Pacheco *et al*., 2003; Li *et al*., 2011). Both cells were prepared as described earlier and inoculated with 10-fold serial dilutions of 200 μL FMDV in PBS containing 1% calf serum. Infectious centers were visualized as plaques by fixation and crystal violet staining. CHO-K1 cells generally express heparan sulfate but not αυβ1 (Lawrence *et al*., 2013). In order to evaluate the relationship between the susceptibility of CHO-K1-αυβ1 cells to FMDV infection and integrin αυβ1, 1 and 2 mg mL^{-1} sodium heparin (sigma) was used to inhibit the expression of heparan sulfate.

RESULTS AND DISCUSSION

Analysis and identification of the lentivirus recombinant plasmid: As shown in Fig. 2a, pOK_{12} fragments (2134 bp) were amplified from the pOK_{12} plasmid using primers POK-F and POK-R containing four restriction enzyme sites (XbaI, MluI, NheI and NotI) and the 585 bp IRES fragment was amplified from pIRES2-EGFP with primers IRES-F and IRES-R. The IRES fragment was then cloned into pOK_{12} to generate pOK-IRES which was confirmed by PCR and digestion with restriction enzyme (Fig. 2b). The αυ fragment containing the NheI and NotI restriction enzyme sites was generated from recombinant pGEM-T containing an *αυ*-coding gene using primers pL-αυF and pL-αυR. Plasmid pOK-IRES was linearized after digestion with NheI and NotI. The PCR product mentioned earlier was digested with NheI and NotI as well and then cloned into plasmid pOK-IRES to generate recombinant plasmid pOK-αυ-IRES which was subsequently confirmed by PCR (Fig. 2c) and digestion with restriction enzyme (Fig. 2d).

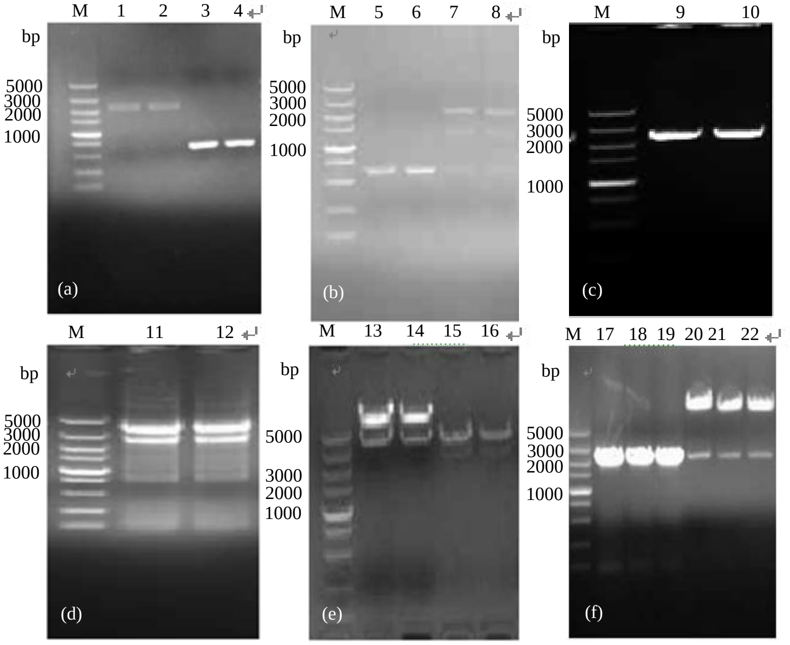

Fig. 2: a) PCR products of the pOK$_{12}$ (Lane 1-2) and IRES (Lane 3-4) fragments; b) PCR (Lane 5-6) and enzyme (Lane 7-8) identification of the pOK-IRES recombinant plasmid; c) PCR (Lane 9-10) identification of pOK-*α*υ-IRES recombinant plasmid; d) Enzyme (Lane 11-12) identification of pOK-*α*υ-IRES recombinant plasmid; e) enzyme (Lane 13-14) and PCR (Lane 15-16) identification of pLVX-*α*υ-IRES recombinant plasmid; f) PCR (Lane 17-19) and enzyme (Lane 20-22) identification of pLVX-*α*υ-IRES-β1 recombinant plasmid; Lane M, molecular weight marker fragment (Takara)

The recombinant plasmid pOK-αυ-IRES was then digested by NotI and XbaI to obtain the αυ-IRES fragment which was subsequently cloned into pLVX-Tight-Puro vector, resulting in the generation of recombinant plasmid pLVX-αυ-IRES. Identification of recombinant pLVX-αυ-IRES vector (Fig. 2e) confirmed the construction of a lentiviral recombinant vector carrying exogenous *αυ* gene. Finally, the β1 fragment containing XbaI and MluI restriction enzyme sites was generated from recombinant pGEM-T containing *β1-coding* gene, using primers pL-β1F and pL-β1R. The amplified β1 fragment was digested with XbaI and MluI and cloned into pLVX-αυ-IRES to generate recombinant plasmid pLVX-αυ-IRES-β1. All the products were sequenced.

Analysis of the inducible expression system: The expression of αυβ1 heterodimer on co-transducing CHO-K1 cells (designated as LV-CHO-K1) was analyzed by IFA. As shown in Fig. 3, LV-CHO-K1 cells were stained (Fig. 3a) while the parental CHO-K1 cells were not (Fig. 3b), indicating that the lentiviral-based gene expression system was successfully constructed in CHO-K1 cells.

Analysis of CHO-K1-αυβ1 cells line: As shown in Fig. 4a and b, the PCR products of the *αυ* (3135 bp) and *β1* (2397 kb) genes were identified by electrophoresis on 1% agarose gel. Sequencing results revealed no mutations in αυ and β1. These data demonstrated that both genes integrated stably into the cellular chromosome of CHO-K1 cells. Furthermore, IFA was used to confirm αυβ1 overexpression in CHO-K1-αυβ1 cells (Fig. 5). The inducibility of αυβ1 expression on CHO-K1-αυβ1 cells was analyzed by flow cytometry. Normal CHO-K1 cells were negative for αυβ1 protein (Fig. 6a). CHO-K1-αυβ1 cells that did not receive Dox treatment were positive for αυβ1 protein (Q2+Q4 = 37.0%; Fig. 6b). However, 500 ng mL^{-1} Dox-treated CHO-K1-αυβ1 cells showed a dramatic decrease in αυβ1 protein expression (Q2+Q4 = 1.7%; Fig. 6c). These data demonstrated the inducibility of αυβ1 expression in CHO-K1-αυβ1 cells.

Analysis of the infectivity of FMDV type Asia I/HN/2006 on CHO-K1-αυβ1: As shown in Fig. 7a, the FMDV RNA level in CHO-K1-αυβ1 cells peaked at 36 h after inoculation which is considerably higher than that in CHO-K1 cells. After the peak was achieved, both viral RNA level and the virus titers began to decline. The level

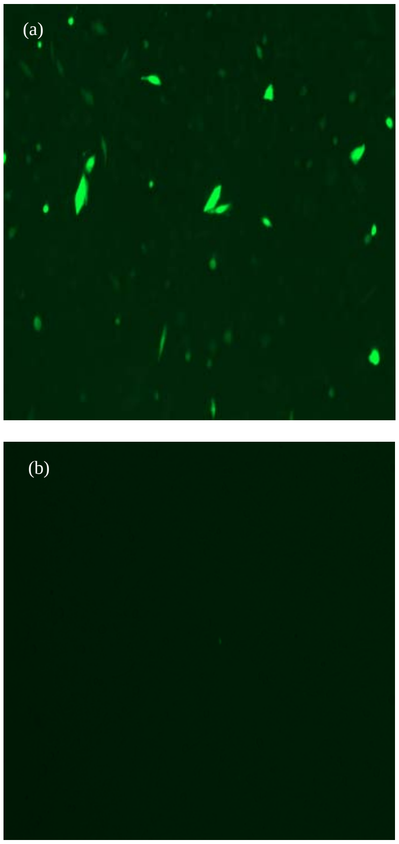

Fig. 3: a) Specific fluorescence of LV-CHO-K1 cell is observed at ×200 magnification; b) No fluorescence detected in parental CHO-K1 cells (control)

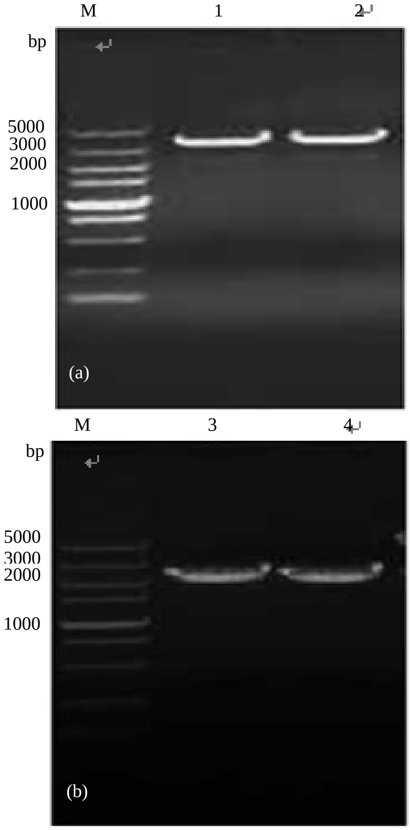

Fig. 4: PCR products from CHO-K1-αυβ1 cells at the 20th passage were electrophoresed in 0.8% agarose gels; a) PCR products of the *αυ* (3135 bp) gene; b) PCR products of *β1* (2397 bp) gene

of FMDV RNA in CHO-K1 was significantly affected by sodium heparin (Fig. 7b). In addition, a standard plaque assay was used to characterize the pathogenicity of FMDV on CHO-K1 and CHO-K1-αυβ1 cells. These results indicated that CHO-K1-αυβ1 cells are susceptible to FMDV infection. CHO-K1-αυβ1 cells were more susceptible to infection than CHO-K1 cells. As shown in Fig. 8, the virus produced bigger and more plaques in CHO-K1-αυβ1 cells (1.05×10^4 PFU mL^{-1}) than in CHO-K1 cells. When sodium heparin was used as the inhibitor, there were significantly fewer plaques on CHO-K1 (from 4.0×10^3-35 and 20 PFU mL^{-1}) while the number and size of plaques on CHO-K1-αυβ1 cells showed no obvious change, indicating that FMDV can utilize αυβ1 which is expressed on CHO-K1-αυβ1 cells as a receptor.

FMDV has been earlier reported to use four αυ integrins αυβ3, αυβ6, αυβ1 and αυβ8 as cellular receptors (Ruiz-Saenz *et al*., 2009). Although, the crystal structure of the extracellular segment of integrin αυβ3 in complex with an RGD ligand is known, the roles of its binding and functional domains in FMDV infection remain not to be fully understood. Since, cells frequently express multiple receptors, the role and function of a single receptor in mediating FMDV infection is unclear. The functional differences among receptors of different hosts cannot be compared. Therefore, it is vital to establish an inducible expression system of a single receptor. In addition, compared integrin αυβ6 (Miller *et al*., 2001; King *et al*., 2011), αυβ1 is difficult to study since its expression appears restricted to specific cells and although, several cell types express both subunits in excess, only a few cells express this heterodimer (Vogel *et al*., 1990; Sheppard *et al*., 1992; Jackson *et al*., 2002). Therefore, it would be of considerable significance to construct a cell line that stably expresses integrin αυβ1 heterodimer.

Nevertheless, previous research has focused on using transient expression of integrin subunits to study receptors and FMDV infection (O'Donnell *et al*., 2005). Fortunately in the study, we were able to obtain a cell line that could stably expressing integrin αυβ1 heterodimer and the expression level could be controlled. This cell

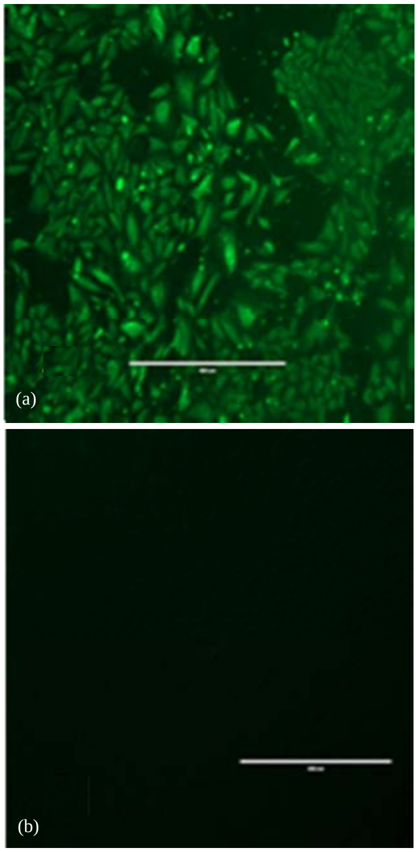

Fig. 5: Expression of the αυβ1 heterodimer detected by immunofluorescence assay; a) Specific fluorescence observed at ×200 magnification; b) No fluorescence detected on parental CHO-K1 cells (controls)

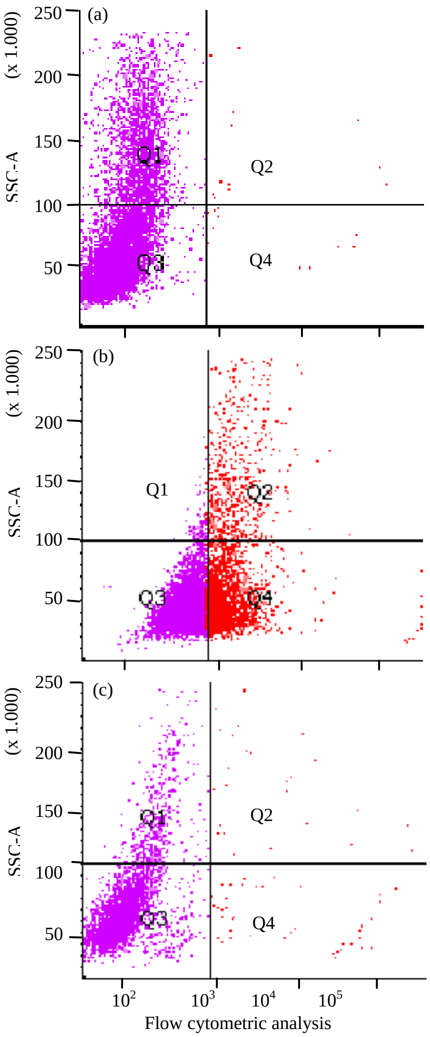

Fig. 6: Flow cytometric analysis of Dox-induced αυβ1 expression; a) Normal CHO-K1 cells were analyzed as the negative control; b) In CHO-K1-αυβ1 cells without Dox, αυβ1 expression was normal; c) In the presence of 500 ng mL^{-1} Dox, there was a sharp decrease in the αυβ1 expression in CHO-K1-αυβ1 cells

line was established by using a lentivirus-based inducible expression system. The inducible expression system has many advantages. For instance, it includes a novel, more highly developed and refined transactivator (Urlinger *et al.*, 2000) and incorporates an improved inducible promoter, P_{Tight}. P_{Tight} consists of a modified tet-responsive element that is composed of seven direct repeats of an altered tetO sequence joined to a modified minimal CMV promoter. P_{Tight} also lacks binding sites for endogenous mammalian transcription factors, so it is virtually silent in the absence of induction. Upon induction in the absence of Dox, tTA-advanced binds to the P_{Tight} promoter on the response vector, activating transcription of the downstream gene. Another advantage in this cell line is that αυ and β1 subunits are connected by the IRES sequence, making both the subunits express within the same cell environment, controlled by the same promoter. This may help form the αυβ1 heterodimer. Furthermore, researchers could use this newly developed cell line and CHO-K1 cells expressing heparan sulfate but not αυ integrins (Lawrence *et al.*, 2013) to study FMDV type Asia 1/HN/2006.

FMDV type Asia 1/HN/2006 was first isolated from naturally infected pigs. It generally infects cattle and sheep but rarely infects pigs (Zhang *et al.*, 2008). The first recorded instance of FMD virus type Asia 1 infection in pigs was reported in Hong Kong, China in March, 2005 (Valarcher *et al.*, 2005). Subsequently, this type of virus was reported in mainland China in April, 2005 (Guo *et al.*, 2006). There were subsequent reports of this virus in pigs

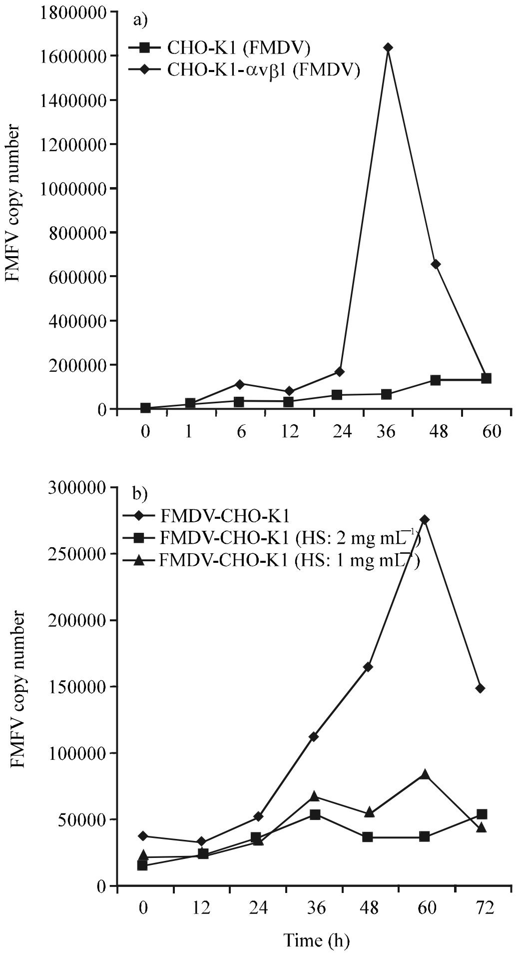

Fig. 7: Time-course of replication and proliferation. The standard curve function ($y = -3.416 \log(x)+42.85$) was used to calculate copy numbers of FMDV to obtain a virus replication curve: a) Copy numbers on CHO-K1 and CHO-K1-αυβ1 cells inoculated with 1.0×105 TCID50 FMDV; b) After adding 1 and 2 mg mL^{-1} sodium heparin, copy numbers on CHO-K1 were calculated, inoculating FMDV at 5.0×106 TCID50

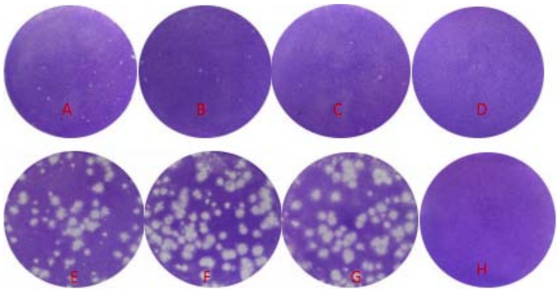

Fig. 8: Differences in plaque formation after inoculation with FMDV: A) Plaques on CHO-K1 cells lacking sodium heparin; B) Plaques on CHO-K1 cells incubated with 1 mg mL^{-1} sodium heparin; C) Plaques on CHO-K1 cells incubated with 2 mg mL^{-1} sodium heparin; D) Uninfected CHO-K1 cells showed no plaques; E) Plaques on CHO-K1-αυβ1 cells incubated without sodium heparin; F) Plaques on CHO-K1-αυβ1 cells incubated with 1 mg mL^{-1} sodium heparin; G) Plaques on CHO-K1-αυβ1 cells incubated with 2 mg mL^{-1} sodium heparin; H) Uninfected CHO-K1-αυβ1 cells showed no plaques

in Russia (2005-2006) and North Korea (July, 2007). By July, 2008, FMD virus type Asia 1 was detected in >15 areas of China and 16 areas of Russia, resulting in severe economic and social consequences and continuing to threaten FMD-free regions (Valarcher *et al*., 2009). Therefore, the study of this unusual phenomenon is of great importance. In addition, the ability to propagate FMDV type Asia 1/HN/2006 plays an important role in laboratory diagnosis and vaccine production to control the spread of this FMDV. Expression of specific receptors is often an important determinant of a cell's susceptibility to viral infection and of virus tropism (Carrillo *et al*., 1984; Mason *et al*., 1994; Fry *et al*., 1999). Field isolates of FMDV initiate infection by attaching to integrin receptors on the surface of a susceptible cell (Jackson *et al*., 2000). To the best of the knowledge, the expression differs strikingly among species, although the inter-species differences are minor. At present, some researches mainly concentrates on integrins receptor of cattle, porcine, sheep (Du *et al*., 2010). While very limited data are available for sucking mice integrins. The FMDV is lethal for suckling mice which promotes their use as an animal model of propagating the virus.

CONCLUSION

In this study, researchers successfully established a CHO-K1 cell line stably expressing αυβ1 from suckling mice. CHO-K1-αυβ1 cells were more susceptible to FMDV infection than CHO-K1 cells. FMDV type Asia 1/HN/2006 FMDV displayed an extended receptor range including αυβ1 and heparan sulfate, although integrins were used preferentially in the presence of both heparan sulfate and integrin receptors, indicating the ability of FMDV to use αvβ1 expressed on CHO-K1-αυβ1 cells as a receptor for infection.

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